THESIS

ANALYZING EARLY CANCER ETIOLOGY IN GOLDEN RETRIEVERS USING GOLDEN RETRIEVER LIFESPAN STUDY (GRLS) DATA

Submitted by

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ABSTRACT

ANALYZING EARLY CANCER ETIOLOGY IN GOLDEN RETRIEVERS USING GOLDEN RETRIEVER LIFESPAN STUDY (GRLS) DATA

Background: Although cancer is a burden in both humans and dogs, humans medicine is characterized by established health care organizations, interdisciplinary networks, and databases from which data and research can be complied and shared. No such organization exists in veterinary medicine. Individual registries provide useful data and information on cancer in dogs, but no mechanism exists to summarize data to detect cancer trends, breed-specific measurements of occurrence, and treatment responses. Therefore, there are vast knowledge gaps related to cancers in dogs, especially among early cases. The Golden Retriever Lifetime Study (GRLS) data collected by Morris Animal Foundation is a unique opportunity to evaluate cancer prevalence in a large number of golden retrievers with known pedigree. Data were evaluated for each state and compared to human cancer prevalence provided by the CDC. Differences in cancer prevalence between young and old dogs was evaluated, along with their resident state, sex status, and cancer type. Golden retrievers were recruited from 2012-2015 to participate in the GRLS cohort study and were confirmed to be free of life limiting conditions by a veterinarian. Owners had to have at least a 3-generation pedigree of their dog to be enrolled. Information regarding the dog's health and condition were recorded annually via owner and veterinarian questionnaire, as well as sample collections, and added to the GRLS study data. The GRLS data

was refined and cleaned in SAS and R studio evaluate state of diagnosis, age at diagnosis, and sex at diagnosis. The highest prevalence of cancer among GRLS participants was in Louisiana (38.5%) with Arizona as the second highest (17.5%). A cluster of higher prevalence regions were observed in the upper east coast, similarly to the CDC's human data. Although the prevalence was highest in Louisiana and Arizona, neither were found to be statistically significant based on the difference of proportion calculations. A statistically significant difference was found in average age at diagnosis between male neutered and intact cancer dogs, but not when comparing female spayed and intact cancer bearing dogs or when comparing all 4 sex statuses. The average age at diagnosis based on tumor types (mammary, hemangiosarcoma, histiocytoma, lymphoma) was significantly different, most likely due to higher numbers of hemangiosarcoma cases in older dogs and histiocytoma cases observed in younger dogs. Older, male neutered dogs were more susceptible to hemangiosarcoma development (85.5% of cases were old), and younger dogs that had been spayed or neutered were more susceptible to histiocytomas (100% of cases were young). **Discussion:** One of the interesting findings of this analysis was that there was a statistically significant difference in average age at diagnosis between intact and neutered male dogs, but not between intact and spayed females. Small sample size of cancer dogs could have impacted the power of statistical test results and been a contributor to statistical insignificance seen throughout the analysis. Dogs moving multiple times throughout the duration of the study can affect interpretations and implications from prevalence by state findings. Prevalence was also calculated using only the total GRLS study population the resided in respective states as the denominator, effecting generalizability of the analysis findings.

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CHAPTER ONE

Overview

The purpose of this chapter is to give a background on cancer, the study of cancer in dogs and humans, and to serve as a literature review of cancer studies and findings related to cancers in dogs and humans. The defining characteristics of cancer, how cancer develops biologically, potential risk factors associated with the development of cancer, and the various forms of cancer are discussed. Through the progression of this chapter, cancers in humans will be discussed for each topic and how it relates to dogs will be discussed thereafter, after which focus on golden retrievers will be reviewed if information is available and breed specification is relevant.

The Biological Development of Cancer

Dogs truly are man's best friend, as the old saying goes, and so much so that we share a similar burden. Cancer (neoplasia) is the second leading cause of death in humans and the leading cause of death in dogs (Sarver et al, 2022, Gardener et al, 2016). Cancer is, essentially, characterized by cells that have evaded death or mechanisms that are meant to control cell growth and size. Normal cells either transition into senescence, where they simply stop growing, or die after they reach a certain number of cell divisions, reflecting the cell's age or likelihood for damage. In its place, a new cell would form. However, if this damaged cell does not stop growing or die, this would then result in what are termed "immortal cells", i.e., cancer cells.

Cancer occurs when there is an abnormality in cells, usually caused by the mutation of DNA. These cells can accumulate and form into tumors, which is a mass of tissue made up of the dividing cancer cells. However, whether a tumor forms or not depends on the type of cancer that forms. Once cancer cells exist, they can spread to other parts of the body (metastasize), where

they can form additional tumors wherever the cancer cells settle. Tumors can either be benign or malignant. Tumors that are benign still grow and accumulate in a way that is abnormal but have a distinct border and do not invade neighboring tissues or spread to other parts of the body (Patel, 2020). Benign tumors are seen to be less fatal, due to their non-invasive nature, however they can still cause issue if grown to a particular size, as they can still cause compression and press on nearby organs (Patel, 2020). Tumors that are malignant, however, can invade neighboring tissue and spread to other parts of the body due to cancer cells traveling through the blood and lymphatic system (Patel, 2020).

There is a list of cancer capabilities and enabling characteristics that are known as the hallmarks of cancer. The most recent update of this framework was in 2022. These hallmarks were formed to describe commonalities that one can find between all forms of cancer cells (Hanahan, 2022). They list a set of functions and capabilities cells have as they develop into a state of malignancy, and how they are able to do so. It is presumed that similar capabilities can be seen in dogs as well, considering similarities between dogs and humans with tumor characteristics such as clinical presentation, histological features, molecular profiles, and response and resistance to therapy, as well as the evolution of drug-resistant metastases (Gardener et al, 2016). There are currently 8 hallmark capabilities and 2 enabling characteristics, and 4 emerging hallmarks:

Table 1a: Hallmark Capabilities of Cancer

8 Hallmark Capabilities:	
Capability	**Meaning**
Sustaining Proliferative Signaling	Able to maintain a signaling system that regulates cell size, shape, and composition as opposed to eventually dying off (apoptosis) as normal cells would. (Duval, 2023)
Evading growth suppressors	Growth suppressors regulate cell growth and prevent them from growing past a certain size, and tumor cells evade this regulation so they can continue growth. (Duval, 2023)
Avoiding immune destruction	Cancer cells can slip under the radar of the immune system, allowing them to avoid being destroyed by the immune system. (Duval, 2023)
Enabling replicative immortality	After a certain number of cell replications, normal cells will senesce (stop growing) or enter a crisis and die, but cancer cells replicate unlimitedly, making them immortal. (Duval, 2023)

Activating invasion & metastasis	Benign tumors are not invasive; however, malignant tumors are invasive and have the ability to spread throughout the body to another site (metastasis). (Duval, 2023)
Inducing angiogenesis or accessing vasculature	Angiogenesis is the process of forming new blood vessels, and is important in wound healing, menstruation, inflammation, growth of embryos, and the formation of tumors. In accomplishing these tasks, the cells and tissues need nutrients and energy while also being able to release metabolic waste and carbon dioxide. Angiogenesis satisfies this need. Forming these new blood vessels ensures the tumor has the nutrients to grow and metastasize. (Samarasinghe, 2013, Eldridge, 2023)
Resisting Cell Death	Normal cells die after a certain number of cellular replications, or when damaged beyond repair. Cells can die and be rid of from the body through processes such as: apoptosis (programmed cell death), autophagy (cell degradation), and necrosis (cell damage), and cancer cells avoid all of them. (Duval, 2023)
Deregulating cellular metabolism	Alterations in the patterns of the cellular metabolic pathways can lead to the exacerbated production of more macromolecules, increased cell proliferation, and resistance to treatment due to alteration in drug processing (Serrano-Carbajal et al, 2020).

Table 1b: Enabling Characteristics of Cancer	
2 Enabling Characteristics:	
Enabling Characteristic	**Meaning**
Genome instability & mutation	A lot of the hallmark capabilities above rely on the alteration of the genomes of cancer cells. Alterations in the genotype of cancer cells enable their outgrowth and can eventually allow them to dominate local tissue environments (Hanahan & Weinberg, 2011).
Tumor-promoting inflammation	Neoplastic lesions (abnormal growth of tissue) contain immune cells at varying densities, from by subtle infiltration of immune cells (both adaptive and innate) that would only be detectable with a cell type specific antibodies test to gross inflammations that can be observed via histochemical staining techniques. Tumor induced inflammatory responses enhance tumorigenesis and progression, helping neoplasia acquire hallmark capabilities (Hanahan & Weinberg, 2011).

Table 1c: Emerging Hallmarks of Cancer	
4 Emerging Hallmarks & Enabling Capabilities (as of January 2022):	
Emerging Hallmark	**Meaning**
Unlocking Phenotypic Plasticity	Increased evidence has shown that a critical component of cancer pathogenesis is being able to alter its appearance/form, which is normally restricted, in order to evade or escape terminal differentiation (permanent cell cycle arrest) (Hanahan, 2022).
Non-mutational Epigenetic Reprogramming	This enabling characteristic is another form of genome instability, but without mutation. Genome reprogramming only involves epigenetic regulation changes in gene expression, i.e., mutationless cancer evolution (Hanahan, 2022).
Polymorphic Microbiomes	Increased evidence that the polymorphic (having multiple forms) microbiomes that exist on barrier tissues within the body can have a great impact on health, disease, and cancer phenotypes. These microbiomes can have either a protective or deleterious effect on cancer development, malignant progression, and responses to therapy (Hanahan, 2022).

Senescent Cells	Senescent cells had been seen as protective against cancers, as cancer cells would be induced to undergo senescence. However, increased evidence has found that it can be the opposite in particular circumstances, and that senescent cells can stimulate tumor development and malignant progression (Hanahan, 2022).

The **hallmark capabilities** were formed and proposed as a means of describing the functional capabilities of human cells as they go from normal to a state of neoplastic growth. These capabilities are known to be crucial for cancer cells to form malignant tumors (Hanahan, 2022). The **enabling characteristics** are more so conditions of neoplasia that enable cancer cells and tumors to adopt their functional traits, rather than the traits and characteristics themselves as described by the hallmark capabilities (Hanahan, 2022). The **emerging hallmarks and enabling characteristics** are newer hallmark capabilities and enabling characteristics that are being proposed and may potentially become some of the core capabilities and enabling characteristics in the future, hence them being described as "emerging" (Hanahan, 2022).

Forms of Cancers in Dogs and Humans

Cancer can present in a multitude of forms, arising in various parts of the body. There are some cancers that have equivocal forms in both species, and other cancers that are unique to specific species. Some examples of cancer that can occur in both species are mammary, testicular, prostate, lymphomas, histiocytomas, lipomas, and leukemia. An example of a form of cancer that only occurs in dogs, or do not have a human equivalent that will also be talked about frequently in this paper are mast cell tumors (MCTs). The next section will go over the most prominent forms of cancer found within each species, and then golden retrievers specifically.

Most Common Cancers in Older Individuals (Young Adults to Seniors)

According to the American Cancer Society and the National Cancer Institute (NCI)'s Surveillance, Epidemiology, and End Result (SEER) Program, breast, lung and bronchus, prostate, and colorectal cancers are estimated to account for nearly 50% of new cancer cases and 45% of cancer deaths in 2023 across both sexes combined.

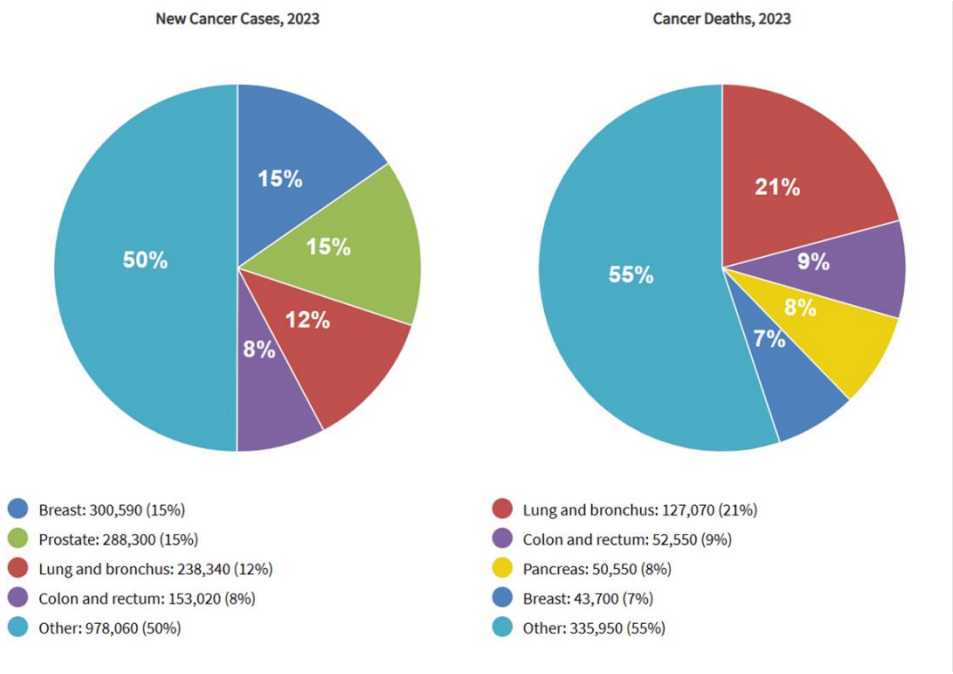

Figure 1 – Estimated New Cancer Cases and Cancer Deaths Percentages in the U.S. (2023)

National Cancer Institute (NCI). (2023a). *Common Cancer Sites—Cancer Stat Facts*. National Cancer Institute Surveillance, Epidemiology, and End Results Program. https://seer.cancer.gov/statfacts/html/common.html

In the years prior between 2016 and 2020, the rate of new cases for both sexes combined was 113.4 per 100,000 for prostate cancer (only men), 67.7 per 100,000 for breast cancer, 50 per 100,000 for lung and bronchus cancers, and 36.6 per 100,000 for colorectal cancer. Therefore, based on the 2016-2020 data, prostate cancer has the highest rate of new cases. Between 2016 - 2020, the death rates across both sexes combined were 35 per 100,000 for lung and bronchus cancer, 13.1 per 100,000 for colorectal cancer, 10.8 per 100,000 for breast cancer, and 11.1 per

100,000 for pancreatic cancer. All of these data were age adjusted to the 2000 US standard population (ACS, 2023). Below is a table visualizing this information in addition to a breakdown of these statistics by sex. Highlighted in blue are the highest counts and rates for each cancer site.

Table 2a: Data on Estimated New Cases and Deaths, and The Incidence and Mortality Rate between 2016 - 2020 in Both Sexes

Site	2023 Estimated New Cases (Count)	2023 Estimated Deaths (Count)	2016-2020 Rate of New Cases (per 100,000)	2016-2020 Mortality Rate (per 100,000)
Lung & Bronchus	238,340	127,070	50	35
Breast	300,590	43,700	67.7	10.8
Colorectal	153,020	52,550	36.6	13.1
Prostate (Men Only)	288,300	34,700	113.4	18.8
Pancreas	64,050	50,550	13.3	11.1

Table 2b: Data on Estimated New Cases and Deaths, and The Incidence and Mortality Rate between 2016 - 2020 in Females Only

Site	2023 Estimated New Cases (Count)	2023 Estimated Deaths (Count)	2016-2020 Rate of New Cases (per 100,000 females)	2016-2020 Mortality Rate (per 100,000 females)
Lung & Bronchus	120,790	59,910	45.3	29.3
Breast	297,790	43,170	126.9	19.6
Colorectal	71,160	24,080	32	11

Prostate (Men Only)	0	0	0	0
Pancreas	30,920	23,930	11.8	9.6

Table 2c: Data on Estimated New Cases and Deaths, and The Incidence and Mortality Rate between 2016 - 2020 in Males Only

Site	2023 Estimated New Cases (Count)	2023 Estimated Deaths (Count)	2016-2020 Rate of New Cases (per 100,000 males)	2016-2020 Mortality Rate (per 100,000 males)
Lung & Bronchus	117,550	67,160	56.4	42.2
Breast	2,800	530	1.2	0.3
Colorectal	81,860	28,470	42.1	15.7
Prostate	288,300	34,700	113.4	18.8
Pancreas	33,130	26,620	15.1	12.7

Of these cancers that have higher incidences, dogs can develop all of them. However, some forms may be rarer in dogs than in humans, such as lung & bronchus, and prostate cancer in male dogs. Lung cancer, especially primary lung tumors (tumors that originate in the lungs), are rare in dogs and are more common in a metastatic form (cancer that spreads to the lungs) (Kuehn, 2018). Increases have, however, been seen over the past 20 years (Kuehn, 2018). Prostate cancer in male dogs is also rare (Davis & Ostrander, 2014; Bryan et al, 2007), which will be discussed in more depth in the risk factors portion of this paper under Sex and Hormones.

Most Common Cancers in Children and Adolescents

Children and adolescents differ from older individuals in the most common form of cancer. In this paper, a child will be defined as an individual who is 0-14 years of age and adolescents (teenagers) as 15-19, consistent with the categorization by the NCI (NCI, 2021). Among children and adolescents, both groups are more susceptible to leukemias, brain and central nervous system (CNS) related tumors, and lymphomas (NCI, 2021). When differentiated by age group, children are most susceptible to leukemia, followed by brain and other CNS related tumors, lymphomas, neuroblastoma, kidney tumors, and malignant bone tumors (NCI, 2021, Siegel et al (ACS), 2021). Leukemias can be either acute (fast growing) or chronic (slower growing), and acute leukemias are the most common in children (ACS, 2019). In addition to being either acute or chronic, leukemias can also either start in myeloid cells or lymphoid cells (ACS, 2019). Myeloid cells are cells such as erythrocytes (red blood cells), megakaryocytes, granulocytes (white blood cells), and macrophages, while lymphoid cells are cells such as T cells, B cells, and natural killer cells. (Kondo, 2010). Therefore, children can develop acute lymphocytic leukemia (ALL) or acute myeloid leukemia (AML) (ACS, 2019). Of the two forms of acute leukemias, children usually develop ALL (ACS,2019). Adolescents most commonly develop brain and other CNS related tumors and lymphomas, followed by leukemias, thyroid cancer, gonadal germ cell tumors (testicular and ovarian), and malignant bone tumors (NCI, 2021, Siegel et al (ACS), 2021).

According to the American Cancer Society's 2021 Statistics, children were estimated to have more cancer diagnoses and resulting deaths than adolescents. There were an estimated 10,500 diagnoses in children with 1,190 deaths in 2021, compared to 5,090 diagnoses and 590 deaths in adolescents. (NCI, 2021, Siegel et al (ACS), 2021). Below is a table summarizing the

most common cancers, as well as the estimated number of new cases and deaths in 2021 by age group.

Table 3: Estimated New Cases and Deaths Based on Most Common Cancers in Children		
Age	Most Common Cancer	Estimated Number of New Cases & Deaths (2021)
Children (0-14)	Leukemia	10, 500 estimated to be diagnosed 1,190 estimated to die
Adolescents (15-19)	Brain and other Central Nervous System Tumors and Lymphomas	5,090 estimated to be diagnosed 590 estimated to die
		Total: 15, 590 estimated to be diagnosed 1,780 estimated to die

Lymphoid cancers (of the lymphatic system) are some of the more frequently diagnosed cancers in dogs, including non-Hodgkin's lymphoma (Bennett et al, 2017). Dogs are also 2-5 times more likely than people to develop lymphoma, affecting any breed at any age (AAHA, 2023). More on lymphomas in dogs will be discussed in the next section of this chapter: Most Common Cancers in Dogs .

There also does not appear to be much literature that makes a distinction between the most common forms of cancer in dogs by age. Most literature only discusses the most common forms of cancer among dogs in general, where some may make a distinction by breed. A proposed reasoning may be due to different breeds of dogs aging differently (AKC Staff, 2019). In such cases, studies involving age associations in dogs can be conducted by breed. There is, however, one more recent study that was published by Rafalko *et al* in February of 2023 that

does analyze the age distribution at cancer diagnosis for cancer types that were represented by dogs 10 and younger (Rafalko et al, 2023). Information on this study will be discussed in the next section after discussion of the most common cancer in dogs in general: Registry/Cohort Data. Also, histiocytomas are known to occur more frequently in younger dogs (Williams et al, 2023). More on histiocytomas in young dogs will be discussed in the section of this chapter that goes over age as a risk factor for cancer: Early Cancer Cases in Dogs.

Most Common Cancers in Dogs

Tumor Classifications

Before discussing different forms of cancer and their frequency among dogs, the different tumor classifications should be understood. A multitude of papers also refer to these different forms of cancer both specifically and generally. That is, some papers may list a tumor classification based on histological type or by body site, which can group some tumor subtypes together. For example, the 1967 Cancer Animal Tumor Registry Study conducted on two counties in California appears to have labeled them based on body site for all of the tumor types listed, except for Leukemia and Lymphomas (Dorn, 1967). Therefore, in circumstances like these, we could say that the skin category could also consist of mast cell tumor cases but may not know the extent at which it contributes to the overall skin body site category depending on what is disclosed in the paper. Below are two tables (Table 1 and Table 2 in the original paper) provided by Dobson *et al* describing classifications of tumors based on histological type and body site (Dobson, 2002).

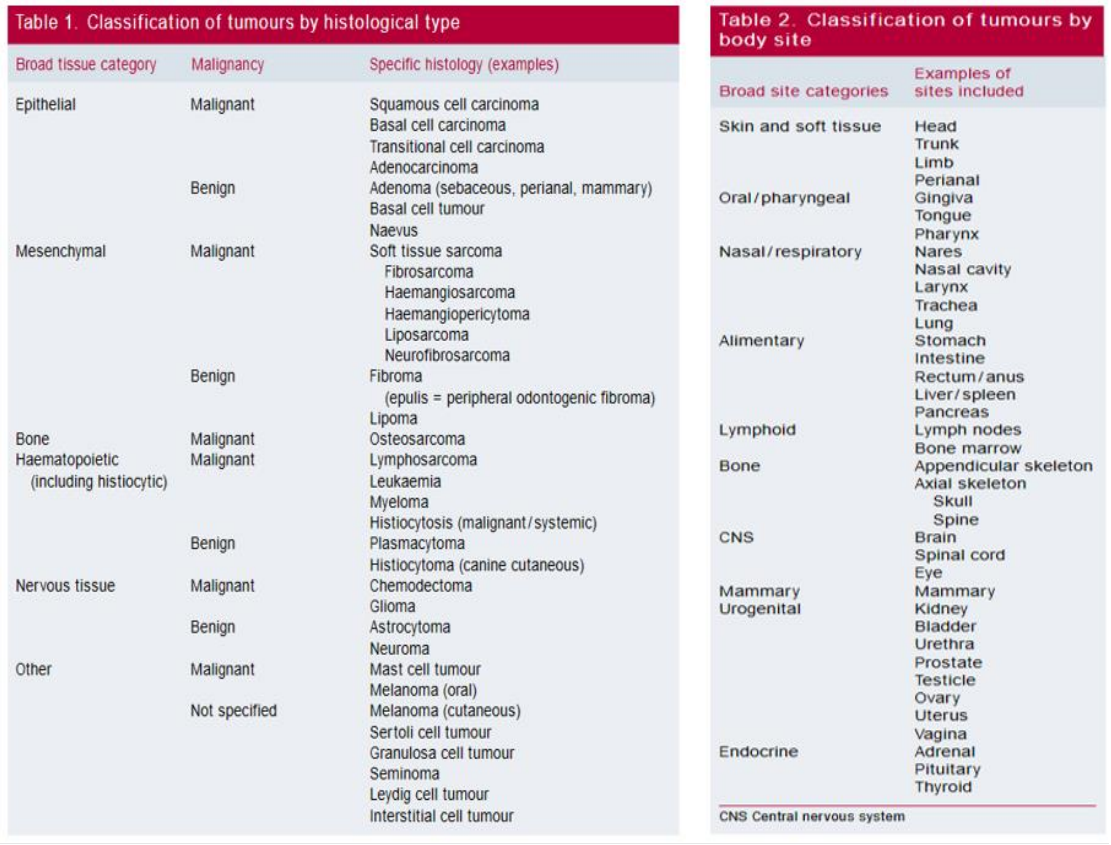

Table 1. Classification of tumours by histological type

Broad tissue category	Malignancy	Specific histology (examples)
Epithelial	Malignant	Squamous cell carcinoma
		Basal cell carcinoma
		Transitional cell carcinoma
		Adenocarcinoma
	Benign	Adenoma (sebaceous, perianal, mammary)
		Basal cell tumour
		Naevus
Mesenchymal	Malignant	Soft tissue sarcoma
		Fibrosarcoma
		Haemangiosarcoma
		Haemangiopericytoma
		Liposarcoma
		Neurofibrosarcoma
	Benign	Fibroma
		(epulis = peripheral odontogenic fibroma)
		Lipoma
Bone	Malignant	Osteosarcoma
Haematopoietic (including histiocytic)	Malignant	Lymphosarcoma
		Leukaemia
		Myeloma
		Histiocytosis (malignant/systemic)
	Benign	Plasmacytoma
		Histiocytoma (canine cutaneous)
Nervous tissue	Malignant	Chemodectoma
		Glioma
	Benign	Astrocytoma
		Neuroma
Other	Malignant	Mast cell tumour
		Melanoma (oral)
	Not specified	Melanoma (cutaneous)
		Sertoli cell tumour
		Granulosa cell tumour
		Seminoma
		Leydig cell tumour
		Interstitial cell tumour

Table 2. Classification of tumours by body site

Broad site categories	Examples of sites included
Skin and soft tissue	Head
	Trunk
	Limb
	Perianal
Oral/pharyngeal	Gingiva
	Tongue
	Pharynx
Nasal/respiratory	Nares
	Nasal cavity
	Larynx
	Trachea
	Lung
Alimentary	Stomach
	Intestine
	Rectum/anus
	Liver/spleen
	Pancreas
Lymphoid	Lymph nodes
	Bone marrow
Bone	Appendicular skeleton
	Axial skeleton
	Skull
	Spine
CNS	Brain
	Spinal cord
	Eye
Mammary	Mammary
Urogenital	Kidney
	Bladder
	Urethra
	Prostate
	Testicle
	Ovary
	Uterus
	Vagina
Endocrine	Adrenal
	Pituitary
	Thyroid

CNS Central nervous system

Figure 2 – Tables 1 and 2 from Dobson *et al* 2002 Paper Listing Classifications of Tumors by Histological Type and Body Site

Dobson, J. M., Samuel, S., Milstein, H., Rogers, K., & Wood, J. L. (2002). Canine neoplasia in the UK: Estimates of incidence rates from a population of insured dogs. *Journal of Small Animal Practice*, *43*(6), 240–246. https://doi.org/10.1111/j.1748-5827.2002.tb00066.x

Most Common Forms of Cancer Among Dogs (All)

According to the American Animal Hospital Association (AAHA), the most common forms of cancer seen in dogs are **lymphomas**, **mast cell tumors**, **osteosarcomas**, **melanomas** (oral), and **hemangiosarcoma** (AAHA, 2023). Colorado State University's Flint Animal Cancer Center also adds **transitional cell carcinomas** (urinary system) (Mingus, 2019). AAHA also states, among other literature, that **mammary gland carcinomas** are a common form of cancer among intact female dogs (AAHA, 2023, Baioni et al, 2017, Merlo et al, 2008).

Canine lymphomas account for up to 20-24% of all new canine cancers and is one of the most frequently diagnosed forms of cancer in dogs (Mingus, 2019; Bennett et al, 2016). Common forms of lymphomas seen in dogs are those that involve 1 or more of the external lymph nodes and appear most often as swollen lymph nodes under the jaw, in front of the shoulders, behind the knee and even the chest and abdomen (Mingus, 2019, AAHA, 2023). **Mast cell tumors (MCTs)** are the most common form of skin tumors in dogs, and account for 16-21% of skin related melanomas in dogs (Mingus, 2019; AAHA, 2023; Mood, 2019, Blackwood et al, 2012). MCTs can practically look and feel like anything and, therefore, can only be identified after cells are taken from the tumor and closely examined through microscope. **Osteosarcomas** are the most common type of bone tumor that can be found in dogs, usually large or giant breeds, and commonly affect the long bones in the limbs, but can also affect other skeletal structures such as the skull, ribs, vertebrae, and pelvis (Mingus, 2019, AAHA, 2023). **Melanomas** most commonly occur in dogs in the oral cavity (mouth) but can also commonly occur on the skin or toenails (Mingus 2019; AAHA, 2023). Malignant melanomas of the oral cavity often spread throughout the body, and usually it has spread by the time its first noticed (AAHA, 2023). Melanomas of the oral cavity often occur in breeds with dark tongues and gums (AAHA, 2023). **Hemangiosarcomas** are a form of cancer that develops from cells lining blood vessels and most commonly attack the spleen but can occur anywhere in the body including the liver, heart, and skin (AAHA, 2023, Mingus, 2019). **Transitional cell carcinomas** most commonly occur in dogs in the urinary system, and are often located in the bladder, but can occur anywhere in the urinary system, including the prostate gland in male dogs (Mingus, 2019). **Mammary gland carcinomas** are a form of cancer that is seen frequently in intact female dogs and appears as small nodules around the nipple (Mingus, 2019; AAHA, 2023). Often it will appear that female

dogs have a higher cancer incidence in comparison to male dogs simply based on intact female dogs often having such a high number of mammary tumors (Baioni et al, 2017; Merlo et al, 2008). However, spaying female dogs before their first heat cycle can heavily decrease the risk of mammary cancer development (AAHA, 2023).

In the next section, Registry/Cohort Study Data, data from studies and registries that analyze common cancer types among various sample populations of dogs will be reviewed in the form of a table to see which forms of cancer were found to be the most frequent in these studies. Studies conducted in the U.S. and Europe will be analyzed.

Registry/Cohort Study Data

While humans have organizations such as NCIs SEER and ACS that collect and summarize information such as incidence, prevalence, morality, and survival of cancer, no such organizations exist in veterinary medicine as of today (Davis & Ostrander, 2014). However, there are individual registries that exist that collect this type of data, one of which is the National Veterinary Cancer Registry originating in the U.S. Other registries also exist in European countries as well, such as the Animal Tumor Registry of Genoa, Italy. In more recent years, most of these registries get their data from pet insurance companies (Davis & Ostrander, 2014). While these registries do provide useful information, there is a lack of publicly available information on cancer trends, breed-specific incidences of cancers, or treatment responses (Davis & Ostrander, 2014). The information provided by these registries is also lacking in resources to link breeders of at-risk populations with geneticists or epidemiologists studying a specified form of cancer. (Davis & Ostrander, 2014). More research and interdisciplinary work need to be completed, so that more information is available to researchers or organizations who lack individual registries,

and so that cancer trends and other measures of occurrence can be observed among dogs. Some of the best resources to gather information on breed-specific cancer trends are highly targeted academic studies and selected veterinary school studies (Davis & Ostrander, 2014).

In European countries from which most of these registries belong, including the United Kingdom (UK), Italy, and Denmark, it appears the most common canine *benign* tumor types are cutaneous histiocytomas, lipomas and adenomas, and the most common *malignant* types are adenocarcinoma, soft tissue sarcomas, mast cell tumors and lymphomas. (Davis & Ostrander, 2014, Dobson, 2013, Brønden et al, 2010, Merlo et al, 2008). Tables 4a and 4b below include descriptions of the most common tumor types found in dogs across the United States and European countries through studies conducted using dog cancer registry data. A maximum of the 6 most frequently occurring cancer types will be given per study.

Table 4a: U.S. Registries & Study Data for Canine Cancers

Literature	Study Population	Method/Study Information	Most Common Canine Tumor Type Reported (Most to Least/ Descending Order)
The Epidemiology of Cancer in Animals https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1502934/ Central Animal Tumor Registry in California - Alameda and Contra Costa counties Year: 1967	Pet Animals, including dogs, seen by 1 of 100 practicing veterinarians in Alameda and Contra Costa Counties (CA).	Data on most common sites in dogs and cats reported to the registry.	1.Skin 2.Mammary gland 3. Leukemia and Lymphoma 4.Mouth and Pharynx 5. Testis 6. Bone

Frequency of Canine and Feline Tumors in a Defined Population https://doi.org/10.1177/030098587801500602 The Tulsa Registry of Canine and Feline Neoplasms Year: 1978	63,504 dogs 715 had one or more tumors for an incidence of 1,126 cases per 100,000 The 715 cases had 899 primary tumors total	1,127 specimens of canine tissues submitted to the registry in the first year. 228 (20%) of dogs' specimens were benign (not neoplastic).	1.Mammary Tumors both benign and malignant (17.5% of benign & 26.4% of malignant respectively) 2.Mastocytoma/sarcoma that are malignant (20.5%) 3. Lipoma/sarcoma that are benign (11.1%) 4. Histiocytoma, which are benign only (10.6%)

Table 4b: European Registries & Study Data for Canine Cancers

Literature	Study Population	Method	Most Common Canine Tumor Type Reported (Most to Least/ Descending Order)
Cancer Incidence in Pet Dogs: Findings of Animal Tumor Registry of Genoa, Italy Cancer Incidence in Pet Dogs: Findings of the Animal Tumor Registry of Genoa, Italy - Merlo - 2008 - Journal of Veterinary Internal Medicine - Wiley Online Library Animal Tumor Registry of Genoa, Italy Year:2008	40,996 biopsies from pets of all 21 Italian regions.	Collected biopsies from July 1, 1985 - December 31, 2002. 6,743 biopsies were from dogs Only biopsies from dogs were considered in this analysis.	By Sex: Male: 1. Non-Hodgkin's Lymphomas (20.1%) 2. Skin Excluding Melanoma (19.2%) 3. Genital Organs (16.8%) 4. Connective and Other Soft Tissues Female: 1. Mammary Cancer (70.5%) 2. Non-Hodgkin's Lymphoma (8.4%) 3. Connective and Other Soft Tissues

			(4.6%) 4. Skin Cancer (3.8%)
Data from the Danish Veterinary Cancer Registry on the occurrence and distribution of neoplasms in dogs in Denmark Data from the Danish Veterinary Cancer Registry on the occurrence and distribution of neoplasms in dogs in Denmark - Brønden - 2010 - Veterinary Record - Wiley Online Library Year: 2010	1,878 cases of neoplasms in dogs.	From May 15, 2005 - April 15, 2008, the cases were reported to web-based Danish Veterinary Cancer Registry.	Malignant 1. Adenocarcinomas (21%) 2. Mast cell tumors (19%) 3. Lymphomas (17%) Benign 1. Lipomas (24%) 2. Adenomas (22%) 3. Histiocytomas (14%) Also reports that, overall, skin (43%) and the female reproductive system, which includes mammary tissue (28%), are the most common locations of neoplasia.
Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs - Dobson - 2002 - Journal of Small Animal Practice - Wiley Online Library BSAVA: British Small Animal Veterinary Association Year: 2002	All dogs insured with a single UK pet insurance from June 1, 1997 - May 31, 1998	Data on all claims for veterinary treatment and veterinary certifications that included diagnosis or nature of the claim were collected. Claims relating to tumor treatment were investigated. 2,546 claims were identified as being related to neoplasia.	1. Canine cutaneous histiocytoma 2. Lipoma 3. Adenoma 4. Soft tissue sarcoma 5. Mast cell tumor 6. Lymphosarcoma

Estimating canine cancer incidence: findings from a population-based tumor registry in northwestern Italy Estimating canine cancer incidence: findings from a population-based tumour registry in northwestern Italy - PMC (nih.gov) The Piedmont Canine Cancer Registry Year: 2017	46 municipalities in northwestern Piedmont 22 veterinary practitioners 10,095 dogs Data on 1,172 tumors collected (618 benign; 554 malignant).	Surveys were carried out to estimate the real size of the dog population and to remove deceased subjects and include the unregistered dogs in the local canine identification and registration system. Veterinary practices provided standardized case reports designed to collect canine cancer cases. Tumor samples were collected and sent to 2 diagnostic labs. Conducted from 2001-2008.	By histopathological diagnosis: 1. Mammary gland 2. Skin and Soft Tissue 3. Testicle 4. Spleen 5. Ovary 6. Gingiva By sex & organs site: Females 1. Mammary gland (n = 585) 2. Skin (n = 229) 3. Ovaries (n = 40) Males 1. Skin (n = 242) 2. Testicles (n = 112) 3. Spleen

Although not of a registry, the Rafalko *et al*. study provides more information on the most common form of cancer found in a study population of 3,452 dogs in the United States (Rafalko et al, 2023). Among this population, the top 6 types of cancer that were found were: lymphomas/lymphoid leukemia (n = 979), osteosarcomas (n = 664), MCTs (n = 565), hemangiosarcoma (n = 292), soft tissue sarcomas (n = 240), and malignant melanomas (n = 128) (Rafalko et al, 2023).

Methods of Screening and Detecting Cancer

This section will go over the screening and detection of cancer from both an epidemiological and clinical standpoint. Below is a diagram from a publication by Fass (2008) that represents the cancer management process, of which screening and detecting cancer falls into.

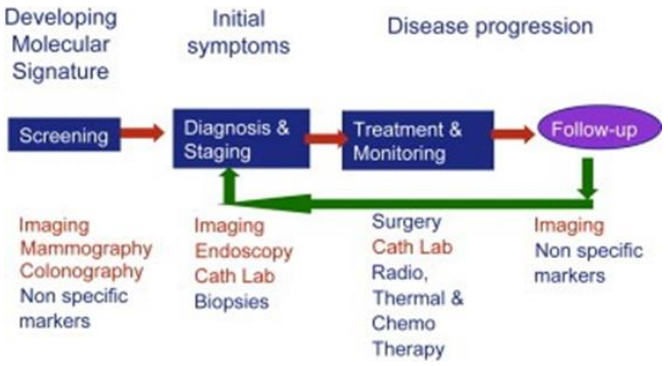

Figure 3 – Cancer Screening and Detection Process Diagram from Fass 2008 Paper

Fass L. (2008). Imaging and cancer: a review. *Molecular oncology*, *2*(2), 115–152. https://doi.org/10.1016/j.molonc.2008.04.001

Definitions

The following section will illustrate distinctions between the process of cancer screening and detection, beginning with the following definitions of screening and detection provided by the World Health Organization (WHO).

Screenings are tests that are conducted on presumably healthy individuals to potentially identify individuals who have developed a disease but have not yet experienced any symptoms.

Detection is done when looking for disease based on symptoms individuals are experiencing.

Sometimes, the distinction between the two can become blurred, as the intention behind the conducting of particular tests or examinations can make the difference between the two. In fact, screening can be seen as a component of overall early detection, as well as early diagnosis (WHO, 2010). Many of the methods that will be discussed in cancer screening and detection can ideally be methods used to both screen for and detect cancer. However, some methods are more traditionally used for screening, and others are traditionally used to detect cancer once symptom onset has appeared to have begun.

Screening

In humans, to monitor individuals regularly and to help potentially detect cancer in its earlier stages, **screening tests** can be performed. The goal of screening tests is to see if there is any sign of disease before symptom onset, therefore these tests are conducted on presumably healthy individuals. According to the World Health Organization (WHO), screenings should only be conducted if:

- Their effectiveness has been shown
- There are sufficient resources available to cover almost the entire targeted group (resources include personnel in addition to equipment)
- Facilities exist to confirm diagnosis as well as for treatment and follow up if there are abnormal results
- Prevalence of disease is high enough that the cost and efforts are a justifiable expenditure

Common Screening Practices in Human Medicine

Common screening tests for cancer in humans are mammograms (breast cancer), Pap smears and HPV testing (cervical cancer), and colonoscopies (colorectal cancer). These tests are meant to be performed annually or every few years, and once an individual has reached a certain age. The following information pertaining to cancer screening and recommended age of conduct of these tests are provided by Centers for Disease Control and Prevention (CDC) and The United States Preventive Services Task Force (USPSTF). Breast, Cervical, Prostate and Colorectal Cancer Screenings are some of the most common screenings individuals undergo. Lung cancer screenings are recommended particularly for individuals at high risk.

Breast cancer screenings are used to check women's breast for cancer without there having been any signs or symptoms. Checking a woman's breast for masses or lumps prior to symptom onset can help to find cancer early if there happens to have been development, as cancer can start to form before any signs or symptoms are experienced. For breast cancer screening via mammogram, it is currently recommended that women who are 50 to 74 years of age and are at an average risk for breast cancer should get a mammogram every 2 years. Women 40 - 49 are recommended to consult health care providers and physicians to weigh their individual benefits of when to start and how often to get a mammogram. In general, consulting health care providers and physicians on their individual risk and benefits should be done if younger than 50 years of age.

Cervical cancer screenings are used to help prevent development of cervical cancer or to potentially find it early via HPV (human papillomavirus) tests or Pap smears. HPV tests are used to see if HPV is detected in the body, which is a virus that can ultimately cause cell changes

in the cervix. Pap smears are used to examine a woman's cervix and vagina, as well as to collect cell and mucus samples to check for any abnormalities. It is currently recommended for women to start getting Pap smears at age 21 and some may even say when they become sexually active, whichever comes first. When an individual is between ages 30 - 65, it can be up to the doctor to perform only an HPV test, only a Pap smear, or a co-testing with both tests depending on the individual health needs and circumstances of patients. With an HPV test only, also known as a primary HPV test, a doctor can potentially instruct patients to come in for another after 5 years if results turn out to be normal. With a Pap smear only test, a doctor may instruct patients to come back for another test after 3 years if results come back as normal. With a co-testing, if results come back as normal, a doctor may instruct patients to come back for another testing after 5 years as well. With screening for cervical cancer, unlike with other screenings, in older ages past 65, patients may not need to get screened anymore if they have tested and have received normal results over the course of several years, have not had cervical precancer in the past, or they have had their cervix removed. If tests come back as abnormal, this is not usually a sign that the individual has cancer, but may be at risk for development, and treatment should begin to prevent progression and potential cancer development right away.

Colorectal cancer screenings are conducted to make sure there are no precancerous polyps present in the colon or rectum. Precancerous polyps are abnormal growths, and colorectal cancer almost always develops from them. By screening and seeing if these polyps are present, if found they can be removed before developing into cancer. Current screening recommendations have patients begin screenings for colorectal cancer at age 45, and once past the age of 75 patients should consult their physician about continuing screening for colorectal cancer. There are a variety of ways to screen for colorectal cancer, including: CT (computed tomography)

colonography, stool tests, flexible sigmoidoscopy, and colonoscopy. It should also be mentioned that some may need to be screened earlier than age 45 if they also have other conditions such as: inflammatory bowel disease, are genetically predisposed to colorectal cancer or polyps, or if they have a genetic syndrome such as familial adenomatous polyposis or Lynch syndrome. These conditions increase one's risk of developing colorectal cancer and, thus, may require earlier screening.

Lung cancer screenings are predominantly recommended for individuals who smoke, usually cigarettes, on a regular basis. It is recommended currently for individuals who smoke or used to smoke within the past 15 years, who have a 20-pack year or higher smoking history, or for individuals who are between ages 50-80 years old. A pack year is a method of measuring the average amount of packs of cigarettes individuals smoke over a certain period of time. A single pack year would be smoking an average of one pack of cigarettes per day over the course of a year. Therefore, someone who has a 20-pack year would be someone who smokes a pack a day over 20 years or smokes 2 packs a day over 10 years. There is currently only one recommended method of screening for lung cancer, and that is through the use of low dose computed tomography (LDCT). During this screening an individual would be scanned through an X-ray machine that uses a low dose of radiation to form images of the lungs. The scan is not painful, however, there are risks to receiving LDCT. In addition to other risks with conducting screenings, which will be discussed later in this chapter, the radiation exposure from conducting LDCTs repeatedly can cause the development of cancer in individuals who would otherwise be healthy. For this reason, it is only truly recommended to get screened for lung cancer if you are at high risk due to reasons such as exposure to smoke (smoking themselves or second-hand) and age.

Prostate cancer screenings are currently recommended in men between ages 55 and 69 if they so choose to. Once in that age range, patients can choose to undergo prostate-specific antigen (PSA) based screening periodically. However, as with the prior screening tests, the patient should go over what is best for themselves, specifically, with a recommendation from a physician. Conducting a prostate screening holds all of the risks that the other screening tests possess, so the benefits and risks should be weighed properly by the physician and disclosed with the patient. The United States Preventive Services Task Force (USPSTF) is against PSA-based prostate screenings of individuals above age 69 (70 and up). Overall, by the recommendations of the USPSTF, men between 55 and 69 have a choice to screen for prostate cancer, but men above 69 years of age should not be screened due to the benefits of PSA-based screening not outweighing the harm at that age range.

In addition to conducting screening tests, an individual's **family medical history** can also give insight on whether one is genetically predisposed to a particular form of cancer. Due to past family members developing a form of cancer, family members thereafter can have similar aspects of their genome that can make them more susceptible to cancer development (Schneider et al, 1983). **Genetic testing** can also be conducted to see if there are abnormalities or changes in the individuals' genes or chromosomes (NCI, 2022). Chromosomes have telomeres at their ends to protect them, enabling cells to seize growth or die after a certain number of replications, controlling the cell's growth. Telomeres shorten with each round of cellular division, which limits cell proliferation to a certain number of cellular divisions by inducing senescence, differentiation, and apoptosis (Jiang & Rudolph, 2007). Eroded telomeres, however, can result in chromosomes sticking together, leading to genome instability and can result in cancer progression (Maciejowski, 2017). That being said, genetic testing can be telling of one's risk of

developing cancer. Telomere length in relation to age and cancer development will be talked about more in depth later in the chapter.

A pattern that can be seen across the common screening practices is the age at which it is recommended to begin the routine screenings. All of these common screening tests do not start to take place until adulthood, the youngest age being 21 with screenings for cervical cancer. It is for this reason that children do not get screened for cancer. Instead, they are more likely to undergo diagnostic tests if they are suspected of having cancer due to signs and symptoms they may be experiencing. Age plays a significant role and correlates considerably with the development of cancer, which will be discussed later in this chapter. Therefore, individuals do not undergo routine screenings as children as they are seen to be at lower risk. Children's lower risk in developing cancer also relates to weighing the benefits and drawbacks of conducting the screenings, as the harm can outweigh the good when conducting screenings that early. The drawbacks of screenings will be discussed later in this chapter: Drawbacks to Screening.

Screening Practices in Veterinary Medicine

It appears dogs, regardless of age, are not traditionally screened for cancer in the same sense as humans are, based on the lack of literature and practices when conducting research and after speaking with multiple faculty throughout the Flint Animal Cancer Center here at Colorado State University. On most occasions, dogs are administered diagnostic testing once symptoms of onset have occurred. When looking for literature, diagnostic procedures were mostly found in reference to cancer procedures in veterinary medicine. Owners can be warned by their veterinarian if their dog is more susceptible to a form of cancer based on risk factors such as breed and age that will be described later in the chapter. In this case, **pedigree** can be useful in

terms of knowing whether or not the dog is predisposed to cancer, as some purebred dogs are more susceptible than others. However, there are not many routine annual tests that are conducted on dogs, like we have for humans. An owner could bring in their dogs annually by choice for a **physical examination (PE)** to make sure there are no palpable masses forming on the dog and may elect to have bloodwork performed at these visits. However, as far as standard practices in health to screen for disease development, like how humans undergo mammograms, pap smears, colonoscopies, etc., this is not necessarily a norm in veterinary medicine. However, there is currently research in the works and developing tests that can be used for screening cancer in dogs, such as the OncoK9 Cancer SAFE Tool. The OncoK9 Cancer SAFE Tool uses data from over 3,000 dogs diagnosed with cancer to help determine which age it is appropriate for an owner to start conducting cancer screening for their dog (PetDx, 2023).

The dataset that was analyzed for this research, that will be discussed in Chapter 2, were dogs who received annual checkups to check for development of cancer. Details of all the tests conducted annually on these dogs will be provided in Chapter 2 as well. Annual examinations and lab work may be an effective way of screening dogs for cancer as a means of potentially detecting cancer early. There are conditions required for consideration when conducting screenings in human health, e.g., effectiveness, prevalence to justify costs and effort, etc., which should also be addressed in cancer screening for dogs. There is, also, still not as much known about cancer etiology in dogs in comparison to humans, which could be the reason why screening is not a common practice among dogs.

Drawbacks to Screening

Although screenings can aid in potentially catching cancers earlier on before symptom onset, there are some drawbacks in conducting them. Even when they are done correctly, routine screenings are flawed in their effects. Screening tests can give a **false positive**, which results in additional testing that may include invasive procedures and increase stress and anxiety to the patient (WHO, 2010). There is also the chance of getting back a **false negative**, which may be an even worse circumstance, as this can delay proper diagnosis when symptoms appear (WHO, 2010). Another drawback to consider in screening tests is **over diagnosing/treating** preclinical cancers that could have never caused symptoms or posed significant health risks, as these treatments can cause unnecessary injury and be more harm than good to the patients' health (WHO, 2010). However, there are a multitude of factors that need to be considered before routine screenings for a specified disease, like different forms of cancer, can be conducted on individuals. While they can be a useful tool to detect silent killers, like cancers in their earlier phases, they have their drawbacks that can cost in monetary value, efforts, people's health, and lead to inaccurate data that can be used in epidemiology to monitor the disease on the epidemiological level. The **sensitivity** and **specificity** in both the screening tests and detection tests deliberate the tests' abilities to accurately detect cancer and show which type of cancer is present. **Sensitivity** specifies how many patients had a true positive test out of all the patients with the condition (Shreffler & Huecker, 2023). **Specificity** specifies, in contrast, how many patients had a true negative out of all patients without the condition (Shreffler & Huecker, 2023). More on sensitivity and specificity will be discussed in the next section on detection and diagnosis of cancer.

Detection and Diagnosis

Types of Imaging Tests

Imaging tests, **biopsies,** and **lab tests** can be conducted to diagnose or detect cancer. **Imaging tests** are scans that return detailed images of the area scanned, making it easy to see if there are any growths or tumors present. Imaging tests are used and both human and veterinary medicine. In Table 6, below, information on the various forms of imaging tests provided by the Center of Disease Control (CDC) and what they do.

Table 5: Imaging Tests Used in Human and Veterinary Medicine

Imaging Test	About
Computed Tomography (CT) Scan	Uses an x-ray machine linked to a computer to take a series of images of the organs at different angles. Images are used to create a detailed 3-D image of the inside of the body.
Magnetic Resonance Imaging (MRI)	Uses strong magnet and radio waves to take pictures of the body in portions. Images are later combined to create a detailed image of the inside of the body.
Nuclear Scan (Umbrella Term)	Uses radioactive material to take images of the inside of the body. Tracer, a small amount of radioactive material, is injected into the body to get to the bloodstream and collects in bones and organs. The scan detects and measures the radioactivity from the tracer that is now in the body to create an image of the bones and organs.
Bone Scan	Nuclear scan that checks for abnormal areas and damage in bones and can be used to diagnose bone cancer or to see if cancer has spread from another part of the body to the

	bones.
Positron Emission Tomography (PET) Scan	Nuclear scan that creates a detailed 3-D image of areas within the body where glucose is uptaken. Tracer, in this case radioactive glucose, is injected into the body. Cancer cells uptake more glucose than normal cells, so they can be identified based on glucose levels.
Ultrasound	Uses inaudible, high-energy sound waves that echo off tissues inside the body. The echoes are used by a computer to create images (sonograms) of the inside of the body.
X-rays (Radiographs)	Uses low doses of radiation to create images of the inside of the body.

Imaging Tests Conducted in Humans and Dogs

These various imaging tests are used at all phases of cancer management and have been an essential part of cancer clinical protocol (Fass, 2008). Imaging tests are used in both human and veterinary medicine, and each method of imaging is used depending on the type and location of the growths or tumors. Choosing which imaging test to utilize may also depend on the affordability to the patient or owner. Each of these forms of imaging tests have their advantages as well as limitations in their use and abilities (Randall, 2023). The following advantages and disadvantages have been provided by Dr. Elissa Randall from Colorado State University's Department of Environmental and Radiological Health Sciences (CSU ERHS) and CSU's Flint Animal Cancer Center. Other supporting literature will be used to further discuss these imaging tests.

Ultrasounds are one of the first imaging tests that are done before others are. In human health they are most commonly used for monitoring pregnancy and growth of a fetus. In veterinary medicine, they are often used to observe structures in the abdomen, and are useful for observing soft tissues, the internal structure of organs, and are often used to observe the gastrointestinal tract (GI tract) in animals. Ultrasounds are more so used to monitor pregnancy in humans and not to look at the GI tract because of the amount of gas that humans accumulate in the GI tract, making it hard to see anything on an ultrasound. Computed tomography (CT) is more so used to observe the GI tract in humans instead. Ultrasounds are also one of the most affordable imaging tests and can enable real-time studies of the heart and reproductive system in both human and veterinary medicine. Some disadvantages to ultrasounds are that they produce 2-dimensional static images which can be hard to interpret, especially if they are images captured by others. Ultrasounds also need to have an acoustic window, which is the location from which an ultrasound probe (transducers) can make its scan. When it comes to the use of ultrasounds in veterinary medicine, they are not best for larger and heavier dogs, as it can be harder to see structures in the body. Also, panting can make it hard to see structures due to the movement.

Radiographs, also known as X-Rays, are used in both human and animal health, providing the best spatial resolution, thus are useful for examining bone and the lungs. Radiographs also do not require a lot of equipment and are affordable. Some disadvantages to radiographs, however, is that a lung nodule must be at least 4-5 mm to see on the radiograph produced images. Having to wait for a mass to reach a certain size is not ideal, as the goal is to catch the mass as early as possible. Radiographs also are not great at capturing complex anatomy or recessed (hidden or deeper) soft tissue structures. The **sensitivity** (the number of true positive patients out of all patients with the condition) of radiographs are also not optimal. While these

imaging tests are great at observing bone and the lungs, the bone must have had a 30-50% mineral loss before signs of bone lysis is detected. There are other imaging tests that can catch bone lysis a lot earlier without the need for such a significant amount of mineral loss, while providing a better view of those recessed soft tissue structures, such as a CT.

Computed Tomography (CTs) is useful for bone and lung imaging like a radiograph but detects bone lysis a lot faster without the need for such a significant bone loss. CT scans can, therefore, catch subtle fractures that are not visible on X-rays (Fayad, 2023). CTs are also adequate for observing soft tissues. CTs hold an advantage in their speed and can create images of a dog in seconds. CTs can also be used for radiation planning in cancer therapy. Although CTs can detect bone lysis better than radiographs, CTs still are not great at detecting subtle disease (low sensitivity) and are not great for observing the brain for lesions. MRIs, however, can accomplish what CTs lack.

Magnetic Resonance Imaging (MRIs) have the advantages of being the best for soft tissue contrast and detail when producing an image and are very sensitive to cancer. Therefore, MRIs will detect cancer and other diseases better. An MRI accomplishes detecting disease via signal change to tissues. However, MRIs are more expensive and less available. MRIs also take longer to acquire the images, unlike CT where you can complete a scan in seconds. The bone and lungs can't be evaluated as well with an MRI as it could with a CT, and MRIs may not be able to be used for radiation planning like CTs can be.

Positron Emission Tomography (PET) scans are nuclear scans where a radioactive glucose tracer is used to measure glucose uptake. The radioisotope FDG (18F-fluoro-deoxyglucose) is commonly used. Tumors and cancer cells have a higher glycolysis rate and

uptake more glucose than normal cells. Therefore, tumors and cancer cells can be identified and located in an image by the amount of glucose uptake. To quantify the uptake of glucose (FDG), a standard uptake value (SUV) is calculated. The SUV is a semi-quantitative measure of intensity of FDG uptake. However, various factors affect the calculated SUV, making it appear as an arbitrary value. Nevertheless, PET scans using the FDG tracer are highly sensitive to most cancers, enabling them to detect cancer well. However, PET scans are not great in producing anatomical resolution, so they are often coupled with other imaging tests that can do so, like an MRI or CT scan. An important fact that should be kept in mind with PET scans is that, while this scan potentially locates cancer in the body due to glucose uptake, seeing increased glucose uptake does not definitely mean cancer is present. Areas of inflammation also have a higher rate of glucose uptake. This is another reason why pairing this test with a CT or MRI is also important, because the CT or MRI would be able to differentiate between benign infection and inflammation from actual malignancy (Rahman et al, 2019). **Bone Scans** are essentially a bone specific PET/CT scan. They are another type of nuclear scan, like a PET Scan, but a tracer like Fluorine-18 (F-18) Sodium Fluoride is used. F-18 Sodium Fluoride is often used in bone scans to check for bone metastasis. The bone scan would be able to detect any osteogenic alteration activity, and produce high resolution, high contrast images of skeletal tissue (Glasgow, 2021).

Overall, imagining tests can give us information on the metabolic, functional, structural, and morphological components of tumors and can be used in tandem with other diagnostic tools, such as *in -vitro* tissue and fluids analysis, to aid in decision-making on the clinical level (Fass, 2008). Imaging tests provide the advantage of giving real time monitoring, accessibility to view the inside of the body and the tumor(s) without inflicting tissue destruction, involve little to no invasiveness of the body, and can be done over wide ranges of time and size scales that are

involved in biological and pathological processes (Fass, 2008). Therefore, a disease that can take years to progress such as cancer can be analyzed on the molecular, cellular, organ, and organismal level (Fass, 2008). Next, biopsies will be discussed using information provided by the National Cancer Institute (NCI) accompanied by supporting literature.

Biopsy Procedures in Humans and Dogs

The above-described imaging tests may detect the presence of a mass, but it is not known what type of mass it is, including if it's benign or malignant. A biopsy can be performed to obtain a sample of the mass for confirmation of malignancy and cancer typing. **Biopsies** take either a sample of tissue or fluid from the suspected growth/tumor (i.e., an incisional biopsy), or they remove the entire tumor or lump (i.e., an excisional biopsy), so it can be examined under a microscope and tested, usually by a pathologist, to see if it is benign (non-cancerous) or malignant (cancerous) based on cellular abnormalities in the sample. A sample of tissue or fluid from the growth/tumor can also be obtained via **needle**. Withdrawal of tissue or fluid as a sample via needle is a method of biopsy often used in biopsies of the breast, liver, and prostate. Biopsy via needle is also used for spinal taps and aspiration procedures such as bone marrow aspiration and fine needle aspiration. The tumor being completely or partially removed would involve a **surgical procedure** or use of **endoscopy**. In surgery, the tumor/growth can be removed completely (excisional surgery) or partially (incisional surgery). In endoscopy, an endoscope is inserted into an open cavity of the body, such as the mouth or anus, and is used to observe and collect a sample of abnormal tissues. The endoscope is a thin tube that has a light and video camera on it so that the area of abnormality can be seen, and tools that are on the endoscope can be used to collect a sample of abnormal tissue. A common method of biopsy via endoscopy is a colonoscopy. In this procedure, an endoscope is inserted in the anus to observe the colon and

rectum and to collect tissue samples from the colon and rectum to test for malignancy (colorectal cancer). Colonoscopies can, thus, be both a screening and diagnostic method. Biopsies are also used in veterinary medicine to detect and diagnose cancer in dogs as well. Biopsy via needle (commonly fine needle aspiration), surgery, and endoscopy are all used on dogs.

Fine needle aspiration (biopsy via fine needle) is a quick, efficient, inexpensive, and tolerable procedure that can be conducted on dogs to gather abnormal cell samples (Ménard et al, 1986). In a study conducted to test the efficacy of fine needle aspiration and cytology (study of cells) in diagnosing malignancy and determination of cell origins, 83 dogs and 19 cats (102 cases) with a total of 97 malignant tumors and 5 benign lesions were examined (Ménard et al, 1986). At the time this study was conducted, studies and literature were limited on the effectiveness of aspiration procedures for cancer biopsy in veterinary medicine. The study had found that malignancy was detected via cytology in 71% of the malignant tumors in dogs and that the cellular origin of the lesions was able to be determined in 72% of the cases in dogs (Ménard et al, 1986). More literature is now available in regard to the use of biopsy methods and their efficacy, including aspiration biopsies. A review in more recent years discussed how biopsies in veterinary medicine are, in fact, seen as a "gold standard" procedure to come to a final diagnosis, especially with neoplastic processes and infectious disease (Glińska-Suchocka, 2013). Fine needle aspiration biopsies are also relatively cheap and have a low complication rate in comparison to other forms of biopsy (Glińska-Suchocka, 2013). On the other hand, based on a veterinarian here at CSU, Danielle Scott, fine needle aspiration may not yield a high diagnostic value. Usually, fine needle aspiration will be attempted first to get a diagnosis, as it is more minimally invasive, and if a diagnosis is not given based on the aspiration, an excisional or incisional biopsy will be conducted. Other literature supports this statement, describing how it is

the least diagnostic biopsy technique because not as much tissue is samples in addition to there being a lack of organized tissue architecture, hindering tumor grading (Orencole & Butler, 2013).

Endoscopy is more minimally invasive, but still invasive nonetheless, and is also frequently used to evaluate internal organs and cavities of animals, like how it is used in humans (Clark, 2012). One of the most common parts of the body that endoscopy is used to evaluate in veterinary medicine is the upper and lower GI tract, but it can still be used to evaluate multiple internal organs and cavities within the body (Clark, 2012). There are two forms of endoscopy: flexible endoscopy and rigid endoscopy. The difference between the two forms is based on the material and malleability of the endoscope. Flexible endoscopes are made of thinner, bendable tubes, while rigid endoscopes are made from metal tubes (Schneider & Feussner, 2017). Flexible endoscopy has been found to be a valuable tool in evaluating disease of the GI tract when done carefully (Jergens et al, 2016). Endoscopies can be used to diagnose cancers in dogs, such as cancer of the GI tract (Williams & Ward, 2023). However, with cancers of the GI tract, tumors do not always affect the inner surface of the stomach and colon, so it is possible to have results that come back as normal, even if cancer is present (Williams & Ward, 2023).

Surgery is usually used to remove an entire (excisional biopsy surgery) or part of an area (incisional biopsy surgery) of abnormal tissue, taking some of the normal tissue surrounding the area with it (NCI, 2023). Surgeries as a method of tumor removal are only applied in the cases of a localized tumor. Once a tumor has metastasized, surgery is no longer a viable option for the complete removal of the cancer. (Nickloff, 2023). Although, even in localized tumors, surgery has the potential to fail and the tumor can grow back if the tumor could not be completely removed, such as if the tumor was located in an area in which the surround tissue is sensitive and limits removal, or if there was undetected metastasis (Nickloff, 2023). As surgery does take

some of the surrounding normal tissue with the removal of the tumor, a lot of tumors cannot be removed if the normal tissue is critical, especially in organs such as the brain and pancreas (Nickloff, 2023). There are also risks of undergoing surgery to remove a tumor such as infection, pain, and other potential complications. Older patients are at increased risk of infection due to their immune systems being weaker (Nickloff, 2023). Dogs are at risk for similar complications post-surgery.

Biopsies are a more invasive route in the analysis of masses/tumors. Biopsies can follow screening when a mass or abnormality is found. This is one of the multiple reasons as to why screening should be done only when physicians find it necessary for patients. Screenings, as mentioned prior, can lead to false positives, and can ultimately lead to unnecessary, more invasive procedures conducted on patients, such as biopsies.

Lab Tests in Humans and Dogs

Lab tests can also be conducted for the diagnosis and detection of cancer. Higher or lower levels of particular substances, i.e., biomarkers, present in our bodies are used as indicators for the potential presence of cancer. Lab tests are a way to help rule in or out a cancer diagnosis once other forms of diagnostic testing have been done, like imaging tests. As mentioned in the imaging test section, results from imaging can be abnormal, yet due to a cause other than cancer, such as what can be seen in PET scan results when inflammation is present (Refer to Page 32). There is no single lab test that can diagnose/detect and monitor all cancers, as all of the following can be used to do so. Below is a table with common lab tests used to diagnose cancer in humans with information provided by the NCI. Common and emerging lab tests used to diagnose cancer

in dogs are provided by the American Kennel Club Canine Health Foundation (AKCCHF) and will be discussed after.

Common Lab Tests in Humans

Table 6a: Lab Tests Used in Human Medicine

Lab Test	Purpose	Significance of Results
Blood Chemistry Test	Measures the amount of metabolites, electrolytes, fats, sugars, and proteins (including enzymes) released into the blood by organs and tissues. Gives information on efficiency of organ function, such as the kidneys, liver, and other organs.	Higher or lower levels of substances can be indicators of disease, such as cancer.
Complete Blood Count (CBC)	Measures the number of red blood cells (RBC), white blood cells (WBC), and platelets in the blood, as well as the amount of hemoglobin (oxygen carrying protein), the amount of your blood that is made up of RBCs, the size of your RBCs, and the amount of hemoglobin in you RBCs. Often part of routine health checkup and used to monitor health during and post treatment.	Helps diagnose some cancers such as leukemias.
Cytogenetic Analysis	Looks for changes in chromosomes in tissue, blood, bone marrow, or amniotic	Changes in specific chromosomes may be indicative of genetic

	fluid samples. Changes may include broken, missing, rearranged, or an extra chromosome(s).	conditions or a form of cancer.
Immunophenotyping	Uses antibodies to identify cells based on antigens or markers that are on the surface of a cell. Often done on blood, bone, body fluid, or tissue samples.	Helps diagnose, stage and monitor forms of blood cancer and disorders including leukemia, lymphomas, myelodysplastic syndromes, and myeloproliferative disorders.
Liquid Biopsy	Looks for cancer cells or pieces of tumor cell DNA in a blood sample.	Helps find cancer at an earlier stage and can also be used to form a treatment plan, see how well treatment is working, or if a cancer has come back.
Sputum Cytology	Look for abnormal cells in sputum, mucus and other matter that is expelled from the lungs when coughing.	Helps diagnose lung cancer.
Tumor Marker Tests	Measure substances that are produced and released by cancer cells or other cells in response to cancer. Most tumor markers are made up of both normal and cancerous cells but are produced in higher levels by cancer cells.	Helps diagnose cancer, decide on treatment, measure how effective the treatment (how well it worked), and to also monitor for signs that the cancer has come back.
Urinalysis	Color description and measurement of contents in urine such as sugar, proteins, RBCs, and WBCs.	Helps diagnose kidney, bladder, and rare urothelial cancers.
Urine Cytology	Looks for abnormal cells shed from the urinary tract into urine to find disease.	Helps diagnose kidney, bladder, and rare urothelial cancers, and can also be used after cancer treatment to see if the cancer has come back.

Common and Emerging Lab Tests in Dogs

In veterinary medicine, there are some lab tests commonly conducted that are also done in human medicine. Common tests that are conducted include CBC, and serum and urine chemistry profiles paired with imaging tests (Modiano & Sharkey, 2012). Physical examinations are also conducted in the diagnostic process in veterinary medicine. There are also some newer and more specialized tests that have emerged in veterinary medicine in more recent years (Modiano & Sharkey, 2012). Some of these tests are still in development and may not yet be widely available in veterinary practices. Below is a table describing a few of the newer and more specialized tests. There are also more tests not listed that are still in the works, as this is an active area of research.

Table 6b: Lab Tests Used in Veterinary Medicine

Modiano, J. F., & Sharkey, L. C. (n.d.). *A Practical Guide to Diagnostic Testing for Veterinary Cancer Patients*.

Lab Test	Purpose	Significance of Results
Clonality testing for lymphoma: PCR for antigen receptor rearrangements (PARR)	Used to distinguish whether a lymphocyte population came from a single cell (monoclonal) or many cells (polyclonal). Uses polymerase chain reaction (PCR) to amplify sequences from all lymphocytes that are in a sample to see if all or most of the sequences are the same (clonal) or if there are different ones (polyclonal).	If the sequences are clonal, they are more likely to have originated from a cancerous process. If they are polyclonal, they are more likely to have occurred from an infectious agent or allergen. Estimated Specificity = 94% Estimated Sensitivity = 75%
OncoPet RECAF test	Used to detect a protein in the blood that is or is similar to the alpha-fetoprotein receptor (RECAF).	RECAF is present on cancer cells and isn't detected in significant levels on health cells or benign tumor cells.

	.	
P-glycoprotein mutation	Identifies dogs that carry a mutation in the ABCB1 gene. The ABCB1 gene encodes for P-glycoprotein (PgP), a transport protein, also known as a multi-drug resistance (MDR) protein. PgP is a protein that is responsible for chemoresistance in cancer.	Aids in planning individualized chemotherapy protocol based on the presence of PgP. If PgP is present, this is an indicator that the dog may be more chemo resistant if they develop cancer and seek treatment.
VCxI-TK test for cancer	Detects levels of Thymidine kinase (TK) in the blood. TK is an enzyme that is expressed in cells undergoing division and is released into the blood, so it is used as a biomarker for cell proliferation.	Higher levels of TK indicate the presence of rapidly dividing cells, which could mean the presence of cancer, but not necessarily. Other conditions cause rapidly dividing cells such as pregnancy, inflammation, and growth.
VDxI canine-specific C-reactive protein test and INCaSe	C-reactive protein (CRP) is produced in the liver as in the earlier phases of systemic inflammation. WBCs responsible for inflammation will produce little to no CRP and, instead, release factors that signal the liver to produce and release CRP.	The presence of CRP can potentially tell if cancer is present, as inflammation is a response to many abnormal states. The cause of the inflammation, however, may not necessarily be due to or related to cancer.

Lab results from healthy individuals will vary, which is why these results are usually given in a range and not a set integer (NCI, 2023). The range that is considered to be normal is formed from a large reference population of individuals who have been tested in the past (NCI, 2023). Lab tests can also return abnormal results, but this does not definitively mean the patient

has cancer (NCI, 2023). The same logic is true in veterinary medicine as well. The various forms of diagnostic testing in both human and veterinary medicine are used in tandem in order to get as accurate results as possible, as results from one form of diagnostic testing may be misleading.

Sensitivity and Specificity

For many of the common diagnostic tests, it is possible for a test to bring back normal results and the individual have cancer, or for the tests to yield abnormal results in the absence of cancer. With any of the methods of diagnostic testing (imaging tests, biopsies, and lab tests), it is possible for the results to not accurately depict if the patient is truly healthy or has cancer. All tests run the risk of returning a false positive, i.e., diagnosing cancer in a patient who does not have it, or a false negative, i.e., returns a normal result when a patient indeed has cancer. **Sensitivity** and **specificity** are used to gauge the accuracy of these tests to return a true positive or a true negative, respectively (Shreffler & Huecker, 2023). Predictive values such as **positive (PPV)** and **negative (NPV) predictive values**, are values that determine how many individuals, out of all the negative or positive individuals, have a true negative or positive (Shreffler & Huecker, 2023). Providers should consider calculations such as sensitivity, specificity, PPV and NPV when choosing a diagnostic test to use to have the proper level of confidence in the accuracy of the results yielded by the diagnostic test (Shreffler & Huecker, 2023).

Cancer Epidemiology

Types of Epidemiological Studies and Study Designs

Descriptive epidemiology is used to monitor (surveillance) and observe trends in various forms of cancer among different populations of humans and animals across the globe. Analytic

epidemiology is used to assess risk factors associated with the development of specified cancers. Organizations such as the American Cancer Society (ACS) provide annual data that estimates the amount of new cases (incidence) and deaths (morbidity) throughout the United States. ACS collects and compiles any current data on population-based cancer occurrence and outcomes (Siegel et al, 2022). Cancer data is also provided through programs and registries such as: the Surveillance, Epidemiology, and End Results Program (SEER), the National Program of Cancer Registries, and the North American Association of Central Cancer Registries which provide data on cancer incidence. These organizations, registries, and programs, therefore, are giving more information on the descriptive epidemiology of cancer.

Publications and study will usually conduct more analytic epidemiology in order to assess association between various risk factors and the development of specified cancers. When conducting an epidemiological study, a study design must be chosen. The study design chosen should be best suited to study the topic of interest. The strength of the scientific evidence the study yields is also an important factor that should be considered when choosing a study design. Below is the Hierarchy of Scientific Evidence that shows study designs that yield the weakest to strongest scientific evidence. The base of the pyramid is the weakest, and the top of the pyramid is the strongest.

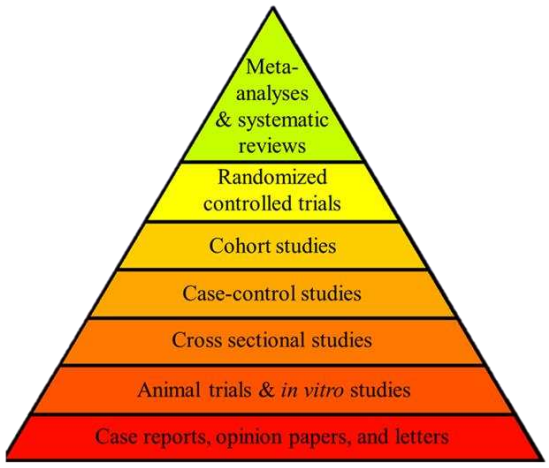

Figure 4 – Hierarchy of Evidence / Study Design Hierarchy

In terms of rare diseases, like cancer, the best study design to use is a **case-control study** (in humans). Case control studies are used to determine how great of an association there is between risk factors and outcomes, which is what is usually the goal in studies with cancer. Risk factors can also be called exposures, as certain exposures, like environmental exposures, can contribute to an outcome, like the development of a cancer. From a case-control study, an odds ratio (OR) calculation can be conducted to see the odds of a particular factor among individuals with the outcome of interest in comparison to those without the outcome (Brighton et al, 2003). The OR, therefore, measures the odds of an outcome occurring in relation to an event or exposure. More preferable forms of observational studies, based on the hierarchy of evidence, like a prospective (looking forward in time) cohort study, aren't appropriate for the study of rare diseases in humans. Being that cancer is a rare disease, it is unknown when the cancer outcome will occur, so the study could take a long time. However, cohort studies can be conducted on dogs for rare outcomes like cancer, as dogs have a significantly shorter life span in comparison to humans.

Through use of a case-control study, beneficial and harmful exposures can be identified. Cases, patients who have the disease, and controls, patients who don't have the disease are gathered and represent the two groups to observe for this type of study. The controls should be a good representative of the cases they are studying from the general population (Munnangi & Boktor, 2023). After the groups are gathered, researchers look retrospectively (into the past) to see if patients in both groups were exposed to potential risk factors. Looking into the past to get exposure status of the patients relies on the recollection of the patients, which makes case-control studies prone to information bias, specifically recall bias. Recall bias is when study subjects have the tendency to inaccurately recall past events or exposures, which can thus bias study results. These study designs are also applicable in epidemiological studies conducted on animals as well, including dogs. An example of a epidemiological study conducted on dogs was a nested case-control study conducted in the UK that aimed to estimate the prevalence of canine diabetes mellitus (DM) to identify potential risk factors associated with DM, and to be able to describe what the survival was of dogs that are affected by DM (Mattin et al, 2014).

Biases and Controlling for Biases

No matter the study design or precautions taken, however, all studies have some form of bias and there is no way to completely eliminate bias. Biases can only be minimized by taking precautions such as adjusting for confounding factors and having an appropriate method of selecting participants. For example, in case-control studies, selection bias can be minimized by appropriately selecting the control group subjects, which as mentioned before is a crucial component to case-control studies. Controls should come from the same source population as the cases and be an appropriate representation of the exposure distribution in the source population (LaMorte, 2021). Also, whether or not an individual is exposed or unexposed should not affect

their enrollment or selection as a control subject in the study, as controls only need to be free of the disease of interest (LaMorte, 2021).

Cancer Causes: Risk Factors for Cancer in Dogs and Humans

Cancer can be caused by a combination of factors over time (CDC, 2021). Research has shown that certain risk factors can potentially increase the risk of developing cancer (NCI, 2015). Some of the most studied risk factors that increase the likelihood of a human developing cancer include age, alcohol use, exposure to carcinogenic substances (environmental factors), chronic inflammation, diet, hormones, immunosuppression, infectious agents (environmental factors), obesity, radiation & sunlight (environmental factors), and tobacco use/secondhand smoke exposure (NCI, 2015). Another factor that can increase one's risk is family history which would make them genetically predisposed to the development of cancer (CDC, 2022).

For children and adolescence, lifestyle related risk factors such as secondhand smoke exposure, diet, body weight, and physical activity are not thought to play a role in childhood cancers like leukemia, as these risk factors usually take years to influence cancer risks (ACS, 2020). Some known risk factors for childhood leukemia include genetic, lifestyle and environmental risk factors (ACS, 2020).

Dogs can have similar risk factors to humans, such as age and environmental factors, where exposure to harmful environmental factors can be caused by their owners (e.g., cigarette smoke). Other potential risk factors include the dog's sex status, meaning whether the dog is spayed or neutered ("fixed"), and their breed. A dog getting spayed or neutered would be removing a sex organ, which would result in a change in their hormone levels (Albright, 2020). Hormones have been found to be associated with cancers such as breast or mammary, ovarian,

endometrial, testicular, and prostate cancer. However, according to the American Veterinary Medical Association (AVMA), the cause of most neoplastic diseases in dogs are not known, making prevention difficult (AVMA, 2022). Below is a table listing risk factors and their common cancer type associations in human, which may also affect dogs:

Table 7: Risk Factors for Cancer Development in Dogs and Humans

Risk Factor	Human Cancer Type Association	Dog Cancer Type Association
Age	Any; Depends on exposures and hormone levels	Any; Depends on exposures, breed, sex status (intact or spayed/neutered)
Sunlight and Radiation	Radiation (ionizing radiation): Leukemia, breast, bladder, colon, liver, lung, esophageal, ovarian, multiple myeloma, and stomach cancer. (United States Nuclear Regulatory Commission, 2020) Sunlight (UV- non-ionizing radiation): Melanoma (Skin cancer) (CDC, 2016), Basal cell Carcinoma (McDaniel et al, 2022)	Skin cancers, including one of the most common forms, mast cell tumors. (Son, 2022)
Hormones	Breast/mammary, endometrial, testicular, prostate, ovarian	Breast/mammary, endometrial, testicular, prostate, ovarian

Diet & Obesity	Depends on the food group and type of diet. A diet consisting of high sugar drinks and highly processed foods can lead to weight gain and obesity, which then increases the risk of developing at least 13 different forms of cancer including colorectal, breast (in postmenopausal women), and endometrial cancer. (CDC, 2022, Avgerinos et al, 2019)	Mast cell tumors, mammary tumors, transitional cell carcinoma of the bladder. (Romano et al, 2016) Larger breeds are also more prone to developing cancer.
Immunosuppression	Depends on the infectious agent that was able to bypass the immune system while immunosuppressed. Cancers that can be caused by an infectious agent such as non-Hodgkin's lymphoma (Epstein-Barr Virus) and liver cancer (hepatitis B and C viruses). Immunosuppression has also been seen to cause lung and kidney cancers as well in organ transplant recipients who need to take immunosuppressive medication so that their new organ does not get rejected, which aren't generally thought to be associated with cancer. (NCI, 2015)	Immunosuppressive therapies can also be conducted on dogs when diagnosed with an immune-mediated disease. (University of Missouri, 2015) Therefore, dogs may be more susceptible to cancer depending on if an infectious agent was able to bypass the immune system while immunosuppressed like in humans, as dogs have a similar acting immune system to humans. (Felsburg, 2002) Not much definitive literature found on association between immunosuppression and risk of cancer development in dogs, but literature found was about immunosuppression characteristic of tumors that drives metastasis and tumor progression in mammary cancer. (Mucha et al , 2016)

		Atopica, a medication used in dogs, is immunosuppressive and is associated with papillomatosis of the gum, which comes from CPV, canine papilloma virus, infection – the dog equivalent of HPV in humans. CPV causes benign warts in dogs and rarely results in carcinomas. (Elanco, 2020, Evenson & Lightman, 2023)
Chronic Inflammation	Can depends on inflammation site (Greten & Grivenikov, 2019) Appears to be a contributor to development of multiple cancers such as: kidney, prostate, ovarian, hepatocellular, pancreatic, colorectal, lung, and mesothelioma. (Pahwa et al, 2022)	Can contribute to the development of certain types of cancer such as liver, bladder and skin cancer (Berg & Hensley, 2023)
Carcinogenic Substances	Depends on the type of carcinogen. Benzene, a commonly known cancer-causing substance, has been found to be associated with development of leukemia and lymphoma. (CDC, 2019, ACS, 2023)	Depends on the type of carcinogen. Benzene may directly contribute to cancer in pets, such as dogs, but there is a lot less research done on these types of associations regarding animals in comparison to humans. However, it has been said to be a multi-organ carcinogen in animals. (Snyder et al,
Infectious Agents	Depends on the infectious	Depends on the infectious

	agent. HPV, a commonly known infectious agent, is commonly associated with the development of cervical cancer. (NCI, 2015)	agent. Canine TVT (transmissible venereal tumors), which are commonly located on the genitalia. (Kutzler, 2022) CPV, the dog equivalent of HPV, causes benign warts in dogs and rarely results in carcinomas. (Evenson & Lightman, 2023) Viral infections, in general, rarely cause cancer in dogs. (Evenson & Lightman, 2023)
Tobacco Use	Many forms of cancer, including lung, and oral cancers (mouth), larynx (voice box), throat, esophagus, kidney, liver, colorectal, bladder, pancreas, cervical, and acute myeloid leukemia cancer. (NCI, 2015)	Dogs can be exposed to secondhand smoke which can result in respiratory compilation, including lung cancer. (Llera & Buzhardt, 2023)
Alcohol Use	Mouth, pharynx (throat), larynx (voice box), esophagus, colorectal, breast/mammary, pancreatic, and stomach. (ACS, 2020, Tramacere et al, 2011)	N/A

While there are potential risk factors that can increase risk of developing a form of cancer, this will not always mean that a human or dog will definitively develop cancer if exposed, or that they will not develop it even after taking preventative measures to avoid it. It is commonly stated how cancer is mostly due to random chance (Wodarz & Zauber, 2015).

Age

Age in Relation to Cancer Development in Humans

In both dogs and humans, cancer is more frequent in those of older age. Age is said to be the most important risk factor for cancer overall, and for many individual cancer types (NCI, 2015). One reason for this may be because of **telomere length**.

Telomeres cover the ends of chromosomes and protect them from degradation, fusion with other chromosomal ends (end - joining), and improper recombination or exchange of genetic materials (Bailey, 2023). They also prevent the ends of chromosomes from being detected as double strand breaks in DNA or DNA damage (Bailey, 2023, Nasir et al, 2001). Humans and dogs have been found to have similar telomere biology when it comes to aspects such as telomere length and telomere shortening (Fick et al, 2012). As humans and dogs age, the telomeres that protect the chromosomal ends erode and shorten. Eroded telomeres can leave chromosomal ends exposed, leading to genomic instability and increased risk of cancer development (Rodier et al, 2005). The telomeres on the chromosomes of humans can be approximately 10-15 kbps in length, while the telomeres on the chromosomes of dogs can be anywhere from 3-25 kbps in length but can also be said to be around 15 kbps as well (Bailey, 2023; Nasir, 2008). Based on the current knowledge of telomeres, they are at their maximum length at birth and progressively shorten over time (Rizvi et al., 2014). This would mean that the complications that come with the shortening of telomeres would, ideally, be apparent later in age. As cell replication occurs, telomeres shorten. As part of the aging process, cells continue to replicate resulting in shorter telomere length with age (Shay, 2016). This also ties into the understanding that more cell replications pose the risk of the accumulation of more mutations in your genetic makeup. Shortening can also occur due to **oxidative stress**, which is an imbalance

of free radicals and antioxidants in the body and naturally accumulates in the body over time and contributes to aging (Eske, 2019). There are multiple factors that contribute to oxidative stress and can lead to excess free radical production such as diet, lifestyle, and environmental factors like sunlight and radiation, which happen to also be risk factors for cancer development as well (Eske, 2019). Shortened telomeres, however, can be found in younger individuals, and can even be present at birth (Johns Hopkins Medicine, 2019). Abnormally shortened telomeres at birth can be attributed to the premature aging and death of immune system cells, like T-cells (Johns Hopkins Medicine, 2019). T-cells are cells that will remember previous invaders of the body so that, if present again, they can recognize them and trigger an immune response quicker (Johns Hopkins Medicine, 2019). Therefore, one does not have to be physically older (lived longer) to have shortened telomeres, but genotypically individuals can be "aged" and this can cause complications such as cancer development.

NCI's SEER Data Showing Correlation Between Age and Cancer Development in Humans

According to the NCI's SEER Program Cancer statistics, the median age of a cancer diagnosis was 66 years old in humans, meaning half of cancer cases occur in people below this age and half occur in people above this age (NCI, 2021). The incidence rate for cancer overall steadily increases with age (NCI, 2021). For individuals aged 20 or younger there can be less than 25 cases per 100,000 where there can be more than 1,000 per 100,000 in individuals aged 60 or older (NCI, 2021). Using 2019 as a year of reference, according to the NCI's SEER Program Cancer statistics, the incidence rate for people aged between 65 and 74 was 1,819 per 100,000 (NCI, 2022). Those 75 and older have an incidence rate of 2,236,6 per 100,000. The incidence rate for people aged 15 and younger is 17 per 100,000 (NCI, 2022).

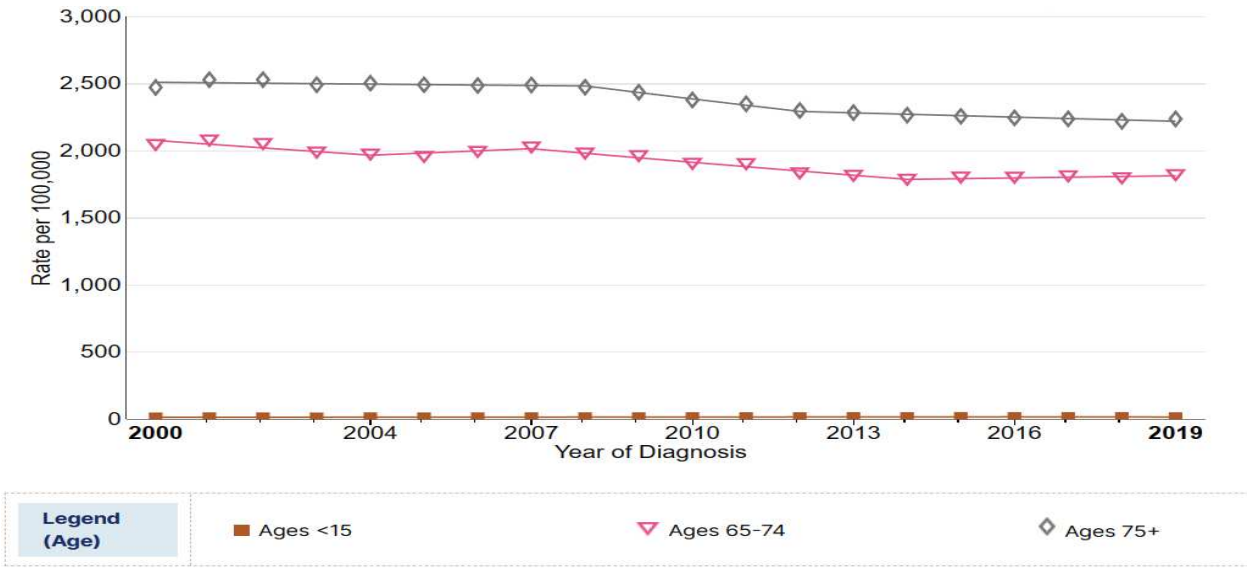

Figure 5 – Comparison of Cancer Incidence Rate Between Difference Age Groups from 2000 - 2019

National Cancer Institute (NCI). (2023d). *SEER*Explorer Application*. National Cancer Institute Surveillance, Epidemiology, and End Results Program. https://seer.cancer.gov/statistics-network/explorer/application.html?site=1&data_type=1&graph_type=3&compareBy=sex&chk_sex_1=1&rate_type=2&race=1&advopt_precision=1&advopt_show_ci=on&hdn_view=0&advopt_show_apc=on&advopt_display=2#resultsRegion0

Childhood Cancer and Early Cases

While not as common as in older individuals, cancer still does occur in children and is considered the leading cause of death by disease past infancy in children in the U.S. (NCI, 2021). As mentioned in a previous section the most common form of cancer diagnosed in children, those age 0-14, are leukemias, usually acute lymphocytic leukemia, and the most common form in adolescence, those age 15-19, are brain and other CNS tumors and lymphomas (NCI, 2021).

According to the NCIs Childhood Cancer Data Initiative National Childhood Cancer Registry Explorer, from 1999- 2015 there appeared to have been a steady increase in childhood and adolescent (<20) incidence with both sexes combined. In 2016, there is a trend change due to a decrease in incidence and has been steadily decreasing from 2016-2019. 2019 is the year of the most recent data available at the moment. The incidence rate went from 201.1 per 100,000 in

2015 to 186.4 per 100,000 in 2019. Below is a graph showing the incidence rates of childhood and adolescent cancer from 1999-2019 provided by the NCIs Childhood Cancer Data Initiative National Childhood Cancer Registry Explorer.

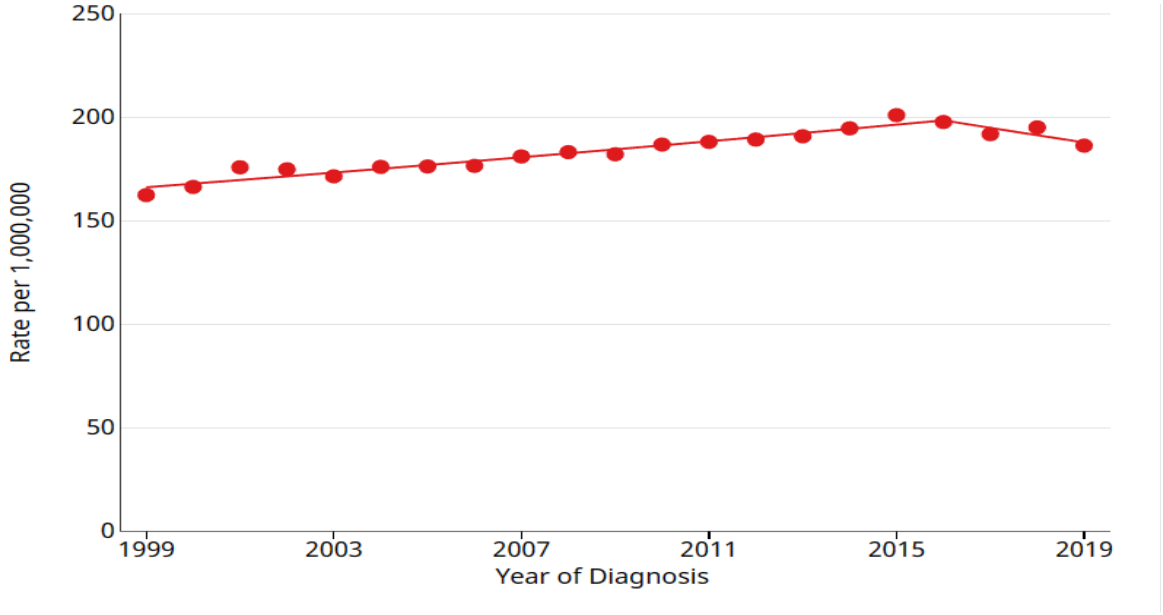

Figure 6 – Cancer Incidence Among Children and Adolescences from 1999 - 2019

National Cancer Institute (NCI). (2023c). *NCCR*Explorer Application*. National Cancer Institute Childhood Cancer Data Initiative National Childhood Cancer Registry Explorer. https://nccrexplorer.ccdi.cancer.gov/application.html

As for survival, the survival rate of children and adolescents who develop cancer has been improving (NCI, 2021). A 5-year survival graph, also provided by the NCIs Childhood Cancer Data Initiative National Childhood Cancer Registry Explorer, shows the 5-year relative survival rate of children and adolescence from 2012-2019 being at 86.1% with a CI of 85.8-86.5. The 5-year survival graph includes those who have survived 5 or more years after diagnosis. Below is the graph depicting survival in children and adolescence with both sexes combined.

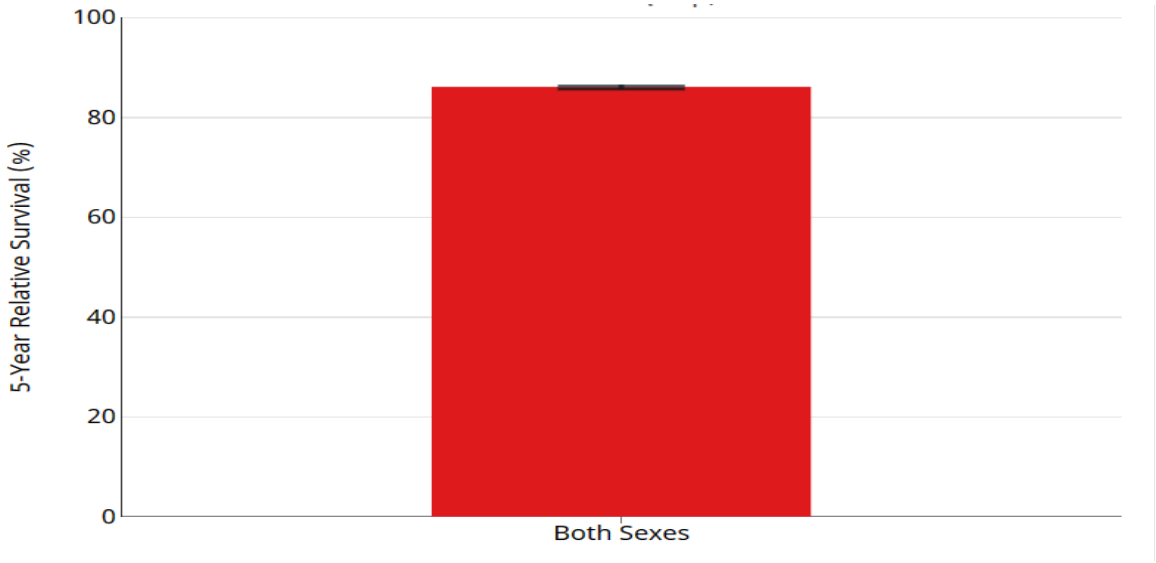

Figure 7 – 5- Year Relative Cancer Survival Percentage Among Children and Adolescences 2013 - 2019

National Cancer Institute (NCI). (2023b). *National Childhood Cancer Registry Explorer (NCCR*Explorer)*. National Cancer Institute Childhood Cancer Data Initiative National Childhood Cancer Registry Explorer. https://nccrexplorer.ccdi.cancer.gov/

Age in Relation to Cancer Development in Dogs

In dogs, cancer is more common in older dogs. However, the age a dog is considered a senior can vary across breeds. Small dogs can be considered to be seniors between ages 8 - 10, while large dogs may be considered seniors at age 5 or 6 (Meyers, 2022). The Animal Tumor Registry of Genoa, Italy was established in 1985 and aimed to estimate the occurrence of spontaneous tumors in dogs. They had collected data from pet animals in European and North American veterinary cancer registries via different methods and reference populations. 6,743 tumor biopsy samples were received from local veterinarians, and of those samples 48.9% were cancerous. All cancer incidence rates increased with age ranging between 23.7 and 763.2 in female dogs and between 16.5 and 237.6 in male dogs aged 3 or younger and 9 - 11 years old (Merlo, 2008).

Oxidative stress has been found to be associated with cancer development and progression in dogs (Macotpet et al, 2013). In a study conducted in 2013, Malondialdehyde

(MDA) was used as a biomarker for oxidative stress and to measure the levels of MDA in cancer-bearing dogs and clinically normal dogs (Macotpet et al, 2013). It was found that MDA levels were significantly higher in cancer bearing dogs than in clinically normal dogs, meaning that there was more oxidative stress present in cancer bearing dogs (Macotpet et al, 2013). While this study does not connect the oxidative stress to telomere length and that being the reason behind cancer development, a connection can still be made between oxidative stress and the shortening of telomeres. Being that humans and dogs have similar telomere biology and have the same potential outcome due to increased oxidative stress, it can be presumed that the reason oxidative stress led to cancer development in these dogs is potentially due to the oxidative stress shortening the telomeres in the dogs as it does in humans.

Early Cancer Cases in Dogs

Similarly, to children, young dogs can still develop cancer despite being young. Also, like with children, there are some cancers that have been found to be more common in young dogs. An example would be **cutaneous histiocytomas**. Cutaneous histiocytomas are benign tumors of Langerhans cells that grow rapidly within the first 1-4 weeks of it appearing, in which they usually ulcerate and become infected (Williams et al, 2023). These are not to be confused with histiocytic sarcomas, which is a more aggressive and fatal histiocytic cancer and effects mostly middle aged and older dogs (MAF, 2023).

As registries for dogs are lacking, like how human children have the NCIs Childhood Cancer Data Initiative National Childhood Cancer Registry, trends of the incidence rate of cancer in young dogs specifically overtime cannot be observed at this time. One of the hopes from the

analysis conducted in this paper is to provide more information on cancer distribution and trend in young dogs, specifically golden retrievers.

Overall, living into older age would mean there has been more opportunity to accumulate exposure, thus increasing the risk of cancer development. Over the span of one's life, including in dogs, there is more time to accumulate the other risk factors such as environmental factors, as well as reap the consequences of the preferred diet and lifestyle choices that were made over the course of life. However, there is the exception of younger individuals being at increased risk of cancer development despite being physically young, but genotypically aged.

Environmental Factors

Environmental factors are components of one environment, including living space. For example, regarding exposure, environmental factors can be harmful substances or matters that one is exposed to that can have an impact on their health. Some examples of environmental factors that one can be exposed to and that can impact one's health and contribute to the development of cancer are carcinogenic substances, infectious agents, radiation & sunlight, and cigarette and tobacco smoke (NCI, 2015).

Carcinogenic Substances

There are some substances that can result in the damaging of DNA, which can ultimately lead to the development of cancer (NCI, 2022). Such a substance can be in the form of a **chemical**. Some examples of chemicals that are known carcinogens include: arsenic, asbestos, benzene (cigarette and tobacco smoke), radon, and formaldehyde (NCI, 2022). Benzene will be focused on, as it is a chemical carcinogen in both humans and animals, and because benzene is one of the 20 most widely used chemicals in the U.S. (ACS, 2023, Snyder et al, 1993).

Benzene is a colorless and flammable liquid that evaporates quickly once exposed to air and is formed from natural processes such as forest fires and volcanoes (ACS, 2023). Benzene is also formed through human activity, which is the form of benzene most people will be exposed to (ACS, 2023). Human activities that result in benzene production include cigarette smoking, vehicle use (motor vehicle exhaust), and the production of plastics, resins, lubricants, rubbers, dyes, detergents, drugs, and pesticides (ACS, 2023). Being that benzene quickly evaporates when it hits air, people are mainly exposed to benzene by inhaling benzene contaminated air (ACS, 2023). Cigarette smoke accounts for nearly half of the exposures to benzene in the U.S. (ACS, 2023). Benzene has been linked to leukemias specifically, as there are studies and evidence that strongly support their association (ACS, 2021). There is not, however, as strong of a finding for the association between benzene and other types of cancer (ACS, 2023). The rate of leukemia, especially AML, has been found to be higher in studies of workers exposed to higher levels of benzene, such as those who work in industries involved with shoemaking, chemicals, and oil refining (ACS, 2023). As mentioned prior, benzene has been found to cause cancer in animals as well and seems to lead to cancers of multiple organs (Snyder et al, 1993). However, not much literature and studies appear to exist regarding the effects of benzene on dogs explicitly in regard to cancer development. Most literature that discusses the effect of benzene on animals, including dogs, discuss petroleum production poisoning, which can cause pneumonia, GI disturbance, impaired reproduction, CNS depression and/or excitation, and death (Mostrom, 2021). However, some sources do include lung cancer as one of the forms of respiratory distress dogs can endure because of exposure to benzene (Llera & Buzhardt, 2023).

Radiation and sunlight can also be classified as cancer causing substances. **Radiation** of certain wavelengths, including radon, x-rays, gamma rays, and high energy forms of radiation,

also known as ionizing radiation, can cause DNA damage and result in cancer in both humans and dogs (NCI, 2015). Humans and dogs can be exposed to radiation in their environments as well as during medical practices. In humans, the effects of radiation on an individual depends on the dose of radiation, dose rate/ how long they were exposed, how much of their body was exposed, and the age at which they were exposed (CDC, 2021). The higher the dose of radiation is, the more likely it is to lead to cancer development (CDC, 2021). Also, a dose that is received over a long period of time is less harmful in comparison to receiving the same dose all at once (CDC, 2021). Regarding age, children and adolescents are more sensitive to the effects of radiation (CDC, 2021). Regarding how much of the body is exposed to radiation, the more of the body that is exposed, the more risk there is in experiencing the effects of radiation. This can be related to concerns toward radiation therapy. A dose to a part of the body is less harmful in comparison to a dose to the whole body (CDC, 2021). Dogs appear to have similar factors to humans in the factors that influence the effects radiation has on them. In a study conducted on dogs, it was determined that the effects of radiation depended on the radionuclide (another form of ionizing radiation), method of exposure, age of exposure, dose rate (amount of radiation absorbed or delivered per unit of time), and total exposure rate (total amount of ionizing radiation per hour that is within an individual's vicinity) (Spatola et al, 2021, EPA, 2023).

Sunlight is a form of non-ionizing radiation and consists of ultraviolet (UV) radiation (CDC, 2023). UV radiation can be beneficial by aiding in production of important nutrients such as vitamin D, but overexposure can result in health complications including skin cancers (CDC, 2023). There are 3 forms of UV radiation: UVA, UVB, and UBC. UBC is absorbed by the earth's ozone layer, so this form does not pose as much of a risk (CDC, 2023). However, UVA and UVB are not absorbed by the earth's ozone layer (CDC, 2023). UVA is not absorbed at all while

UVB is partially absorbed but can still reach earth's surface and affect us (CDC, 2023). UVA is weaker in comparison to UVB, but still penetrates the skin deeper and is more constant year-round than UVB (CDC, 2023). In humans, overexposure to sunlight can lead to development in melanoma in humans and can lead to mast cell tumors in dogs (CDC, 2023, Son, 2023).

Infectious Agents

Infectious agents such as viruses, bacteria, and parasites can lead to the development of cancer or increase one's risk. The way in which these agents can lead to or increase risk of cancer development is by disrupting signals that would normally aid in keeping cell growth and proliferation (viruses), weakening the immune system, and causing chronic inflammation (NCI, 2015). Some viruses can disrupt important signals that regulate the growth and proliferation of cells, thus causing them to grow out of control. Some infections can weaken the immune system so that the body is unable to defend itself against cancer causing infections. Some viruses, parasites or infections can cause inflammation which, as mentioned prior, can ultimately lead to cancer development as well. Two infectious agents that have been associated with cancer in humans and dogs in Human Papillomavirus (HPV) and Canine Papillomavirus (CPV), the dog variation of HPV. HPV in humans is mostly known to be associated with the development of cervical cancers but has also been found to cause most anal cancers as well as oropharyngeal (throat), vaginal, vulvar, and penile cancers (NCI, 2015). CPV in dogs, however, causes benign wart and rarely results in malignancies in dogs although it is possible (Evenson & Lightman, 2023).

Lifestyle and Diet

Lifestyle choices as well as diet have been found to be correlated with the development of various cancers. Lifestyle can be considered a culmination of one's day to day activities (including occupation), habits, and diet, which all can affect the risk of cancer in humans and dogs. In regard to everyday activity and habits, this can consist of exercise or lack thereof, smoking cigarettes, what one's occupation is and alcohol consumption.

Lack of exercise is understood to increase one's cancer risk, as there would then be lack of mitigation of other risks and complications related to cancer as well, such as weight gain (obesity), deep vein thrombosis (blood clots in veins deep inside the body), cognitive impairment, depression, muscle pain and inflammation (arthralgia), and fatigue (Thomas et al, 2021). A meta-analysis that was conducted on 126 studies found that the total cancer risk was decreased by 10% in people who participated in the most leisure time physical activity (Liu et al, 2016). Physical activity has also been found to decrease the likelihood of cancer recurring in individuals, and improve mortality, as shown from another meta-analysis and systematic review (Morishita et al, 2020). The risk ratio (RR) had been calculated for each report to investigate the effect that exercise had on mortality and the recurrence of cancer (Morishita et al, 2020). Of 2868 articles, exercise had significantly reduced the risk of mortality in patients with cancer and survivors (Morishita et al, 2020). Lack of exercise in dogs can also lead to the development or progression of cancer, due to the correlation with obesity and weight gain, which will be discussed in the next section (Canadian & American Pet Cancer Foundation, 2022).

As mentioned in the previous section discussing environmental factors, **cigarette smoking** increases one's risk of developing cancer, partially due to the chemical benzene being in cigarettes. Dogs can also be at increased risk of respiratory distress and cancer from

secondhand smoke exposure. Previously, it was also mentioned how chemical and radiation exposures can increase the risk of cancer development, which individuals can be more exposed to at work depending on the occupation (industrial workers, fire fighters, etc.). However, alcohol consumption has not yet been discussed. **Alcohol consumption** can also increase the risk of cancer development due to the presence of ethanol and the damage alcohol can cause to internal organs and tissues, and their function (ACS, 2020). Aldehyde, a metabolite of ethanol, can cause DNA damage and block DNA synthesis and repair. Ethanol itself can induce inflammation and oxidative stress, which leads to the oxidative degeneration of lipids and further DNA damage (Rumgay et al, 2021). Alcohol can also disrupt absorption of vital nutrients like folate, a vitamin that cells need in order to stay healthy (ACS, 2020). Alcohol increases hormone levels and weight, which increases the risk of cancer development (ACS, 2020). A meta-analysis conducted had also found evidence of cancer risk increasing with the amount of alcohol consumed daily. For example, for cancer of the oral cavity and pharynx, an increased RR from 1.13 to 5.13 was seen with an increase of alcohol consumption from light drinkers (up tp 12.5 grams/day) to heavy drinkers (more than 50 grams/day) (Rumgay et al, 2021).

Obesity

Obesity has been found to be a risk factor for developing cancer in humans and dogs, and the size of dogs has been found to be correlated with the development of cancer. Obesity falls under lifestyle and contributes to how one's lifestyle can increase their risk of cancer development. Obesity can be defined as having an unhealthy amount and/or distribution of body fat and can be measured using the body mass index (BMI) in humans (NCI, 2022). A BMI of 30-39.9 is considered obese and any BMIs higher than 39.9 are considered severely obese in

humans (NCI, 2022). In dogs, body condition score (BCS) is used to classify and assess the fatness as well as muscle composition of the dogs and is used to classify most animals (Mullins et al, 2019). A BCS can be on a 5- or 9-point scale. On a 5-point scale: 1 would be considered very thin, 3 would be an ideal weight, and 5 would be obese. On a 9-point scale: 1 would be considered emaciated as well, but 4-5 would be considered an ideal weight, 8 would be obese, and 9 would be severely obese (Williams & Buzhardt, 2023). BMI is determined by calculation, while BCS is determined by physical examination of the hips, ribs, and waist of the dog (NCI, 2022, Williams & Buzhardt, 2023). Both scales are used regardless of age.

There are some mechanisms that have been suggested to explain how obesity increases cancer risks. Some of these mechanisms involve the effects fat cells have on the body as well as secondary effects that obesity can cause. Fat cells (adipose tissues) can produce excess estrogen, which higher levels of have been linked to increased risk of endometrial, breast, and ovarian cancers (NCI, 2022). Hormones and them increasing the risk of specified cancer development will be talked about more in depth in the next section. Another hormone, known as adipokines, can stimulate or inhibit cell growth. A specific kind of adipokine called leptin increases in the blood with increased body fat, and higher levels of leptin can promote abnormal cell proliferation (NCI, 2022). Fat cells can also have both direct and indirect effects on cell growth and metabolic regulators such as mTOR, which regulates a multitude of processes including aging and autophagy (NCI, 2022, Murugan, 2019). Obesity is also associated with increased levels of insulin in the blood, which can lead to the development of type 2 diabetes, another risk factor of cancer development (NCI, 2022). Lastly, obesity can result in chronic inflammatory conditions such as gallstones or non-alcoholic fatty liver disease, which can cause oxidative stress and lead to DNA damage, increasing the risk of cancer development as well (NCI, 2022).

Similar mechanisms are thought to be the explanation of cancer being a greater risk in obese dogs as well (Marchi et al, 2022).

Sex & Hormones

Sex is in reference to one's sexual/physical anatomy. Biological sex influences the amount of particular sex hormone production in the body. Both sexes have overlapping hormones, specifically sex hormones, that are present in both males and females (Campbell & Jialal, 2022). The level of each type of hormone is what makes the differences between the external and internal functions of the body (Campbell & Jialal, 2022). The main sex hormones are testosterone, estrogen, and progesterone. Estrogen and testosterone can be produced by the testes (in males) and ovaries (in women), however estrogen is produced in smaller amounts in men in comparison to women and men produce more testosterone than women. In females, progesterone is the hormone that thickens the lining of the uterus so it is prepared to accept eggs after ovulation (Endocrine Society, 2022). However, progesterone can be found in both males and females. Androgens are male sex hormones, and the main androgen is testosterone, but also includes dihydrotestosterone (DHT) (NCI, 2021). Androgens are hormones that are responsible for the maintenance and development of male sex characteristics, including the growth and function of the prostate gland which is the gland in males that is responsible for semen production (NCI, 2021). These hormones are considered known carcinogens, as they can increase the risk of either sex to be at risk of breast/mammary, endometrial, prostate, and testicular cancers. This means that the sex of a human, anatomically male or female, or the sex status of male or female dogs, intact, spayed (female) or neutered (males), can be risk factors in the development of those cancers.

When comparing females from both species, similarities can be seen in the development of breast cancer and mammary cancer. Breast cancer is one of the most common forms of cancer seen in women and in intact female dogs or female dogs that were spayed after 2 years; the most common site of tumor developments has been found to be the mammary gland (Dobson, 2013, ACVS, 2023). The importance of sex hormones, and thus sex/reproductive anatomy, can be seen through breast, endometrial (uterine), and ovarian cancer in women, and mammary cancer in female dogs. Focus will be brought upon the effects of estrogen and progestin/progesterone in the development of breast and ovarian cancer in women, estrogen in the development of endometrial cancer in women, and the nature of mammary cancer in female dogs.

For women, hormones have been said to be a risk factor for cancer, as estrogens, which have physiological roles in both men and women, are known human carcinogens and have been associated with an increased risk of specific cancers, such as breast, endometrial (uterine), and ovarian cancer (NCI, 2015). Examples of these incidences are usually seen in women who undergo menopausal hormone therapy (MHT), a form of treatment that can be recommended to women by their doctors in order to help relieve symptoms that come with menopause, and to help with the long-term biological changes that come with menopause (NCI, 2018). During MHT, women receive a combination of estrogen and progestin (a synthetic form of progesterone), while estrogen alone is used in women who have undergone a hysterectomy in the past (NCI, 2018). The reason for this specified treatment due to hysterectomy history is because estrogen alone has been associated with an increased risk of endometrial cancer, but estrogen plus progesterone has not (NCI, 2018). A woman who has undergone a hysterectomy has had their uterus removed, eliminating their risk of developing endometrial cancer, a cancer of the uterine lining.

Estrogen + Progestin in Breast and Ovarian Cancer Development

Studies have found that menopausal hormone therapy can increase a woman's risk of developing breast and ovarian cancer, due to the use of estrogen and progestin (a synthetic version of the female hormone progesterone) (NCI, 2015; Greisere et al, 2007). Two randomized, prospective, placebo-controlled clinical trials conducted by the Women's Health Initiative (WHI) had evaluated estrogen and progestin in 16,608 postmenopausal women, and evaluated estrogen alone in 10,739 postmenopausal women who had received a hysterectomy in the past (Chlebowski & Anderson, 2015). The age of the women being evaluated had been between ages 50 - 79 (Chlebowski & Anderson, 2015). In the first trial evaluating estrogen and progestin, it had been found that invasive breast cancer incidence had significantly increased in those who used estrogen and progestin, so much so that the trial was stopped early after 5.6 years mean follow up when it was seen that the harm outweighs the benefit in the estrogen and progestin use (Chlebowski & Anderson, 2015). The hazard ratio was 1.24 (95% CI 1.01-1.54; p-value = 0.003) for invasive breast cancer and had been commonly diagnosed among the women who received both estrogen and progestin (Chlebowski & Anderson, 2015). The breast cancers were also larger and in more advanced stages in comparison to those in the placebo group (Chlebowski & Anderson, 2015). Furthermore, it had been seen through this study that estrogen and progestin use could also hinder diagnosis, as it was found that there was decreased sensitivity, specificity, and positive predictive value of mammography (Chlebowski & Anderson, 2015). As for the evaluation of estrogen alone on women who had received a hysterectomy in the past, the breast cancer findings had been quite different from most of the observational reports that had demonstrated an association between increased breast cancer risk and duration of use of

estrogen alone. Less invasive breast cancers had been seen at the end of intervention, having a hazard ratio of 0.80 (95% CI 0.62-1.04; p-value =0.09), where other studies in the past had mixed outcomes (Chlebowski & Anderson, 2015, Stefanick et al, 2006). The grave majority of older observational studies had reported modest increases in breast cancer diagnoses (Stefanick et al, 2006). However, the reduction in breast cancer incidence when comparing the estrogen alone and placebo group that was seen in this analysis was found to not be statistically significant, more than likely due to the CI including the null value of 1.0 (Chlebowski & Anderson, 2015, Stefanick et al, 2006). A potential explanation was suggested after an exploratory study was conducted, and the findings suggested that estrogen might decrease breast cancer incidence in certain subgroups (Stefankick et al, 2006). A meta-analysis based on 42 studies and 12,238 cases also found an increased risk for ovarian cancer, having an OR/RR of 1.110 (Greiser et al, 2007).

Estrogen + Progesterone in Breast Cancer Development

According to the NCI, studies have also shown that the risk of breast cancer is related to estrogen and endogenous progesterone that is being made in the ovaries (NCI, 2015). However, another paper in more recent years has claimed that the link between endogenous progesterone in breast cancer development is undefined (Trabert et al, 2020). Endogenous progesterone refers to the progesterone that naturally occurs within the body as opposed to the synthetic form progestin. It appears, from review of literature, that estrogen drives the first stage of pubertal development in the breast, while progesterone is responsible for cellular proliferation in the mammary gland (Trabert et al, 2020).

Estrogen in Endometrial Cancer Development

Estrogen can increase a woman's risk of developing endometrial (uterine) cancer. Excess estrogen, which in a normal menstrual cycle causes tissue growth (thickening of uterine lining) in anticipation of pregnancy (Rodriguez et al, 2019). Progesterone inhibits estrogen-induced endometrial growth. The balance between estrogen and progesterone is usually dominated by estrogen in endometrial cancer development (Rodriguez et al, 2019). In animal models, it was found that higher levels of estrogen that was unopposed by progesterone lead to endometrial cancers (Rodriguez et al, 2019). Many endometrial cancer risk factors involve excess estrogen or estrogen signals that are unopposed by progesterone (Rodriguez et al, 2019).

Mammary Cancer in Female Dogs

In intact female dogs or female dogs spayed after 2 years, mammary cancer appears to be the most prominent and frequent. In a study conducted on the Animal Tumor Registry of Genoa, Italy, female dogs were found to have three times higher incidence of all cancers than male dogs, but the difference could be explained by a high rate of mammary cancer observed in female dogs (Merlo, 2018). In this study, mammary cancer was found to be the most frequently diagnosed cancer in female dogs, being that it accounted for 70% of all the cancer cases (Merlo, 2008). This shows that, among the various forms of cancer female dogs can develop, they tend to develop mammary cancer.

In the U.S., it is commonly recommended in veterinary medicine that female dogs be spayed before their first heat at about 6 months of age, as this will reduce their risk of mammary cancer development to about 0.5% (ACVS, 2023). Once the dog is past the first heat cycle without being spayed, their risk jumps up to about 8%, and after their second heat cycle without

being spayed, the risk jumps up to about 26% (ACVS, 2023). In northern Europe, however, spaying and neutering (surgical removal of the gonads) is considered mutilation under the Animal Welfare Act (Von Heimendahl, 2011). As in humans, mammary cancer is more common in females than male dogs. Male dogs possess mammary glands as well, and on rare occasions can develop mammary tumors (ACVS, 2023). However, even then, the tumors appear to usually be benign (Saba et al., 2008). This, as well as other studies findings, suggest that a potential cause of the higher frequency in female dogs is due to differences in hormones, like in humans. Similarly, progesterone and estrogen are linked to the development of mammary cancer in female dogs (Son, 2023).

Prostate and Testicular Cancer in Male Dogs

Hormonal stimulation and imbalance in males pose a risk in the development of prostate and testicular cancer. Androgens have been found to be a crucial component, also, for the growth and development of prostate and testicular cancer (NCI, 2021, Ferlin & Foresta, 2014). Therefore, normal, and cancerous prostate cells need androgen for growth and development (NCI, 2021). There are currently hormone therapies for men that lower the androgen levels or block androgen action in order inhibit the growth of prostate cancers (NCI, 2021). However, some prostate cancer cells can become resistant to therapy and continue to progress, despite androgen levels being lowered or androgen action being blocked (NCI, 2021). Testicular germ cell tumors, which are the cells that represent 95% of testicular cancers, are generally assumed to be controlled by androgen and estrogen level imbalance and/or activity (Ferlin & Foresta, 2014). This imbalance of androgen and estrogen levels as well as their activity are understood to, thus, aid in the progression and development of testicular germ cell tumors (Ferlin & Foresta, 2014).

In male dogs, however, prostate cancer is uncommon, but has shown to develop more frequently across male dogs that have been neutered in comparison to intact male dogs (Davis & Ostrander, 2014, Bryan et al, 2007, Kutzler, 2020). In a study conducted in 2007, it was found that neutered male dogs had a significantly increased risk of developing prostate cancer, having an odds ratio of 8.00 and CI of 5.60-11.42 for prostate transitional cell carcinoma (Bryan et al, 2007). This is an opposite effect of spaying female dogs in order to prevent the development of mammary and endometrial cancers. Testicular cancer, on the other hand, is fairly common in intact male dogs. The testicles are one of the most common anatomical sites for tumor development in intact male dogs (Gazin et al, 2022). Two factors that have been found to predispose male dogs to testicular cancer are age, a factor that is a common risk factor across most cancers, and cryptorchidism (Ortega-Pacheco et al, 2006, Gazin et al, 2022). Cryptorchidism is when a male dog's testicles fail to descend into the scrotum after birth, as the testicles should descend no later than 6 months (Williams et al, 2023). Usually, it is a case where only one has failed to descend or “drop” (unilateral cryptorchidism). Cryptorchidism affects 1-3% of dogs, and the risk of developing testicular cancer is at least 10% greater in dogs with the condition (Williams et al, 2023). Therefore, it is recommended to neuter male dogs experiencing cryptorchidism, as they are at increased risk for testicular cancer development (Williams et al, 2023). Neutering in intact dogs is also recommended, as it eliminates the risk of the dogs developing testicular cancer (Williams & Ward, 2023).

Considering that these hormones have been found to have a correlation with the development of the respective cancers mentioned above, it has been strongly recommended by veterinarians for years to spay female dogs before the first estrous cycle and neuter/castrate male dogs at an early age if they have undescended testicles in the U.S. (Kelsey, 1998). Overall, it is

seen as standard practice to have dogs spayed or neutered within the first year of life in the U.S., (Hart et al, 2020). Having this procedure performed can potentially reduce the likelihood of mammary and testicular cancer specifically. However, there appears to also be potential drawbacks.

Drawbacks to Spaying and Neutering

While spaying female dogs and neutering male dogs is known to significantly decrease the risk of mammary and testicular cancer development in female and male dogs respectively, it can also increase the risk of other more aggressive cancers (Katz, 2007). Some of the more aggressive cancers include osteosarcomas, lymphomas, hemangiosarcomas, and mastocytomas (Kutzler, 2020). Also, as mentioned prior when discussing neutering in males, neutering increases the risk of developing prostate TCCs in male dogs, obesity, and orthopedic disease (Davis & Ostrander, 2014, Bryan et al, 2007, Kutzler, 2020, Katz, 2007). In larger breeds, spaying and neutering prematurely can increase the risk of developing osteosarcomas, mastocytomas, lymphomas, and can increase the risk of developing splenic and cardiac hemangiosarcomas in some breeds (Katz, 2007, Kutzler, 2020). Hemangiosarcoma development risks may be higher in female spayed dogs (Kutzler, 2020). Also, in golden retrievers, male dogs that are neutered have been found to be 3 times more likely to develop lymphomas than intact goldens and about 1 in 10 will develop lymphomas (Kutzler, 2020).

Breed

With dogs, the susceptibility to a particular form of cancer can be based on breed. There are over 175 different breeds of dogs, and some breeds are more susceptible to a particular form

of cancer than others (Dobson, 2013). Although this difference in susceptibility across breeds is well recognized, there are few large-scale epidemiological studies that study incidence of different cancer types while considering breed variations in the dog population (Dobson, 2013).

Most of the literature that lists breeds of higher cancer susceptibility generally agree on the breeds but differ in the ranking among the most susceptible breeds (Dobson, 2013). Below is a table of literature, and breeds they reported to be the most susceptible.

Table 8: Studies Reporting Most Susceptible Breeds

Article	Study Population	Method	Reported Higher Risk Breeds & Ranking
Kennel Club/BSAVA Study in UK Adams et al , 2010 Methods and mortality results of a health survey of purebred dogs in the UK - Adams - 2010 - Journal of Small Animal Practice - Wiley Online Library	Dogs of larger breed clubs in 169 UK Kennel Club recognized breeds. 15,881 deaths recorded	Cross sectional Study Approx. 58,363 questionnaires sent out to breed club members in 2004 Reported age at death and causes of death of all dogs that dies within last 10 years 13,741 responses on 15,881 deaths included in analysis	From Highest to Lowest (Descending Order): Irish Water Spaniel Flat-coated Retriever Hungarian Wirehaired Vizsla Bernese Mountain Dog Rottweiler Italian Spinone Leonberger Staffordshire Bull Terrier Welsh Terrier Giant Schnauzer
Bonnett et al, 1997 Mortality in insured Swedish dogs: rates and causes of death in various breeds - PubMed (nih.gov)	222,000 Swedish dogs enrolled in life insurance in 1992 and 1993	No Access to Full Literature to Observe Methods	5 breeds with highest mortality from tumor-related deaths: Bernese Mountain Dog Irish Wolfhound Flat-coated Retriever Boxer

			Saint Bernard

These studies both provided large study populations; therefore, they were chosen in an attempt to visualize what the most common breeds that develop cancer are. However, the number of each breed of dog that happened to end up participating and being included in the study can skew results on which breeds are the most susceptible. The size of the study populations, on the other hand, is still an important aspect that can help reduce bias. Of the most common cancers that occur in dogs, golden retrievers are most susceptible to hemangiosarcomas and lymphomas.

Comparative Oncology: Dogs as Sentinels

Analysis on dogs, like in this thesis paper, can be potentially related to humans, as dogs are a great cancer model for humans, due to commonalities in tumorigenesis and progression, the appearance of tumors and tumor characteristics being similar for certain cancers, as well as in responses and resistance to therapy (Gardner et al, 2015). Although lab animals such as rats and mice are more commonly used as animal models, dogs are better models due to the molecular and biological commonalities they have with humans. Dogs are also companion animals, and experience and share more common environments as well as environmental exposures in comparison to other animals (Kelsey et al, 1998, Thamm, 2019). Therefore, the findings of research conducted in dogs can potentially help us learn more about cancer in humans.

Purpose of Research & Research Objectives

The analysis conducted in this paper is for exploratory purposes. Given that such organizations that human medicine has to observe cancer incidence, prevalence, and other trends does not exist in veterinary medicine, an analysis was conducted on the GRLS study data to make an attempt to do so. The GRLS study data offers breed-specific information from an 8-year cohort study that follows golden retrievers to see if they do or do not develop cancer, while also recording other factors and characteristics of interest, such as their states of residence, sex status, spay or neuter date, the form of cancer they develop, and age at diagnosis or visit. With these factors and characteristics, prevalence can be calculated by state, and various statistical tests can be ran on the age at diagnosis or visit, as early cancer cases are of interest, in relation to sex status and the form of cancer the dogs develop.

The overall goal of this analysis is to explore the GRLS dataset and observe information on cancer trends among the GRLS dogs, and factors and characteristics of the GRLS cancer dogs by age/age category ("Young" or "Old"). The objectives of this analysis that will aid in accomplishing this goal are to:

- Observe cancer prevalence across the U.S. in golden retrievers using GRLS study data.
- Compare cancer prevalence among the GRLS dogs and humans using CDC data.
- Test to see if there are any statistically significant differences in age proportions by state and by sex status.
- Test to see if there are any statistically significant differences in average age at diagnosis based on sex status.
- Observe the most frequent cancers among the GRLS population.

- Observe cancer dogs by case type to analyze characteristics of case groups.
- Test to see if there are any statistically significant differences in average age at diagnosis based on case group/type.

CHAPTER TWO

Introduction

Cancer is a burden that impacts humans and animals alike, including companion animals and animal athletes. Although cancer is a cross-species disease, more is known about cancer in humans than any other species. Human cancer patients have the advantage of having a well-established medical system and methods of gathering more accurate data on exposures, outcomes, prevalence, incidence, mortality, and morbidity. Data collection and referencing is facilitated among humans, as we have established organizations and interdisciplinary networks and databases from which data and research can be compiled and shared. No such organization exists for other species, including dogs. The closest to these practices that are in veterinary medicine are individual registries that have been created across mostly Europe, but in the United States as well. Most of the data on cancer trends on animals are from academic and select veterinary school studies (Davis & Ostrander, 2014).

Currently, most of the knowledge on cancer in dogs is about association to cancer development type and breed (a potential indicator of genetic predisposition), age, size, certain environmental factors, and sex and reproductive status (i.e., spay or neuter status). The types of cancer that dogs can develop have been more well established as well, including the types of cancer dogs are more susceptible to based on breed. There is still, however, vast knowledge gaps considering the lack of organizations and synchronized data collection between registries related to cancers in dogs. As mentioned in the prior chapter, registries that exist currently for dogs do provide useful information, but there is not a lot of publicly available information on cancer trends, breed-specific incidences of cancers, or treatment responses. These registries are also

lacking in resources to link at-risk populations' breeders with geneticists or epidemiologists studying a specified form of cancer (Davis & Ostrander, 2014). Early cancer cases in dogs are an especially lacking sector of cancer study in dogs, as the etiology of these cancers are unknown.

The age at which a dog is considered a senior can vary based on size and breed (Meyers, 2022). However, overall, dogs that are 2-6 years old can be considered mature adults and dogs that are 7 years or greater can be considered older dogs or of "old age" (Meyers, 2022, Harvey, 2021). This age classification will be used in the following analysis on early cancer cases in golden retrievers among an overall sample population of 3,044 golden retrievers across the U.S. Dogs that are younger than 6 will be considered "young" and the dogs that are 6 and older will be considered "old". Being that 6 is the last year in the adult years (on the cusp), it was added to the old age category for this analysis. Information gathered on dogs in general, like in this analysis, also has the potential of giving insight into human health as well, as dogs share a common environment to humans as companion animals. Also, like in humans, dogs can still develop cancer at a younger age and the etiology (causes) is unclear. Analysis of the GRLS dataset will be a first attempt at better understanding early cancer epidemiology in dogs so that further epidemiological work can be done to understand it even more in the future. The objective of this analysis is to explore the GRLS dataset. In doing so, information will be gathered on cancer prevalence in golden retrievers, the association, and statistical differences of average age at diagnosis or age category across the GRLS population based on sex status and cancer case type will be assessed, and the frequencies of cancer types and the characteristics of the cancer dogs grouped by the most frequent cancer types will be evaluated.

This GRLS data is one of the largest collections of information on a vast population of dogs. The large sample size increases the chances of reliable findings that accurately represent

the population for this particular breed of dogs. A specific breed was focused on to help reduce genetic variability: golden retrievers. The GRLS dataset was analyzed to gather information on the cancer prevalence among these golden retrievers and how this compares to human cancer prevalence. The most frequent neoplasia that were developed among these dogs were also analyzed to cross reference with current knowledge and potentially find any new findings on types of cancers developed in golden retrievers. Age and sex status will be considered to see how diagnoses vary across the GRLS population based on these characteristics.

Methods

Dog Study Population

Data from the Golden Retriever Lifetime Study (GRLS), coordinated by the Morris Animal Foundation (MAF), was the study population for this analysis. Methods used to recruit the study population are described elsewhere (Labadie et al, 2022). Briefly, dogs were recruited between the years 2012 - 2015 within the United States through advertisements with social media, and organizations and clubs such as: the Golden Retriever Club of America, the Golden Retriever Foundation, regional golden retriever clubs, and veterinary professional organizations. Golden retrievers enrolled were between 6 months to 2 years of age and were screened for enrollment via the completion of an owner profile, which obtained information for registration and other demographic information. Owners had to be able to provide at least 3 pedigrees (dogs family history) to be enrolled, and dogs had to be confirmed to be free of life limiting conditions by veterinarians. The final enrolled cohort for this dataset consists of 3,044 golden retrievers.

Gathering Information from Participants

The data collected for the GRLS study data was obtained through a prospective, observational, population-based cohort study design. Information on the dogs used to compose

the GRLS study data were gathered through **the annual owner's questionnaire**, **the annual veterinarian questionnaire and veterinary examination**, and **sample collection**, which also was collected by the veterinarians and sent to a laboratory for tests and examination to be conducted on the sample. Once the dogs had been screened for enrollment and the owners had completed the written informed consent, they could fill out their first (baseline) annual owner questionnaire and then go to the baseline veterinary visit for examination and sample collection. At the baseline veterinary visit, the veterinarians confirmed the dogs were free of life-limiting conditions and also completed written informed consent before completing the baseline annual veterinarian questionnaire and collecting samples.

The annual owner questionnaire is completed by the dog's owner and serves the purpose of gathering detailed information on the dogs' environment and lifestyle including at home oral hygiene regimen, reproductive history, over the counter medications and flea, tick, and heartworm preventatives, and behavior as well as information on the dogs' living conditions and exposures (including environmental exposures). Every year, the annual owner questionnaire is made available to the owners to fill out 1 month prior to the anniversary date until 10 months after the anniversary date. Once the owners have completed their annual questionnaire, they are sent sample collection kits and are instructed to schedule their annual veterinary visits (study visit). Veterinarians then complete the annual veterinarian questionnaire, full physical examination (PE), and sample collection (whole blood, serum, urine, feces, hair clips, and toenails) at the study visit.

The annual veterinarian questionnaire is completed by the study veterinarians and collects data on the dogs' medical history, PE examination findings (including height at the wither, weight, and body condition score (BCS)), a map of superficial masses, vaccination

history, and prescribed medication history. The **sample collection** is conducted using the sample collection kits that are sent to the owners and includes detailed and comprehensive instructions on how to collect the samples as well as how to ship the samples for laboratory analysis and biobanking. Details on the amount and storage protocol can be seen in Table 1 of Labadie *et al.* (Page 4). Laboratory tests that are run on the samples include: CBC, serum biochemistry profile, fecal evaluation for ova and parasites, urinalysis, heartworm antigen test, and thyroid hormone (T4) level. The results from these tests are shared with the study veterinarians and added to the database. More details on the methodology and about the laboratory tests conducted can be found in the S1 Table of Labadie *et al.*

Follow - Ups for Cancer and Death, and Endpoint Tracking

In addition to the annual follow-ups that are conducted by veterinarians, veterinarians were instructed to contact MAF when a cancer diagnosis was suspected or confirmed among any of the participants. If malignancy was suspected, veterinarians were sent a biopsy kit to collect a sample and for submission to MAF's diagnostic laboratory where further testing (histology) could be conducted on the sample. Additional testing, for lymphoproliferative disorders, could also be run such as flow cytometry and polymerase chain reaction (PCR) for antigen receptor rearrangement (PARR) at CSUs Clinical Immunology Laboratory. Clear instructions were given to the veterinarians for sample collection, which gave details on how to collect and submit the samples for histopathology, flow cytometry, PARR, and bio-banking. It should be mentioned, in addition, that not all malignancies were diagnosed histologically due to issues such as cost constraints, invasiveness of sampling, or the owner's preference. Therefore, in the data, a tier of confidence was given for each cancer diagnosis. Tier 1 would mean that there was confirmation of the diagnosis through histology or cytology conducted by a board-certified pathologist. Tier 2

would mean that the diagnosis was a presumption based on visualization or imaging without microscopic confirmation. Tier 3 would mean that the diagnosis was a presumption based on clinical suspicion alone.

Endpoint tracking questionnaires were used to track cancer diagnoses and causes of death throughout the study. As of February 8, 2022, the day in which the most up to date form of the data set was given for this analysis, there were 322 golden retrievers diagnosed with some form of cancer, malignant or benign, and 372 forms of cancer identified among these 322 dogs.

Human Comparison Study Population

To understand if there were similar geographic trends in dog cancers and human cancers, we conducted a spatial analysis of GRLS data and human cancer data by state. CDC data on human cancer prevalence were used to gather information for the population of humans. The following link gives a breakdown by state, the human prevalence of all types of cancer as of January 1, 2020: USCS Data Visualizations - CDC. It should also be noted that this was information gathered over the 5 years prior to the January 1, 2020 date ("5-year Limited Duration"). Therefore, the prevalence includes data from January 1, 2015 to December 31, 2019. This source was chosen to gather data to represent the human population in this analysis because it comes from a reliable organization (the CDC) and because the data is inclusive of all races and ethnicities from across the United States, as the GRLS data is based on golden retrievers across the United States as well. The CDC data (2015-2019) and the GRLS data (2012-2020) were also collected during similar years in time.

GRLS Dataset Cleaning & Analysis: SAS On Demand and R Studios

Data Cleaning Process

The GRLS dataset was imported into SAS On Demand, an online platform used to code in SAS, as well as R studio. After being imported into SAS and R studio, the data were cleaned, and specific datasets were created that derived from the original GRLS dataset. This was to make the dataset more user-friendly and make it easier to use to generate findings. Cleaning was conducted with R studio as well and not only SAS, as certain commands that aid in maintaining data accuracy as far as dog count (tally) could be used and to use packages that could be used to create data visualizations (ggplot2). Other functions that were unique to R studios were also needed in order to create a dataset that included the cancer dogs only (**MAF.cleaneddata**). **MAF.cleaneddata** was created from the original GRLS dataset and gives information on the 322 cancer dogs that are in the sample population for this analysis. The MAF.cleaneddata dataset was refined so there was one observation per cancer dogs and gave their sex status at diagnosis, diagnosis date, age at diagnosis and state of first diagnosis. Although the MAF.cleaneddata dataset gave information on each cancer dogs, it only gives one observation per cancer dog and is mostly used to obtain the state, age and sex of the cancer dogs for their first cancer diagnosis. Therefore, the dataset from which the MAF.cleaneddata dataset derived, **MAF.cancer_dogs_data**, was used to evaluate all of the cancer dogs recorded data. MAF.cleaneddata did not include a spay_neut_date or date_of_birth (later named “DOB”) variable, which gives the date in which the dogs were spayed and neutered and their dates of birth, respectively. Therefore, MAF.cleaneddata was merged with a temporary dataset labeled “x” that contained the DOGID, date of birth, and spay or neuter date of all of the cancer dogs and

was named **MAF.cleaneddata3** so that the age in which each of cancer dogs got spayed and neutered could be calcualted using the YRDIFF function in SAS.

In SAS, a cancer indicator variable was created and added to the MAF.cleaneddata dataset after importing it into SAS from R studio, so that it could be merged to a dataset of all of the dogs (**MAF.lastobs_alldogs**) and subset the cancer free dogs to create a dataset of the cancer free dogs only (**MAF. cancerfree_dogs**). MAF.lastobs_alldogs was created from the original dataset and gives the last observation from each of the 3,044 dogs participating in the GRLS study. The MAF.cancerfree_dogs dataset can be used to get the last documented pieces of information on the cancer free dogs and was used to mostly observe the last age, and sex status that was observed for the cancer free dogs. Overall, R studio was mostly used to refine the bigger dataset into smaller, more organized datasets to use for the analysis, and then were imported into SAS to conduct the analysis. Datasets that were created would be checked multiple times through observation number and spot checking to make sure data accuracy was being maintained in SAS. Also making sure that datasets were properly sorted by DOGID and AVQ_DATE (the date in which the annual veterinary questionnaire was completed) to make sure that the correct rows were being selected from when gather the last observations for the cancer free dogs. From the cleaneddata3 and cancerfree_dogs dataset, counts to create Table S1 could be gathered. The lastobs_alldogs dataset was used to find the number of study years each dog participated in the study as well as their study status (participation). The cleaneddata dataset was used to get the first diagnosis of each cancer dog for this analysis.

The cleaneddata3 dataset was also subsetted into 4 datasets that were specific to grouped cancer types: mammarycases_cleaneddata, hermangiocases_cleaneddata, lymphcases_cleaneddata, and histiocases cleaneddata. Each dataset has mammary,

hermangiosarcoma, lymphoma, and histiocytoma cases subsetted so cases could be analyzed separately and against one another in the data analysis process.

Data Analysis Process

Table S1 Counts

As mentioned prior, the condensed datasets that show data on the cancer and cancer free dogs, cleaneddata3 and cancerfree_dogs, were used to get the counts that comprise Table S1. IF-THEN statements in SAS were used to subset these datasets into smaller datasets by characteristics of interest such as age categories (Young or Old) and sex status (Female Spayed, Female Intact, Male Neutered, Male Intact). From these smaller datasets, PROC FREQs could be conducted to get counts on sex status, as well as how many dogs were of each subgroup. For age, the subgroups were “Young” and “Old”. Dogs less the 6 years old were considered to be “Young” and dogs greater than or equal to 6 were considered old in this analysis. For sex status, the subgroups were “Female Spayed”, “Female Intact”, “Male Neutered”, or “Male Intact”. PROC MEANS were used to get the average age of the dogs that comprised each subgroup for age across all the GRLS dogs, all of the cancer dogs and all of the cancer free dogs. The output from these procedures would be saved as a permanent data table in the “MAF” SAS library that was created to store datasets and tables produced for this analysis, and the results would be saved and organized in a Google Sheet to build Table S1. Google Sheet would also be frequently used to assure account accuracy after adding data.

Summary Statistics and Normality Testing on Age at Diagnosis

The procedure PROC UNIVARIATE in SAS OnDemand was used to gather summary statistics and test for normality simultaneously on the age_at_diagnosis_or_visit_yrs, as this was the variable of interest for statistical analysis. Summary statistics such as mean, median, standard deviation, and interquartile were statistics that were especially of interest. A normality test was conducted to see whether parametric or non-parametric statistical testing should be used in conducting analyses on the variable. A histogram was also created to visualize the distribution of the variable across the dataset.

Prevalence Calculations

To calculate the cancer prevalence by state, the state in which the cancer dogs received their first diagnosis was used, as multiple dogs moved through the duration of the study. The prevalence was calculated by state by dividing the number of dogs that have cancer in a particular state by how many dogs reside in that state, both those that have cancer and are cancer free. In other words, the numerator for the cancer prevalence calculation in each state was the number of cancer dogs in each state, and the denominator was the total population of dogs in each state enrolled in the study (including cancer free dogs). The amount of cancer free dogs that resided in each state was determined based on where they resided upon enrollment. These counts were found separately and then added together to get the amount of dogs in the GRLS population that reside in each state. The counts for each state were then totalized to make sure all 3,044 dogs were still accounted for. The “Function” feature in Google Sheets was used to simultaneously divide (“DIVIDE”) the number of cancer dogs in each state over the total number of dogs in the study population that reside in each state to get the cancer prevalences. The list of states and their

corresponding prevalence percentages were taken and made into a separate Excel file, and uploaded into R studio so that the map of state prevalences could be created.

The U.S. map, as in the template of the U.S. map, was created using the maps package in R studio. From here, the cancer prevalence information was put into the map and visualized using the ggplot2 package and the geom_point function from the ggplot2 package in R Studio. This map shows differences in prevalence by state using dot size and a color gradient of blue. The higher the prevalence, the darker and larger the dot will be over the state on the map.

Differences of Proportions (Cancer Prevalence) Between Age Categories

The difference of proportions was calculated to compare the cancer prevalences among the young and old cancer dogs by state and sex status. From the difference of proportions calculations, a lower (LCI) and upper (UCI) confidence interval was calculated to conclude if there is a significant difference between the two proportions.

From the Table S1 that was created in Google Sheets, the young dog proportion was calculated by using the number of young cancer dogs that were of a certain state or sex status in the numerator and using the number of young dogs overall that were of a certain state or sex status in the denominator. The same was repeated for the old dogs. The standard deviation and difference between the proportions had to be found to obtain the LCI and UCI. The following was the formula structure used to calculate the difference: (proportion 1 (young) - proportion 2 (old)). The square root function was used in Google Sheets (=SQRT) to calculate the standard deviations. The following was the formula structure used to calculate the standard deviation: =SQRT((((proportion 1*(1-proprotion 1))/n1)) + (((proportion 2*(1-proportion2))/n2))), where n1 is the numerator (young cancer dogs) for the young dogs proportion and n2 (old cancer dogs)

is the numerator for the old dogs proportion. From here, the LCI and UCI could be calculated using the difference and standard deviations calculated. The LCI was calculated using the following formula structure: difference + (1.96*standard deviation). The UCI was calculated using the following formula structure: difference - (1.96*standard deviation). These calculations on the difference of proportions were repeated for each state observed in this analysis and the 4 different sex statuses the dogs could fall under in this analysis.

After calculating the LCI and UCI, they were observed to see if 0 fell within the two intervals. If 0 was within the two intervals, it can be interpreted that there was not a significant difference between the two proportions.

Age at Diagnosis Analysis

A **Kruskal-Wallis (KW) test** was used to see if there was a difference in the average age at diagnosis among the cancer dogs by state. Since the age at diagnosis variable is not normally distributed, the KW test was used in place of an ANOVA test. The KW test was run in SAS On Demand using the PROC NPAR1WAY arguments. The CLASS statement for this test used the state_at_dx variable from the cleaned dataset on the cancer dogs, which is a variable that lists the subject's state in which they received their first cancer diagnosis. The VAR statement used the variable age_at_diagnosis_or_visit_yrs, which gives the age at which the dogs received their first cancer diagnosis.

The KW test was also run among the young cancer dogs and among the old cancer dogs, separated, by subsetting the cleaneddata dataset into 2 datasets and running the KW test on these two datasets: cleaneddata_youngdogs and cleaneddata_olderdogs. Cleaneddata_youngdogs was

comprised on all of the young cancer dogs (<6) and the cleaneddata_olderdogs was comprised of all of the old cancer dogs (>=6).

Sex at Diagnosis Analysis

A **Wilcoxon Rank Sum (WRS) test** was used to determine if there was a difference in the average age at diagnosis between sex status categories. Since the age at diagnosis variable is not normally distributed, the WRS test was used in place of an independent t-test. The WRS test was run in SAS On Demand as well, using the PROC NPAR1WAY procedure, specifying Wilcoxon in the argument. A char_sex variable was added to the MAF.cleaneddata dataset to assign the dogs to either the Male or Female sex, grouping the intact and spayed female dogs together under female, and the intact and neutered dogs together under male. The WRS test was then run using the char_sex variable in the CLASS statement, and the age_at_diagnosis_or_visit_yrs variable in the VAR statement to get results.

The WRS test was also run among the females and then among the males, separately, to see if there was a difference in the average age at diagnosis between intact and spay/neuter status in the female and male cancer dogs. Two columns were added to the MAF.cleaneddata dataset: Fem_status and Male_Status. Fem_status relayed the females' status (either sprayed or intact), and if the dog was male, the cell was left blank in the Fem_status column. The same was done for the males, relaying the males' status (either neutered or intact) under the Male_status. If the dog was female, the cell was left blank in the Male_status column. For the test among the females, the Fem_status variable in the CLASS statement and the age_at_diagnosis_or_visit_yrs variable in the VAR statement. For the test among the males, the Male_status variable in the CLASS statement and the age_at_diagnosis_or_visit_yrs variable in the VAR statement.

Analysis of Cancer Dogs by Case Type

To analyze some of the most common cancers in dog among the GRLS cancer dog population, the cancer dogs that were diagnosed with mammary, hemangiosarcoma, lymphomas, and histiocytomas were subsetted from the MAF.cleaneddata dataset so all dogs that were diagnosed within each cancer type could be analyzed individually. Then, KW, WRS, and Fisher's exact tests were used to test if there were statistically significant differences in average age at diagnosis between the case types and if there was a statistically significant association between age category and case type (group). The KW and WRS tests were ran in SAS using the PROC NPAR1WAY procedure, specifying "Wilcoxon" in the argument if a WRS was being ran. Fisher's exact tests were also ran in SAS and were done using the PROC FREQ procedure and specifying "fisher" within the TABLES argument.

Data Visualizations

Data visualizations such as bar charts, pie charts, and histograms were created in Google Sheets and SAS OnDemand to depict various characteristics of the data. Bar charts and pie charts were predominantly used to visualize the amount of neoplasia types across the cancer dog population and how many dogs (both cancer and cancer free dogs) were of a particular sex status at diagnosis or by their last recorded observation if they were cancer free. Such visualization were also used to evaluate the cancer dogs within specific cancer types (mammary, hemangiosarcoma, lymphoma, and histiocytoma) to evaluate how many were of a particular age group, how many were spayed or neutered prematurely or past maturity (if at all), how many of the cases were of a particular subtype, and how many were spay or neutered, or intact. A U.S. map was created in R studios to visualize the state prevalence of cancer among the GRLS

population, which will then be compared to the state prevalence of cancer in humans from the CDC.

Results / Analysis

Table S1 - Sample Population Descriptive Statistics

Descriptive statistics on the sample population used for this analysis are provided in Table S1. Statistics include the state of diagnosis (cancer dogs) or the first state the dog resided in upon enrollment (cancer free dogs), average age at diagnosis (cancer dogs) or last age recorded (cancer free dogs), and sex status at diagnosis (cancer dogs) or last sex status recorded (cancer free dogs). Only 3,043 dogs are being considered, instead of the total 3,044 dogs, in the state counts due to one cancer free dog being lost when refining the dataset. The dog was identified, and it was found that it was because their state of residence was not included in the last observation for this particular dog for some reason. However, all 3.044 were considered for Age Category, Average Age at Diagnosis, and Sex Status counts and calculations.

All of the states in the U.S. are present, except for Hawaii and Alaska, as there were no participants from Hawaii or Alaska. Therefore, only 48 states and 1 capital district (Washington, D.C.) were considered. There was also a cancer dog that came up as NA for the state of diagnosis. The dog was identified, and their state of residence had been omitted and was a young histiocytoma case.

State of Diagnosis/Residence & Prevalence

State Counts

Of the 48 states and 1 capital district, the highest contributing states of the overall sample population were California (277) and Colorado (252). All of the states that contributed 100 participants or more (aside from California and Colorado) include: Florida, Illinois, Massachusetts, Michigan, New York, North Carolina, Pennsylvania, Virginia, and Washington state. Most of the dogs that developed cancer came from California (27) and Colorado (25), which could be due to the higher participation from those states. Other states that had 20 or more dogs develop cancer include Florida (23) and Illinois (20), which were states with higher participation states.

Prevalence

To calculate the cancer prevalence in each state, the number of dogs with cancer in a specified state (numerator) was divided by the total number of dogs that were enrolled in the GRLS study (denominator). For example, 13/3044 of the GRLS dogs were resided in Louisiana either at diagnosis (if a cancer dogs) or when they initially enrolled into the study (if a cancer free dog). Of those 13 dogs, only 5 developed cancer. Therefore, the prevalence in Louisiana would be calculated as 5/13, making the state prevalence 38.5%.

Figure 8a: State Prevalence of Cancer in GRLS Population

Prevalence was highest in Louisiana (38.5%) with Arizona being the second highest (17.5%). There also appears to be higher prevalences along the upper east coast region. The two states with the higher prevalences in the upper east coast were Maine (17.1%) and Massachusetts (17%).

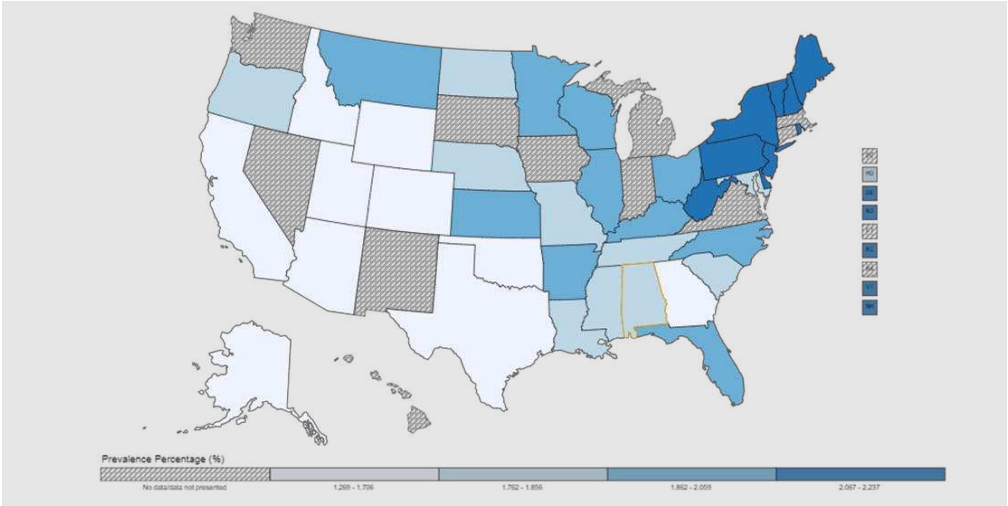

Figure 8b: State Prevalence of Cancer in Humans in the U.S.

Differences of Proportions (Cancer Prevalence) Between Young and Old Dogs

By State

After calculating the difference of proportions between young and old dogs by state, it was found that all but 4 states had an LCI and UCI that contained zero. Therefore, for most of the states, there was not a significant difference between the young and old dog proportions. The states where there was a significant difference between the young and old dog proportions were Arkansas, Delaware, Kentucky, and New Hampshire.

By Sex Status

After calculating the difference of proportions between young and old dogs by sex status, it was found that all of the sex statuses had an LCI and UCI that contained zero. Therefore, there was not a significant difference between the young and old dog proportions for any of the sex statuses.

Average Age at Diagnosis by State Analysis

Summary Statistics on Age at Diagnosis: Average Age, Median, Standard Deviation, & Interquartile

Below are the results from running a PROC UNIVARIATE on the age at diagnosis variable. Given are the average, median, and standard deviation of the age at diagnosis among cancer dogs, among other statistics. The average at at diagnosis among the cancer dogs was approximately 5.6 years old. Table 1 can be reviewed for the average age at diagnosis among the

cancer dogs based on their age category. The median age at diagnosis was 5.98 and the standard deviation was approximately 2.2. The interquartile range was 3.2.

Table 9: Summary Statistics of Age at Diagnosis Variable

Summary Statistics on Age at Diagnosis Variable	
n	322
Mean	5.5889059
Standard Deviation	2.19986758
Median	5.987700
Interquartile Range	3.20330

Normality Testing and Distribution of Age at Diagnosis

In addition to calculating summary statistics, PROC UNIVARIATE was used to test for normality. Multiple normality tests were output using PROC UNIVARIATE, however, the Shapiro-Wilks Test (SWT) was focused upon for results. The null hypothesis for the SWT states that the population is normally distributed. The p-value returned from the test was <0.0001, meaning that we reject the null hypothesis. Therefore, the age variable is not normally distributed. Also, the skewness returned was -0.46, as shown in the results above, meaning the data is left skewed. Non-parametric statistical tests are, therefore, appropriate for further analysis of the age at diagnosis variable.

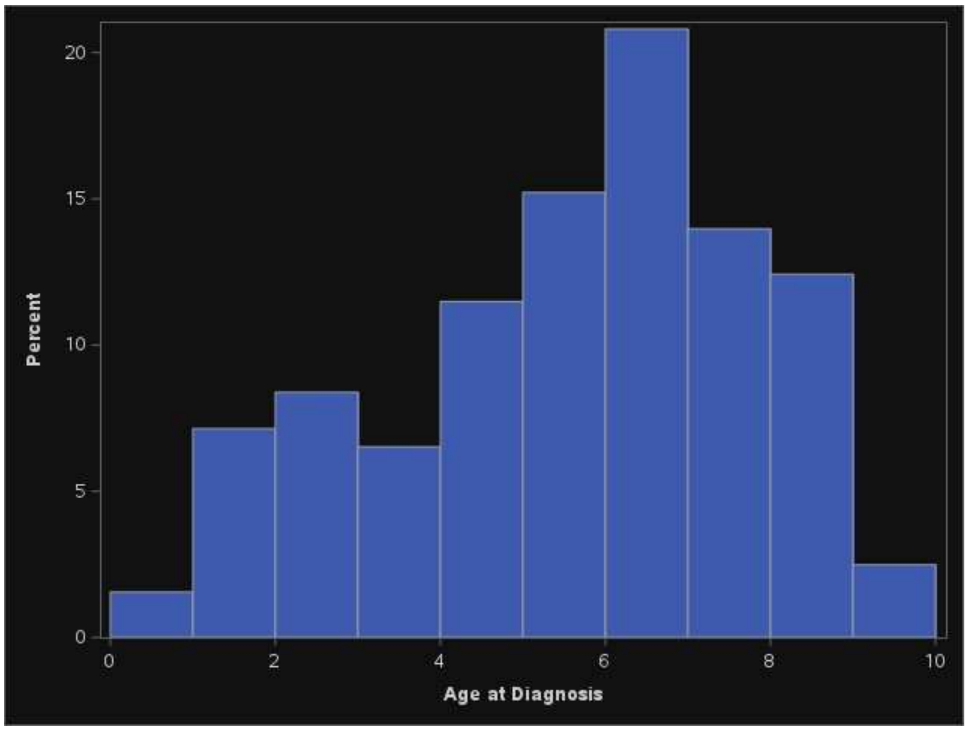

Figure 9: Histogram of Distribution of Age at Diagnosis

Testing For Difference in Average Age at Diagnosis by State

To test the variability of the age at diagnosis data among the cancer dogs across the U.S., Kruskal Wallis tests were run to see if there was a significant difference in the average age at diagnosis by state.

All Cancer Dogs

When testing to see if there was a significant difference in average age at diagnosis by state, a p-value > .05 was given ($p = 0.75$). We fail to reject the null, and the difference in average age at diagnosis by state is not statistically significant.

Table 10a: Results of Kruskal-Wallis Test Conducted on Age at Diagnosis by State for All Cancer Dogs

Kruskal-Wallis Test		
Chi-Square	DF	Pr > ChiSq
37.3081	44	0.7521

Young Cancer Dogs (<6)

When testing to see if there was a significant difference in average age at diagnosis by state among the young dogs, a p-value > .05 was given (p = 0.60). We fail to reject the null, and the difference in average age at diagnosis by state among the young dogs is not statistically significant.

Table 10b: Results of Kruskal-Wallis Test Conducted on Age at Diagnosis by State for Young Cancer Dogs

Kruskal-Wallis Test		
Chi-Square	DF	Pr > ChiSq
33.0479	36	0.6098

Old Cancer Dogs (>=6)

When testing to see if there was a significant difference in average age at diagnosis by state among the old dogs, a p-value > .05 was given (p = 0.87). We fail to reject the null, and the difference in average age at diagnosis by state among the old dogs is not statistically significant.

Table 10c: Results of Kruskal-Wallis Test Conducted on Age at Diagnosis by State for Old Cancer Dogs		
Kruskal-Wallis Test		
Chi-Square	DF	Pr > ChiSq
27.3310	37	0.8771

Average Age at Diagnosis by Sex at Diagnosis Analysis

Testing For Difference in Average Age at Diagnosis by Sex

To test the variability of the age at diagnosis data among the cancer dogs across the different sex categories the cancer dogs belonged to at diagnosis, Kruskal Wallis tests were run to see if there was a significant difference in the average age at diagnosis by sex at diagnosis.

Males Vs. Females

The average age at diagnosis among the female cancer dogs in this study was 5.72 and the average age at diagnosis among the male cancer dogs was 5.47. When testing the difference between these averages, a p-value > .05 ($p = 0.19$) was returned was testing for a difference in average age at diagnosis between male and female cancer dogs. Therefore, we fail to reject the null and there is not a statistically significant difference in the average age at diagnosis between the 2 groups.

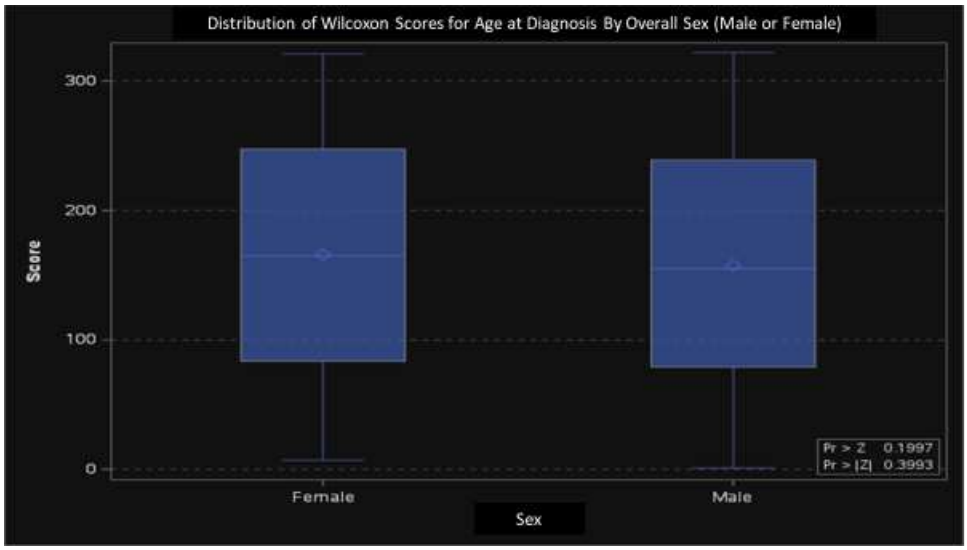

Figure 10a: Results of Wilcoxon Rank Sum Test Conducted on Age at Diagnosis by Sex (Male or Female)

Females: Intact Vs. Spayed

The average age at diagnosis among the spayed female cancer dogs in this study was 5.72 and the average age at diagnosis among the intact female cancer dogs was 5.67. When testing the difference between these averages, a p-value > .05 ($p = 0.27$) was returned was testing for a difference in average age at diagnosis between intact and spayed female cancer dogs. Therefore, we fail to reject the null and there is not a statistically significant difference in the average age at diagnosis between the 2 groups.

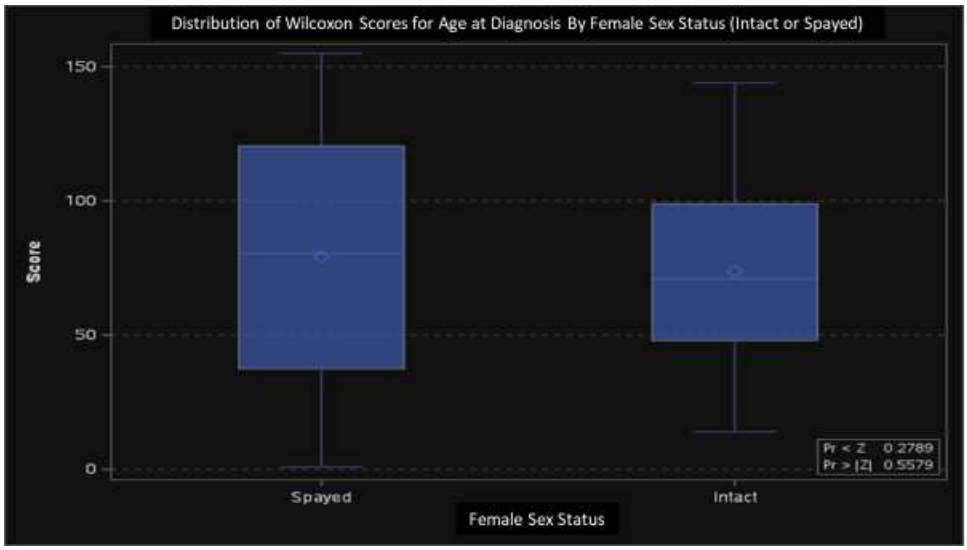

Figure 10b: Results of Wilcoxon Rank Sum Test Conducted on Age at Diagnosis Among Females by Sex Status (Intact or Spayed)

Males: Intact Vs. Neutered

The average age at diagnosis among the neutered male cancer dogs in this study was 5.75 and the average age at diagnosis among the intact male cancer dogs was 4.86. When testing the difference between these averages, a p-value < .05 ($p = 0.01$) was returned was testing for a difference in average age at diagnosis between intact and neutered male cancer dogs. Therefore, we can reject the null and state that there is a statistically significant difference in the average age at diagnosis between intact and neutered male dogs.

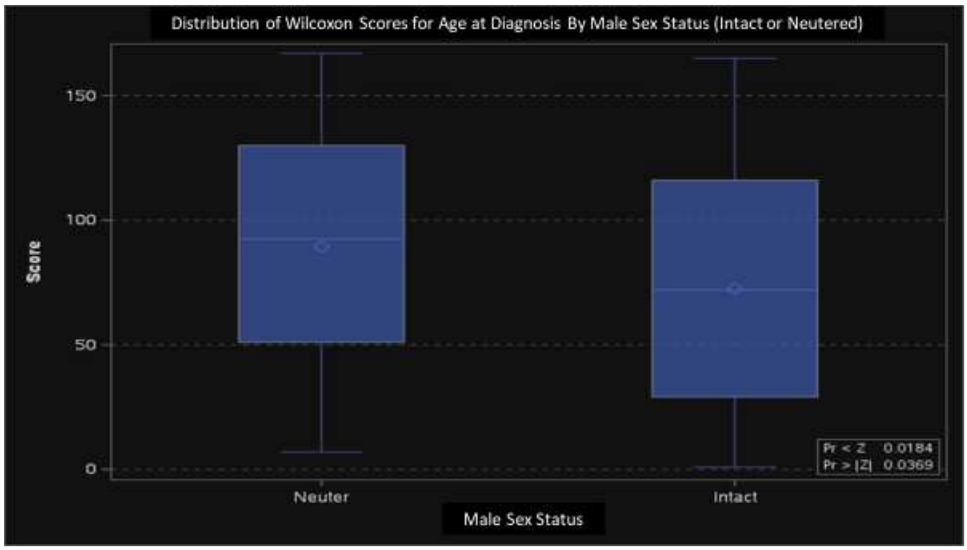

Figure 10c: Results of Wilcoxon Rank Sum Test Conducted on Age at Diagnosis Among Males By Sex Status (Intact or Neutered)

As these results differs from what was found among the female dogs and when grouping their spay/neuter and intact status by sex (male or female), a Kruskal Wallis Test was run to compare the all 4 sex status categories (female or male intact, or spayed/neutered).

Table 11: Results of Kruskal-Wallis Test Conducted on Age at Diagnosis Among All Sex Statuses

Kruskal-Wallis Test		
Chi-Square	DF	Pr > ChiSq
5.5342	3	0.1366

To reiterate, the average age among female spayed and intact cancer dogs was 5.72 and 5.67, respectively. The average age among male neutered and intact cancer dogs was 5.75 and 4.86, respectively. When testing the difference between these averages, a p-value > .05 (p = .07) was returned was testing for a difference in average age at diagnosis between all 4 sex categories

in the cancer dogs. Therefore, we fail to reject the null and there is not a statistically significant difference in the average age at diagnosis between the 4 groups. It appears the significant difference in average age at diagnosis can only be seen when comparing the two possible male sex statuses.

Analysis of The Frequency of Cancer Types in Golden Retrievers

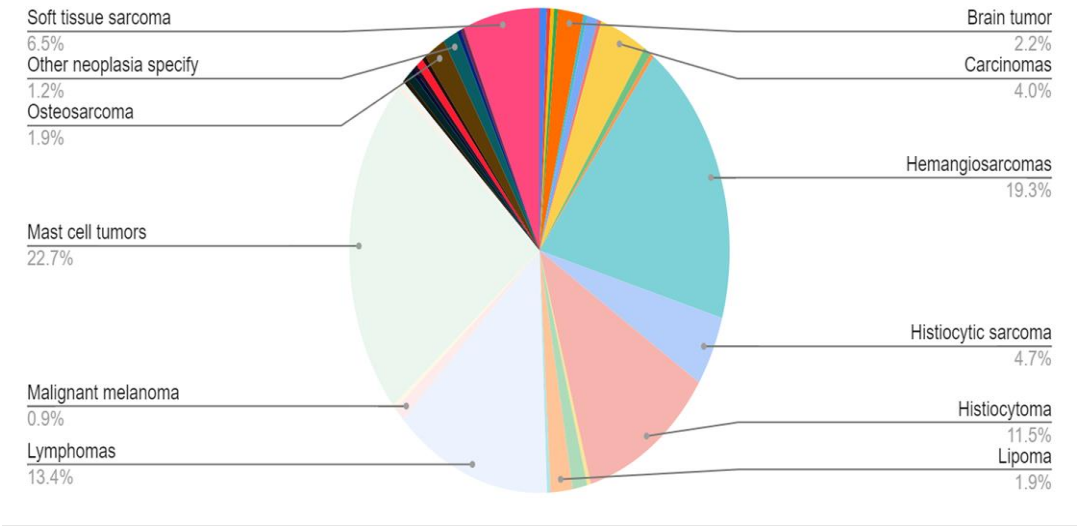

Figure 11a: Most Frequent Cancer Diagnosis Among All Cancer Dogs Grouped By Subtypes(n=322)

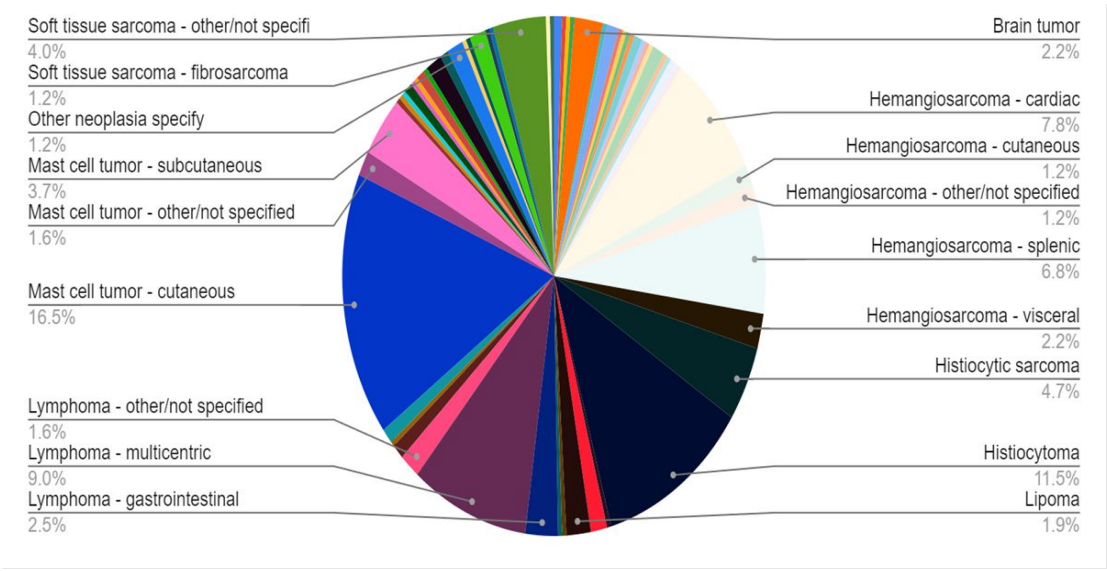

Figure 11b: Most Frequent Cancer Diagnosis Among All Cancer Dogs by Subtypes(n=322)

In Figure 11a, the cancer types found among the GRLS cancer dog population are grouped by subtype to better visualize the cancer types and see how much they contribute to the amount observed in the population. The same structure in pie charts will be repeated when analyzing the most common cancer types by age category ("Young" or "Old"). Among all of the cancer dogs, the most common cancer types (contributing 10% or more) were MCTs, hemangiosarcomas, lymphomas and histiocytomas. Golden retrievers are more susceptible to hemangiosarcomas and lymphomas, so these findings were expected (Mingus, 2019, AAHA, 2023). MCTs are also a common cancer type in dogs in general (Mingus, 2019, AAHA,2023).

In Figure 11b, the cancer types are broken down into subtypes. Among the MCTs, the most common types observed were cutaneous and subcutaneous MCTs. Among the lymphomas, the most common type was multicentric. Among the hemangiosarcomas, the most common types were splenic and cardiac. The common subtypes for hemangiosarcomas being splenic and cardiac was expected, as this aligns with literature mentioned in chapter one that expressed how

common sites for hemangiosarcomas in dogs are the spleen, but also other sites like the heart (Mingus, 2019, AAHA, 2023).

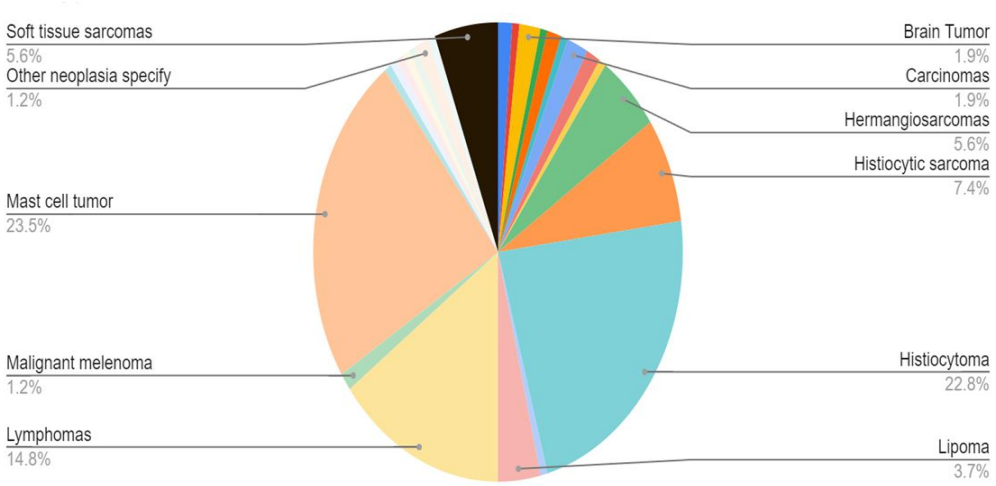

Figure 11c: Most Frequent Cancer Diagnosis Among the Young Cancer Dogs Grouped by Subtype

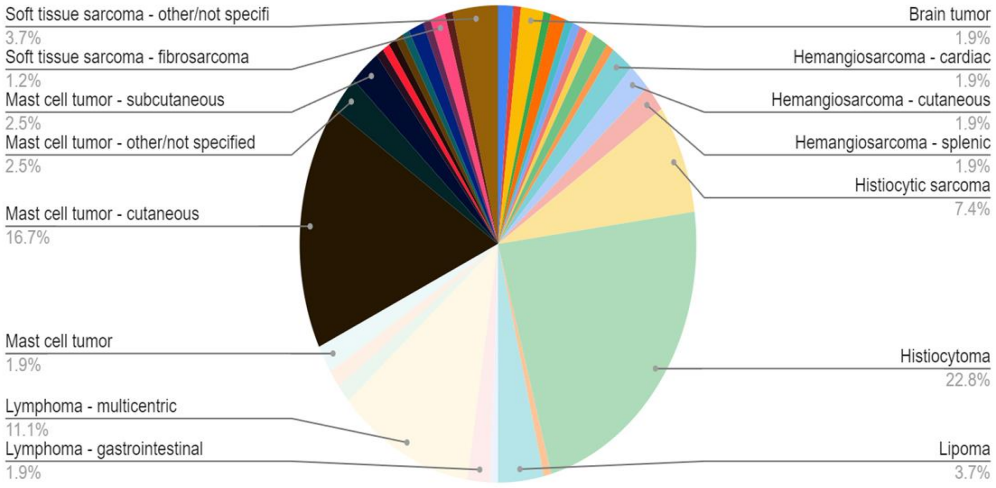

Figure 11d: Most Frequent Cancer Diagnosis Among the Young Cancer Dogs by Subtype

When looking at the grouped subtypes of cancer in the young cancer dogs in the GRLS population, the most common are histiocytomas, MCTs, and lymphomas. According to the VCA, they are mostly common in young dogs, usually younger than 3 (Williams et al, 2023). Referring to Table 1, the average age at diagnosis among the young cancer dogs was around 4 years (3.82). The average age among histiocytoma cases specifically will be observed later in the analysis.

When looking at the cancer types by subtype, among the MCTs the most common subtype was cutaneous. Among the lymphomas, the most common subtype was multicentric. Histiocytomas were not broken down by subtype and were the most commonly observed cancer type among the young cancer dogs.

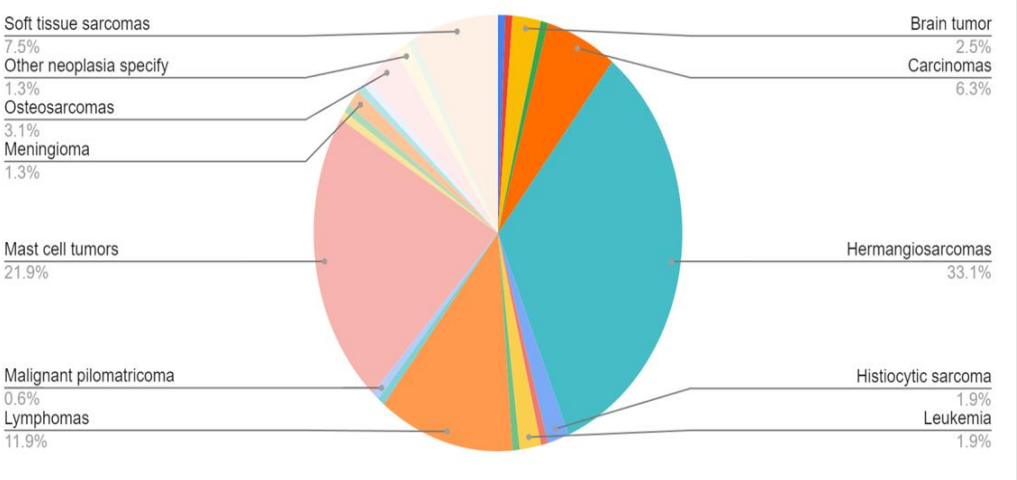

Figure 11e: Most Frequent Cancer Diagnosis Among the Old Cancer Dogs Grouped by Subtype

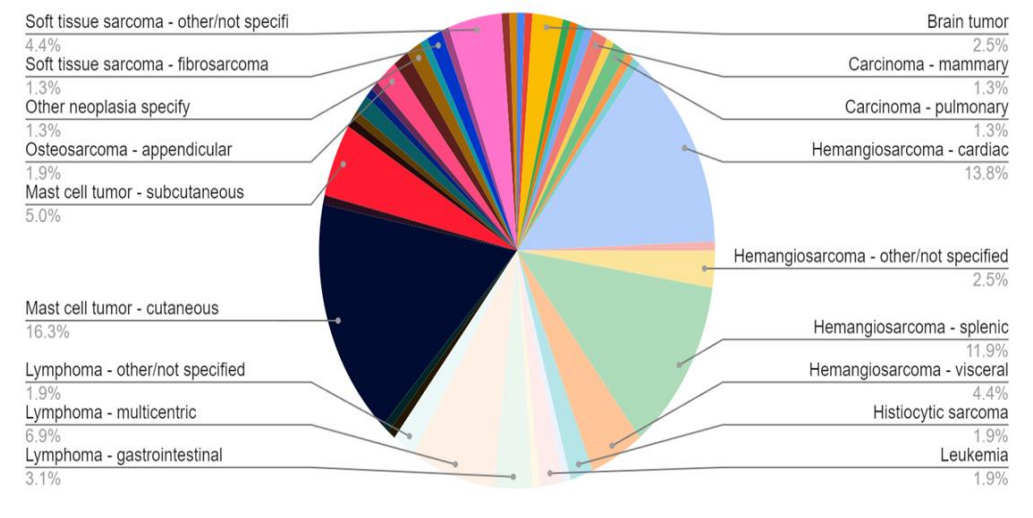

Figure 11f: Most Frequent Cancer Diagnosis Among the Old Cancer Dogs by Subtype

When looking at the grouped subtypes of cancer in the old cancer dogs in the GRLS population, the most common are hemangiosarcomas, MCTs, and lymphomas. Hemangiosarcomas, as mentioned prior, are one of the cancer types most commonly seen in golden retrievers, so these findings are expected (Mingus, 2019, AAHA, 2023).

When looking at the cancer types by subtype, among the hemangiosarcomas, the most common subtypes were splenic and cardiac. As mentioned prior, these are common sites for hemangiosarcomas in dogs (Mingus, 2019, AAHA, 2023). Among the MCTs, the most common subtype was cutaneous. Among the lymphomas, the most common subtype was multicentric.

When comparing the most common cancer types in young and old dogs, it was noticed that older dogs had, apparently, significantly more cases than young dogs. Also, only young dogs comprised the histiocytoma cases. Also, mammary related cancers were not a common cancer type found among this population. These findings prompted the analysis of the cancer types by case groups.

Analysis of Cancer Dogs by Case Groups

Mammary, hemangiosarcoma, lymphoma and histiocytoma cancer cases are grouped by their cancer diagnosis so further analysis can be conducted on the cancer dogs that developed one of these specific cancer types.

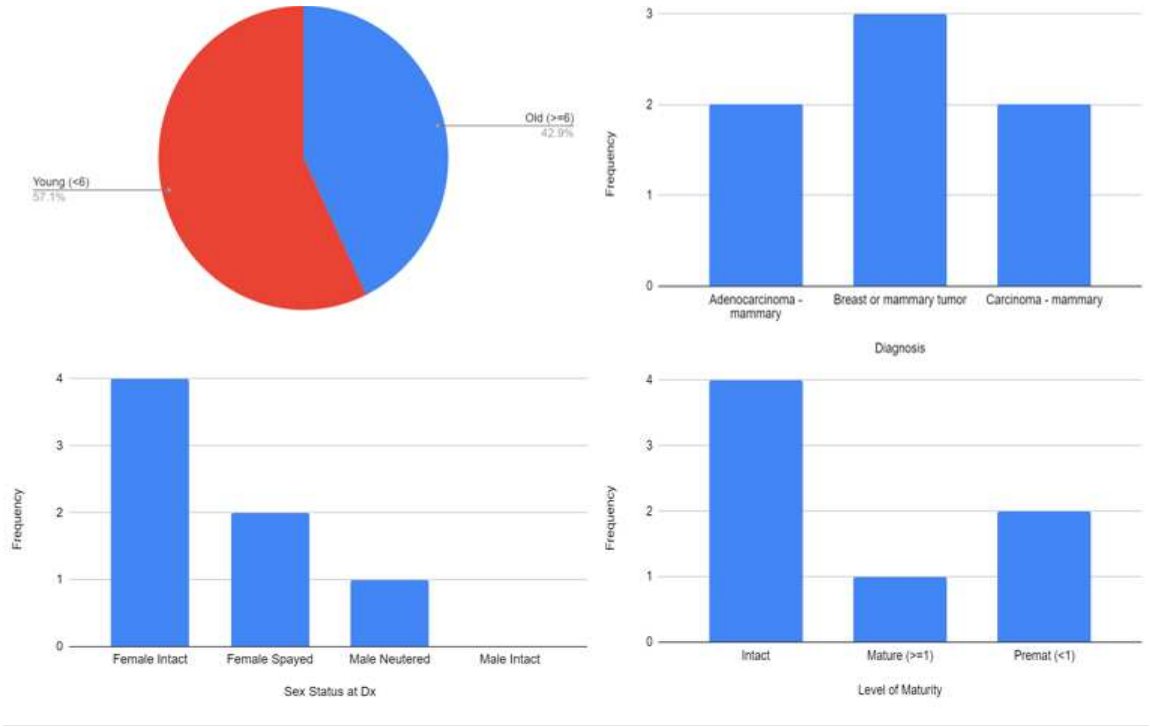

Figure 12a: Statistics on the Mammary Cases

There were only 7 mammary related cases in total. The mammary related cancer cases were mostly young but are fairly close to being split between young and old dogs. The mammary cases were also mostly intact, female dogs, which is expected as intact female dogs are most susceptible to mammary tumor development (Mingus, 2019, AAHA, 2023). Therefore, most of the mammary cases were intact, but when comparing the dogs that were spayed or neutered, there were more dogs that were spayed prematurely (before 1). The most frequent mammary

cancer type that was observed was breast or mammary tumor, but it is only most frequent by 1 case when comparing it to the other types.

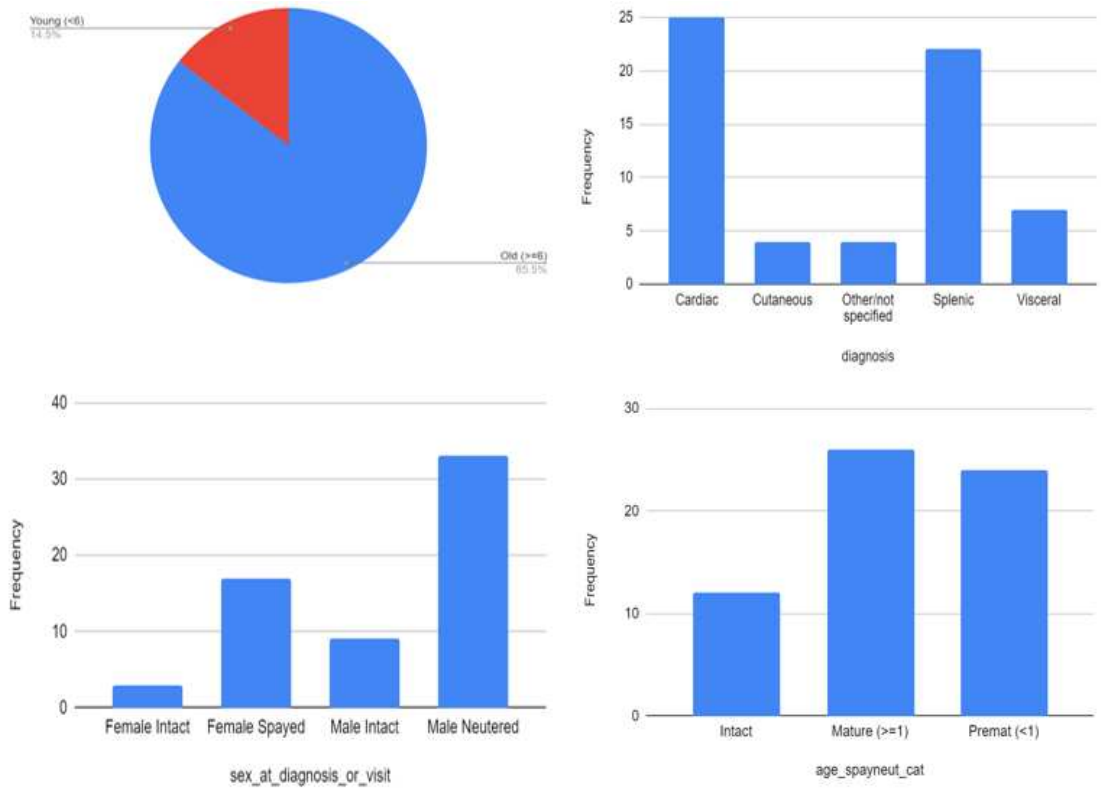

Figure 12b: Statistics on the Hemangiosarcoma Cases

There were 62 hemangiosarcoma cases in total. The hemangiosarcoma cancer cases were predominantly older dogs. The hemangiosarcoma cases were also mostly neutered, male dogs. When looking at when the hemangiosarcoma cases were spayed or neutered, most of them had been spayed or neutered after maturity, but there were was an almost even split between the dogs being spayed or neutered at or after maturity and being spayed or neutered prematurely. The most frequent subtypes that were observed were splenic and cardiac.

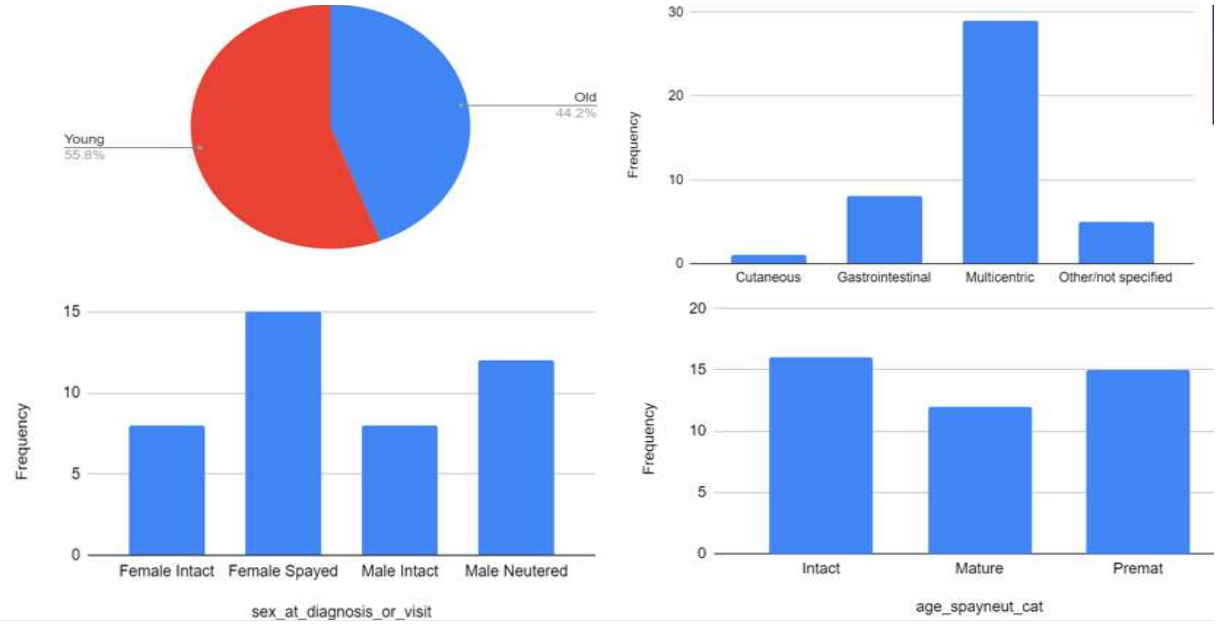

Figure 12c: Statistics on the Lymphoma Cases

There were 43 lymphoma cases in total. The lymphoma cancer cases were mostly young dogs but is fairly close to being split between young and old dogs like we observed in the mammary related cases. The lymphoma cases were also mostly spayed, female dogs (15). When looking at when the lymphoma cases were spayed or neutered, most of them had been spayed or neutered prematurely (15). The most frequent subtypes that were observed were multicentric.

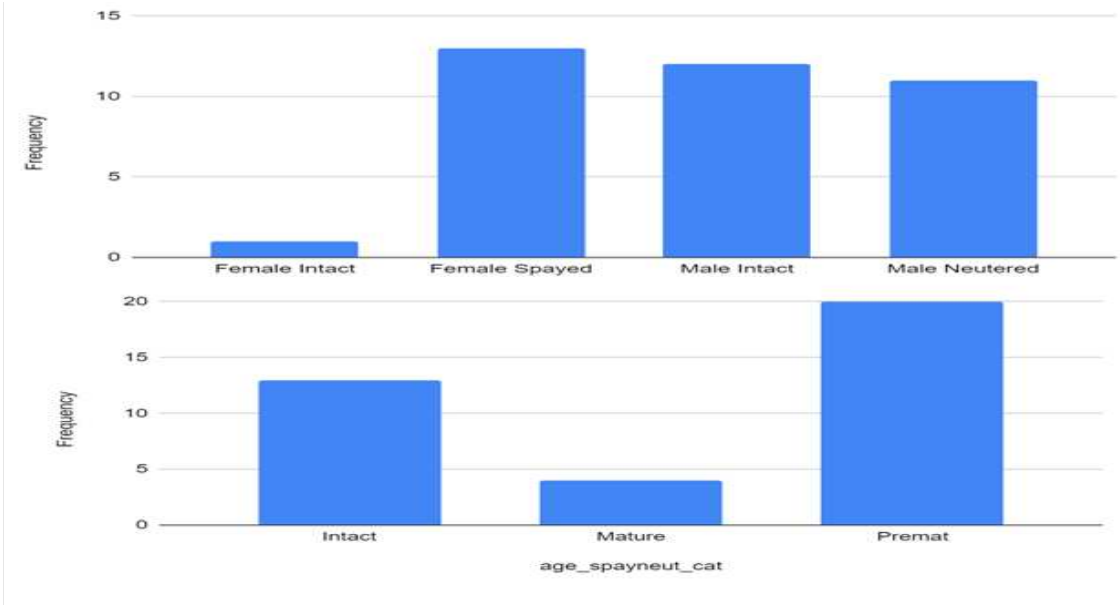

Figure 12d: Statistics on the Histiocytoma Cases

There were 37 histiocytoma cases in total. The histiocytoma cancer cases were all young dogs, so a pie chart was not depicted. Most of the histiocytoma cases were split between being a spayed female, or a spayed or intact male dog. However, only 1 was an intact female. When looking at when the histiocytoma cases were spayed or neutered, most of them had been spayed or neutered prematurely (20). Histiocytomas were not broken down by subtypes.

After taking a deep dive into the cancer types by case groups, older dogs predominating the hemangiosarcomas and only young dogs composing the histiocytoma cases piqued interest. Also, the fact that the hemangiosarcoma cases were most neutered, male dogs, and that there was a lack of female intact dogs among the histiocytoma cases. Therefore, further statistical testing was conducted to compare the difference in the average age at diagnosis between the case types (groups) and to observe potential associations between age category and case types.

Testing Difference in Average Age at Diagnosis Between 3 of the Most Common Cancer Types Found in Golden Retrievers

Older cancer dogs appeared to have comprised a majority the Hemangiosarcoma cases. However, the average age at diagnosis among the Hemangiosarcoma cases was slightly below 6 (5.7). A KW test was conducted to observe the difference in average age at diagnosis between mammary, hemangiosarcoma, and lymphoma cases to see if there was a significant difference in average age at diagnosis between these case types. A p-value < 0.0001 was returned. Therefore, we can reject the null and there is significant difference in average age at diagnosis between mammary, hemangiosarcomas, and lymphomas.

Table 12 - Results of Kruskal-Wallis Test Conducted on Age at Diagnosis Among the Mammary, Hemangiosarcoma, and Lymphoma Cases

Kruskal-Wallis Test		
Chi-Square	DF	Pr > ChiSq
24.7271	2	<.0001

A Fisher's exact test was also conducted to see if there was an association between age category and the case type (group), and the p-value was also < 0.0001. Therefore, we can reject the null and there is a statistically significant association between the age category the dogs fall under, and the case type they are.

Table 13 - Results of Fisher's Exact Test Conducted to Test Association Between Age Category and Mammary, Hemangiosarcoma, and Lymphoma Cases

Fisher's Exact Test	
Table Probability (P)	<.0001
Pr <= P	<.0001

After observing these results, another Kruskal Wallis test was run between mammary and lymphoma cases alone, to see if there was a statistical difference in average age at diagnosis between those two case types. If there is not a statistically significant difference between the two, the statistical difference observed when observing all 3 case types was likely due to the hemangiosarcoma cases. The p-value returned from this test was > .05, meaning we fail to reject the null and the difference in average age at diagnosis between mammary and lymphoma cases is not statistically significant. These findings suggest the statistical significance we see when we include the hemangiosarcomas is likely due to the hemangiosarcoma cases' average age at diagnosis.

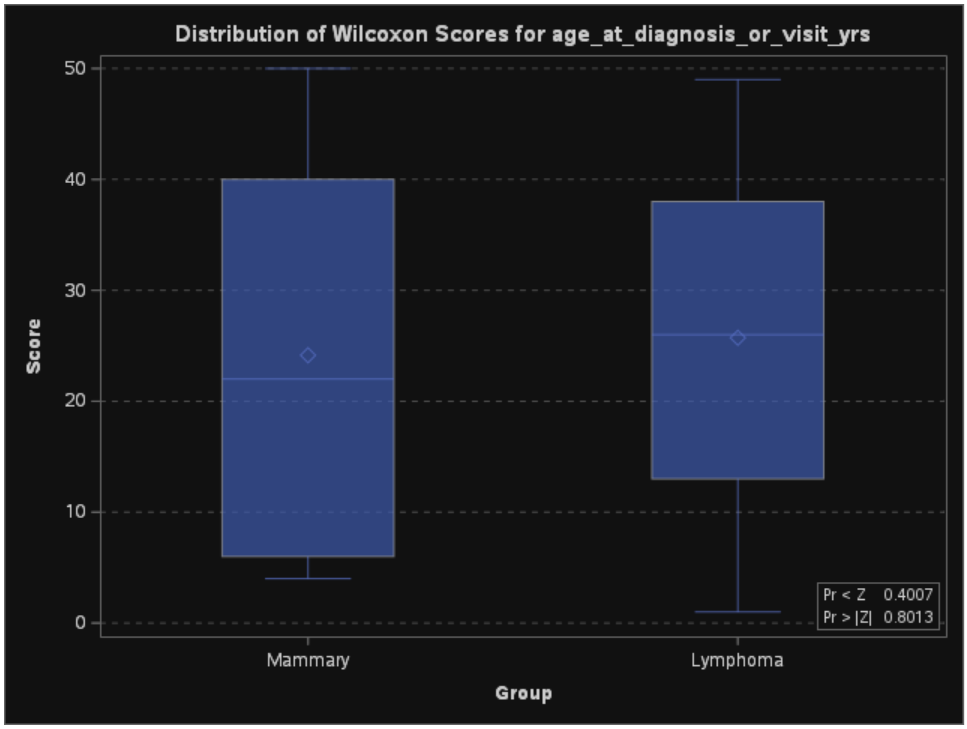

Figure 13 - Results of Wilcoxon Rank Sum Test Conducted on Age at Diagnosis Among the Mammary and Lymphoma Cases

Testing Difference in Average Age at Diagnosis Between 2 of the Most Common Cancer Types Found in Golden Retrievers While Considering Histiocytomas

All of the histiocytoma cases were of the young age category and the average age at diagnosis among histiocytoma cases was 1.83, which is an apparently low average age, especially when compared to the average age at diagnosis among the mammary, hemangiosarcoma, and lymphoma cases. Therefore, another KW test was ran which compared the differences in average age at diagnosis between mammary, lymphoma, and histiocytoma cases. Histiocytomas were compared to mammary cases and lymphoma cases due to the mammary and lymphoma cases being similar in average age (no statistical difference) at diagnosis among their groups. Therefore, any potential statistical significance observed is likely due to the histiocytoma cases' average age at diagnosis. The p-value returned from this test was <.0001. Therefore, we reject the null hypothesis and the difference and average age at diagnosis was statistically significant between mammary, lymphoma, and histiocytomas cases; presumably from the histiocytoma cases.

Table 14 - Results of Kruskal-Wallis Test Conducted on Age at Diagnosis Among the Mammary, Histiocytoma, and Lymphoma Cases

Kruskal-Wallis Test		
Chi-Square	DF	Pr > ChiSq
57.7483	2	<.0001

Testing Difference in Average Age at Diagnosis Between 3 of the Most Common Cancer Types Found in Golden Retrievers While Considering Histiocytomas

All 4 case types (mammary, hemangiosarcoma, lymphoma and histiocytoma) were compared using a KW test to see if there was a significant difference in the average age by case type (group), although it was presumed it would be due to the hemangiosarcomas and histiocytomas. As expected, a p-value < .0001 was returned. Therefore, we can reject the null, and there is a statistically significant difference in average age between all 4 case types.

Table 15 - Results of Kruskal-Wallis Test Conducted on Age at Diagnosis Among the Mammary, Hemangiosarcomas, Histiocytoma, and Lymphoma Cases

Kruskal-Wallis Test		
Chi-Square	DF	Pr > ChiSq
94.3611	3	<.0001

Lastly, a Fisher's exact test was run to see the association between age category and group type again, now considering all of the 4 case types, and a p-value < .0001 was also returned. Therefore, there is a statistically significant association between age category and case type when considering all of the case types as well.

Table 16 - Results of Fisher's Exact Test Conducted to Test Association Between Age Category and Mammary, Hemangiosarcoma Histiocytoma, and Lymphoma Cases

Fisher's Exact Test	
Table Probability (P)	<.0001
Pr <= P	<.0001

CHAPTER THREE

Discussion

Finding Highlights

Analysis of Cancer Prevalence in Golden Retrievers and Prevalence Comparison to Humans

Among all of the states in the United States (not considering Alaska, Hawaii, the District of Columbia), Louisiana had the highest overall prevalence of cancer. When observing cancer prevalences by age, Louisiana had the highest proportion of young and old cancer cases also. However, the difference of proportions between young and old dog cancer prevalence was found to be statistically insignificant in Louisiana. Further, there were only 13 cases of cancer in the GRLS found in Louisiana, leading to unstable estimates of prevalence.

Analysis of Average Age at Diagnosis by State and Sex Status

There was no statistically significant difference found in average age at diagnosis between states when looking at all of the cancer dogs, the young cancer dogs only, and the old cancer dogs only. There was also no statistically significant difference found in average age at diagnosis between males and females among the cancer dogs, or when comparing intact and spayed female cancer dogs only. However, an interesting finding was that there was a statistically significant difference in average age at diagnosis when comparing intact and neutered male cancer dogs. The average age at diagnosis among the neutered male cancer dogs had been higher (5.75) than that of the intact male cancer dogs (4.86).

Analysis of Cancer Type Frequency

Based on the findings from this analysis of the GRLS study data, one of the notable overall observations was that most of the hemangiosarcoma cases consisted of older, neutered male dogs. This finding can potentially relate to the significant difference in the average age at diagnosis between the intact and neutered male cancer dogs that was also found. The findings in relation to the hemangiosarcoma cases were expected and confirm the current understanding of cancer in golden retriever because golden retrievers are more susceptible to hemangiosarcomas and older dogs, in general, are more susceptible to cancer in general. After conducting this analysis and finding that hemangiosarcomas were most common among male dogs that were neutered, literature on these types of finding were searched for and it was found that in prior research, neutered males were at a greater risk at developing hemangiosarcoma in general and in of the splenic subtype (Robinson et al, 2020). Therefore, all of the findings among the hemangiosarcoma cases align with current knowledge and understanding of hemangiosarcoma cases in dogs.

Another notable observation was histiocytomas were only found in young cancer dogs and mostly found in those that had been spayed or neutered. It was interesting that only 1 female dog was intact among the histiocytoma cases. Although literature directly addressing this sort of finding was not found, another study had found there to be an increased risk in male and female dogs that had been spayed or neutered for cancers such as: MCTs, hemangiosarcomas (as seen in this analysis as well), lymphomas (as seen in this analysis as well), and osteosarcomas (Belanger et al, 2017).

The findings for the hemangiosarcoma and histiocytoma cases had been confirmed using statistical testing, and, notably, a significant difference in the average age at diagnosis was found when comparing all of the cancer types analyzed, but when looking at lymphomas and mammary cases alone there was not a significant difference. This finding suggests that the significant difference was due to the hemangiosarcoma and histiocytoma cases, as when they were added back in (individually and together), a significant difference was found. There was also a statistically significant association found between age category and case type (group) when histiocytomas and hemangiosarcomas were considered.

Limitations

Detailed limitations within the data collection process can be found in Labadie *et al.* As for the limitations of this analysis of the data, one limitation was that some dogs moved 1-4 times. Therefore, their state for this analysis was determined by the state they were diagnosed if they were a cancer dog by the end of the study data or the state they resided in upon enrollment if they were a cancer free dog by the end of the study data. Therefore, the findings in regard to prevalence may be questionable in terms of trying to make interpretations, considering the dogs did not reside in one place throughout the duration of the study.

Another limitation in this analysis is how the prevalence has to be calculated. Since there is no way of knowing, at least currently, the number of golden retrievers that were in Louisiana throughout the study, the denominator used to calculate the state prevalences were only the total amount of dogs that were enrolled into the study that resided in that state. Not being able to use the true total amount of golden retrievers in each state during this study, therefore, limits the generalizability of the analysis findings, as the only dogs being considered are the dogs of this

study. Another aspect that could affect the generalizability of the findings, is that these dogs can come from a specific group of people in terms of economic classes. Purebred dogs tend to be considerably expensive, suggesting that the individuals/families that own these dogs may be of a higher economic status.

Another limitation is that there was a relatively small sample size of cancer dogs. While there were 3,044 dogs enrolled in total, only 322 ended up developing cancer and therefore information on cancers in golden retriever that was gathered from this analysis is being based on a smaller sample size. This can result in some power issues when conducting more statistical analysis in regard to the cancer dogs because sample size affects power.

Also, since there were 3,044 dogs and there was data taken over the course of 8 years (2012-2020) there were over 200,000 observations. There were also 22 variables to consider. Given the amount of data that had to be cleaned and the unique structure of this dataset, there is the risk of there being errors in the data that was pulled despite being thorough and double checking for data accuracy throughout the analysis. There is also the potential for missing observations for certain variables, as the data is dependent on questionnaire fulfillment from the owners and veterinarians. With surveys, there is a risk of questions being unanswered or answered inaccurately (information bias).

Future Steps

The GRLS study data offers a multitude of variables to study and among a large overall population of dogs. Some more potential research and future steps that can be taken using this dataset is analyzing the body condition scores and weights among the dogs, as that did not make it into this analysis. Also, the GRLS study is an amazing example of how breed specific studies

can be conducted in dogs to learn more about canine oncology. More breed specific analysis should be conducted regarding cancer, and even other diseases, so there is less genetic variability. That way, more can be discovered about individual breeds, as breeds vary in susceptibility.

As for me, I plan on taking all I have learned about the data analysis process and using it to contribute to studies in health disparities. I hope to make an impact in communities of the underrepresented and overlooked, so that their health and well-being is being properly monitored and prioritized, and their questions about their health and the environments they live in are answered.

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Table S1: Descriptive Statistics of the GRLS Dog Population

	All Dogs			Cancer Dogs			Cancer Free Dogs		
Population Characteristics	Dog Population	Young (<6)	Old (>=6)	All Cancer Dogs	Young (<6)	Old (>=6)	All Cancer Free D	Young (<6)	Old (>=6)
State of Dx/Residence									
Alabama	22	12	10	1	1	0	21	11	10
Arizona	57	25	32	10	6	4	47	19	28
Arkansas	22	11	11	2	0	2	20	11	9
California	277	106	171	27	8	19	250	98	152
Colorado	252	111	141	25	14	11	227	97	130
Connecticut	50	23	27	6	4	2	44	19	25
Delaware	22	4	18	3	0	3	19	4	15
District of Colom	1	1	0	0	0	0	1	1	0
Florida	167	76	91	23	12	11	144	64	80
Georgia	62	22	40	5	3	2	57	19	38
Idaho	16	7	9	1	0	1	15	7	8
Illinois	122	53	69	20	8	12	102	45	57
Indiana	61	26	35	6	4	2	55	22	33
Iowa	38	16	22	2	1	1	36	15	21
Kansas	24	11	13	3	2	1	21	9	12
Kentucky	25	12	13	2	0	2	23	12	11
Louisiana	13	4	9	5	1	4	8	3	5
Maine	35	17	18	6	3	3	29	14	15
Maryland	92	37	55	9	6	3	83	31	52
Massachusetts	112	43	69	19	10	9	93	33	60
Michigan	129	54	75	9	3	6	120	51	69
Minnesota	68	32	36	6	4	2	62	28	34
Mississippi	8	3	5	0	0	0	8	3	5
Missouri	52	23	29	3	3	0	49	20	29
Montana	30	15	15	2	1	1	28	14	14
NA	1	1	0	1	1	0	0	0	0
Nebraska	24	9	15	2	1	1	22	8	14
Nevada	24	10	14	3	2	1	21	8	13
New Hampshire	29	12	17	2	0	2	27	12	15
New Jersey	53	28	25	7	3	4	46	25	21
New Mexico	12	1	11	1	0	1	11	1	10
New York	121	49	72	9	4	5	112	45	67
North Carolina	108	37	71	14	9	5	94	28	66
North Dakota	7	1	6	1	0	1	6	1	5
Ohio	99	39	60	12	6	6	87	33	54
Oklahoma	15	7	8	1	1	0	14	6	8
Oregon	46	17	29	4	3	1	42	14	28
Pennsylvania	188	72	116	15	8	7	173	64	109
Rhode Island	23	14	9	1	1	0	22	13	9
South Carolina	31	11	20	5	0	5	26	11	15
South Dakota	8	4	4	0	0	0	8	4	4
Tennessee	39	14	25	5	3	2	34	11	23
Texas	90	36	54	7	3	4	83	33	50
Utah	17	10	7	1	1	0	16	9	7
Vermont	21	7	14	0	0	0	21	7	14
Virginia	110	52	58	14	6	8	96	46	50
Washington	108	55	53	13	9	4	95	46	49
West Virginia	17	6	11	1	1	0	16	5	11
Wisconsin	90	45	45	8	6	2	82	39	43
Wyoming	5	0	5	0	0	0	5	0	5
Total*	3043	1281	1762	322	162	160	2721	1119	1602
Age	Dog Population	Young (<6)	Old (>=6)	All Cancer Dogs	Young (<6)	Old (>=)	All Cancer Free D	Young (<6)	Old (>=6)
Young (< 6)	1282	--	--	162	--	--	1120	--	--
Old (>= 6)	1762	--	--	160	--	--	1602	--	--
Total	3044	--	--	322	--	--	2722	--	--
Average Age (at dx)	5.95	4.15	7.25	5.59	3.82	7.38	5.99	4.20	7.24
Sex Status (at dx)									
Female Intact	329	226	103	31	18	13	298	208	90
Female Spayed	1175	401	774	124	58	66	1051	343	708
Male Intact	494	256	238	53	33	20	441	223	218
Male Neutered	1046	399	647	114	53	61	932	346	586
Total	3044	1282	1762	322	162	160	2722	1120	1602

Table S2: State Prevalence (Proportion) by Age Category*

*Highlighted in orange are the highest prevalences for each age category

	State Prevalence% Of Cancer			Young Dogs State Proportion		Old Dogs State Proportion
0.04545454545	4.5	Alabama	0.08333333333	8.3	0	0.0
0.1754385965	17.5	Arizona	0.24	24.0	0.125	12.5
0.09090909091	9.1	Arkansas	0	0.0	0.1818181818	18.2
0.09747292419	9.7	California	0.07547169811	7.5	0.1111111111	11.1
0.09920634921	9.9	Colorado	0.1261261261	12.6	0.0780141844	7.8
0.12	12.0	Connecticut	0.1739130435	17.4	0.07407407407	7.4
0.1363636364	13.6	Delaware	0	0.0	0.1666666667	16.7
0	0.0	District of Colom	0	0.0	#DIV/0!	#DIV/0!
0.1377245509	13.8	Florida	0.1578947368	15.8	0.1208791209	12.1
0.08064516129	8.1	Georgia	0.1363636364	13.6	0.05	5.0
0.0625	6.3	Idaho	0	0.0	0.1111111111	11.1
0.1639344262	16.4	Illinois	0.1509433962	15.1	0.1739130435	17.4
0.09836065574	9.8	Indiana	0.1538461538	15.4	0.05714285714	5.7
0.05263157895	5.3	Iowa	0.0625	6.3	0.04545454545	4.5
0.125	12.5	Kansas	0.1818181818	18.2	0.07692307692	7.7
0.08	8.0	Kentucky	0	0.0	0.1538461538	15.4
0.3846153846	38.5	Louisiana	0.25	25.0	0.4444444444	44.4
0.1714285714	17.1	Maine	0.1764705882	17.6	0.1666666667	16.7
0.09782608696	9.8	Maryland	0.1621621622	16.2	0.05454545455	5.5
0.1696428571	17.0	Massachusetts	0.2325581395	23.3	0.1304347826	13.0
0.06976744186	7.0	Michigan	0.05555555556	5.6	0.08	8.0
0.08823529412	8.8	Minnesota	0.125	12.5	0.05555555556	5.6
0	0.0	Mississippi	0	0.0	0	0.0
0.05769230769	5.8	Missouri	0.1304347826	13.0	0	0.0
0.06666666667	6.7	Montana	0.06666666667	6.7	0.06666666667	6.7
1	100.0	NA	1	100.0	#DIV/0!	#DIV/0!
0.08333333333	8.3	Nebraska	0.1111111111	11.1	0.06666666667	6.7
0.125	12.5	Nevada	0.2	20.0	0.07142857143	7.1
0.06896551724	6.9	New Hampshire	0	0.0	0.1176470588	11.8
0.1320754717	13.2	New Jersey	0.1071428571	10.7	0.16	16.0
0.08333333333	8.3	New Mexico	0	0.0	0.09090909091	9.1
0.07438016529	7.4	New York	0.08163265306	8.2	0.06944444444	6.9
0.1296296296	13.0	North Carolina	0.2432432432	24.3	0.07042253521	7.0
0.1428571429	14.3	North Dakota	0	0.0	0.1666666667	16.7
0.1212121212	12.1	Ohio	0.1538461538	15.4	0.1	10.0
0.06666666667	6.7	Oklahoma	0.1428571429	14.3	0	0.0
0.08695652174	8.7	Oregon	0.1764705882	17.6	0.03448275862	3.4
0.07978723404	8.0	Pennsylvania	0.1111111111	11.1	0.06034482759	6.0
0.04347826087	4.3	Rhode Island	0.07142857143	7.1	0	0.0
0.1612903226	16.1	South Carolina	0	0.0	0.25	25.0
0	0.0	South Dakota	0	0.0	0	0.0
0.1282051282	12.8	Tennessee	0.2142857143	21.4	0.08	8.0
0.07777777778	7.8	Texas	0.08333333333	8.3	0.07407407407	7.4
0.05882352941	5.9	Utah	0.1	10.0	0	0.0
0	0.0	Vermont	0	0.0	0	0.0
0.1272727273	12.7	Virginia	0.1153846154	11.5	0.1379310345	13.8
0.1203703704	12.0	Washington	0.1636363636	16.4	0.07547169811	7.5
0.05882352941	5.9	West Virginia	0.1666666667	16.7	0	0.0
0.08888888889	8.9	Wisconsin	0.1333333333	13.3	0.04444444444	4.4
0	0.0	Wyoming	#DIV/0!	#DIV/0!	0	0.0

Table S3: Results of Difference of Proportion Calculations by State and Sex Status**

**Highlighted in orange are the states that had a significant difference of proportions due to the CI passing through zero

Difference of Proportions (By State)										
	Proportion 1 (Yo	Proportion 2 (Ol	n1	n2	diff	s (standard devi	LCI (lower confience interval)		UCI (upper confidence interval)	
Alabama	0.083	0.000	12	10	0.083	0.07978559231	0.240	23.97130943	-0.390	-39.0052072
Arizona	0.240	0.125	25	32	0.115	0.1035083028	0.318	31.78762735	-0.520	-51.9529193
Arkansas	0.000	0.182	11	11	-0.182	0.1162912998	0.046	4.611276585	0.026	2.59102787
California	0.075	0.111	106	171	-0.036	0.03515447545	0.033	3.326335889	-0.030	-3.00417079
Colorado	0.126	0.078	111	141	0.048	0.03876964473	0.124	12.41004454	-0.204	-20.4467228
Connecticut	0.174	0.074	23	27	0.100	0.09373722515	0.284	28.35639307	-0.462	-46.2048071
Delaware	0.000	0.167	4	18	-0.167	0.08784104612	0.006	0.550178372	0.077	7.70575500
District of Colom	0.000	#DIV/0!	1	0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Florida	0.158	0.121	76	91	0.037	0.05401202964	0.143	14.28791941	-0.226	-22.6031190
Georgia	0.136	0.050	22	40	0.086	0.08087408705	0.245	24.4876847	-0.399	-39.908453
Idaho	0.000	0.111	7	9	-0.111	0.1047565602	0.094	9.421174683	-0.080	-7.98984636
Illinois	0.151	0.174	53	69	-0.023	0.06708381527	0.109	10.85146307	-0.146	-14.5604860
Indiana	0.154	0.057	26	35	0.097	0.08090850445	0.255	25.52839654	-0.419	-41.9448067
Iowa	0.063	0.045	16	22	0.017	0.07506204585	0.164	16.41670644	-0.247	-24.6705400
Kansas	0.182	0.077	11	13	0.105	0.1377884612	0.375	37.49604889	-0.597	-59.713409
Kentucky	0.000	0.154	12	13	-0.154	0.1000682516	0.042	4.228761935	0.017	1.71845177
Louisiana	0.250	0.444	4	9	-0.194	0.2725983167	0.340	33.98482562	-0.394	-39.3504265
Maine	0.176	0.167	17	18	0.010	0.1275335156	0.260	25.97696121	-0.382	-38.1614924
Maryland	0.162	0.055	37	55	0.108	0.06789465361	0.241	24.06902287	-0.404	-40.3858194
Massachusetts	0.233	0.130	43	69	0.102	0.07612074959	0.251	25.13200261	-0.416	-41.6486501
Michigan	0.056	0.080	54	75	-0.024	0.04419257852	0.062	6.217300946	-0.078	-7.76665200
Minnesota	0.125	0.056	32	36	0.069	0.06982438503	0.206	20.63002391	-0.335	-33.4524083
Mississippi	0.000	0.000	3	5	0.000	0	0.000	0	0.000	
Missouri	0.130	0.000	23	29	0.130	0.07022372636	0.268	26.80732863	-0.455	-45.5199914
Montana	0.067	0.067	15	15	0.000	0.09108400681	0.179	17.85246533	-0.259	-25.8824313
NA	1.000	#DIV/0!	1	0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Nebraska	0.111	0.067	9	15	0.044	0.1229718872	0.285	28.54693434	-0.437	-43.6548025
Nevada	0.200	0.071	10	14	0.129	0.1440055878	0.411	41.08223807	-0.661	-66.1206278
New Hampshire	0.000	0.118	12	17	-0.118	0.0781424899	0.036	3.551222138	0.009	0.853853599
New Jersey	0.107	0.160	28	25	-0.053	0.0937685725	0.131	13.09292593	-0.163	-16.2852775
New Mexico	0.000	0.091	1	11	-0.091	0.0866784172	0.079	7.898060681	-0.068	-6.81235721
New York	0.082	0.069	49	72	0.012	0.04926967965	0.109	10.87567807	-0.164	-16.3893610
North Carolina	0.243	0.070	37	71	0.173	0.07679220719	0.323	32.33334341	-0.557	-55.6941323
North Dakota	0.000	0.167	1	6	-0.167	0.1521451549	0.132	13.15378369	-0.106	-10.5869005
Ohio	0.154	0.100	39	60	0.054	0.06955490633	0.190	19.01737703	-0.303	-30.3185683
Oklahoma	0.143	0.000	7	8	0.143	0.1322600143	0.402	40.20867708	-0.656	-65.5830056
Oregon	0.176	0.034	17	29	0.142	0.09847236551	0.335	33.4993666	-0.558	-55.8115219
Pennsylvania	0.111	0.060	72	116	0.051	0.04313425457	0.135	13.53094225	-0.222	-22.2072213
Rhode Island	0.071	0.000	14	9	0.071	0.06883029369	0.206	20.63359471	-0.336	-33.5588162
South Carolina	0.000	0.250	11	20	-0.250	0.09682458366	-0.060	-6.022381604	0.215	21.4863263
South Dakota	0.000	0.000	4	4	0.000	0	0.000	0	0.000	
Tennessee	0.214	0.080	14	25	0.134	0.1223529283	0.374	37.40974538	-0.611	-61.0878081
Texas	0.083	0.074	36	54	0.009	0.05824126686	0.123	12.34121423	-0.184	-18.3646532
Utah	0.100	0.000	10	7	0.100	0.09486832981	0.286	28.59419264	-0.466	-46.5577846
Vermont	0.000	0.000	7	14	0.000	0	0.000	0	0.000	
Virginia	0.115	0.138	52	58	-0.023	0.06334831107	0.102	10.16162706	-0.136	-13.5819579
Washington	0.164	0.075	55	53	0.088	0.06168369036	0.209	20.90648966	-0.348	-34.8083119
West Virginia	0.167	0.000	6	11	0.167	0.1521451549	0.465	46.48711702	-0.759	-75.9002338
Wisconsin	0.133	0.044	45	45	0.089	0.05925925926	0.205	20.5037037	-0.343	-34.2613333
Wyoming	#DIV/0!	0.000	0	5	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Difference of Proportions (By Sex Status)										
	Proportion 1 (Yo	Proportion 2 (Ol	n1	n2	diff	s (standard devi	LCI (lower confience interval)		UCI (upper confidence interval)	
Female Intact	0.0796460177	0.1262135922	18	13	-0.04656757453	0.1120523357	0.1730550034	17.30550034	-0.2661901525	-26.6190152
Female Spayed	0.144638404	0.08527131783	58	66	0.05936708616	0.05757509283	0.172214268	17.22142681	-0.05348009579	-5.34800957
Male Intact	0.12890625	0.08403361345	33	20	0.04487263655	0.08515461364	0.2117756793	21.17756793	-0.1220304062	-12.2030406
Male Neutered	0.1328320802	0.0942812983	53	61	0.0385507819	0.05977647972	0.1557126822	15.57126822	-0.07861111836	-7.86111183