DISSERTATION

VETERINARY CANCER EPIDEMIOLOGY: USING CANINE SPONTANEOUS TUMOR MODELS FOR THE STUDY OF HUMAN CANCERS

Submitted by

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ABSTRACT

VETERINARY CANCER EPIDEMIOLOGY: USING CANINE SPONTANEOUS TUMOR MODELS FOR THE STUDY OF HUMAN CANCERS

Cancer is the second leading cause of death in humans worldwide and the most common cause of death for humans in developed countries. Cancer is also the leading cause of mortality in dogs. Animal models, primarily genetically engineered mice, have been used historically in research aimed at discovering causes and treatments for human cancer types, but the canine model has been underutilized in this research area. Though the field of canine cancer epidemiology is relatively new, it has great potential to produce answers to research questions pertaining to cancer prevention, development, and treatment relevant to both dogs and humans. In fact, the canine spontaneous tumor model is actually a better model for use in human cancer epidemiology research than other animal models or even human populations. This is due to the fact that dogs spontaneously produce many different types of tumors that are molecularly indistinguishable from human tumors. Canine DNA shares a large amount of ancestral sequence with human DNA, but dogs have greater genetic homogeneity – even across breeds – than do humans, which simplifies disease mapping at the genomic level. Dogs live in the same environments as humans, too, so they share many similar exposures to environmental factors that may contribute to the development of cancer. Tumors in dogs progress at a rapid rate as compared to humans and many tumor types that are rare in humans occur frequently in dogs. These facts, when considered along with the existence of an accelerated aging process in dog, support how use of the canine spontaneous tumor model will allow us to gain a greater

understanding of genetic and environmental contributions to human disease and do so at a rapid pace.

The primary aims of my dissertation were to examine the current body of evidence produced through canine cancer epidemiology research, produce new research using study designs similar to those used in human cancer epidemiology research, and show how we can advance knowledge of cancer risk and pathogenesis in both fields using the canine spontaneous tumor model.

- Through utilization of systematic review methodology I was also able to identify a lack of consistency in study design and statistical methods used in veterinary cancer research even when exploring the same research question.
- Then, with a study population of nearly 68,000 dogs admitted to hospitals across the United States over a 20-year time frame, I was able to show there are several breed-specific and hormone-dependent risks associated with development of lymphoma, many of which had not been previously reported, likely due to the use of small samples sizes in veterinary cancer research.
- I also discovered differences in the geographic distribution of dogs diagnosed with two different subtypes of lymphoma in the US. This implies molecular characterization of some cancers, which is commonly done in human epidemiologic research, may be a necessary component of future veterinary epidemiologic research in order for us to truly identify risks for disease occurrence.
- Lastly, I utilized a study population composed only of Bernese mountain dogs, a breed known to be predisposed to developing a cancer type that is rare in humans, to investigate exposure variables associated with disease outcome. The results of this project suggested

a mechanism of disease pathogenesis not previously reported in either the veterinary or the human literature.

As a body of work, these individual studies contribute to advancing the concept of using canine spontaneous tumor models in lieu of using other animal models for comparative research. In addition, the canine model can be superior to use of human subjects in many instances, including when determining the etiology of rare cancer types or when determining the pathogenetic basis of disease. In order to continue positively contributing to the field of cancer epidemiology, veterinary epidemiologists must increase the rigor with which we are conducting studies, report research in a transparent manner by conforming to accepted reporting guidelines, and ensure we are investigating appropriate research questions.

DEDICATION

The process of obtaining a PhD is absolutely grueling and I certainly would not have completed this degree without the love and support of my husband, Dave, and our three amazing daughters, Logan, Eloise, and Molly. It is their spirit, spunk, and never-ending faith in me that has fueled my progress in this work. The drive and passion for this endeavor, however, is inspired almost solely from the life and death of my son, Aidan. It was his brief presence here on this earth and the pain experienced as a result of his departure that sparked the interest necessary to start down this path. It is my sincere and humble hope that this and all future work I complete will honor his life in some small way. Thus, this work is truly dedicated to him.

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Chapter 1 - A systematic review of the epidemiology and etiology of canine lymphoma[1]

Overview

Background: Canine lymphoma (CL) is one of the most commonly diagnosed neoplasms in dogs and there is evidence suggesting the incidence rate of the disease is increasing. However, the complete etiology of the disease remains unclear despite several published reports investigating this question.

Objective: To determine which exposure variables have reported associations with the outcome of CL and formally assess the quality of evidence related to each reported risk.

Methods: Systematic review methods were utilized to obtain published reports investigating relationships between exposure variables and the occurrence of CL. Data from all research deemed relevant to the review were grouped based upon the similarity of exposure variable(s) studied. The quality of individual studies was evaluated and the strength of the body of evidence for individual exposure variables was assessed using guidelines developed by the GRADE working group.

Results: In total, 38 studies evaluating 10 specific exposure variables met the inclusion criteria for this review. Only one exposure variable, breed of the dog, was found to have high quality of evidence to support the association between the variable and the outcome of CL. The majority of individual exposure variables examined were deemed to have moderate or low quality evidence to support the association with the outcome of CL. Factors reported by reviewers to have decreased the overall quality score associated with individual exposure variables included risk of bias in study design or statistical methodology utilized and inconsistency of results reported in different research articles.

[1] A. Ruple-Czerniak, R. L. Page, and P. S. Morley

Conclusions: Reported evidence suggests several exposure variables are likely associated with the occurrence of CL, but the true effect of these exposure variables may be substantially different from the reported effect estimates due to the risk of bias and inconsistent results reported in the veterinary literature. Increased rigor, consistent use of study designs that produce high quality evidence, transparency in reporting, and use of similar methodologies allowing for ease of comparison of results will help to increase the overall quality of evidence produced in veterinary cancer research.

Introduction

Canine lymphoma (CL) is one of the most commonly diagnosed neoplasms in dogs.[1,2] The incidence of CL, when calculated from data collected in tumor registries during the 1960s and 1970s, was reported to be about 25 to 30 cases per 100,000 dogs per year.[3,4] A more recent age-adjusted incidence rate calculated from data collected by a pet insurance company in the UK was 107 cases per 100,000 dogs per year (95% CI: 90, 125).[5] Despite the apparent increase in the number of cases of CL being diagnosed each year the etiology of the disease remains undetermined. Most published research investigating the etiology of CL consists of observational studies, the case-control study design being most frequently used.[6] However, study methods utilized for observational studies are prone to bias and, according to the Oxford Centre for Evidence-Based Medicine (OCEBM), the findings are in many cases considered to be a lower level of evidence than is produced through randomized control trials.[6,7]

A study design that is considered by groups such as the OCEBM to produce a higher level of evidence is systematic review. Systematic reviews are different from narrative reviews in that the purpose is to provide an objective summary of the published literature on a particular topic.[8] The formalized method utilized to synthesize data from different studies allows for an

appraisal of the quality of the body of evidence for a particular subject rather than relying solely upon the evidence produced from individual studies.[9] Guidelines for the assessment of evidence quality based on multiple reports have been developed by The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Working Group.[9] In these assessments, the quality level determined for a body of evidence reflects the level of confidence there is that the estimates of effect determined are close to the true effects. Thus, the purpose of this study was to determine which exposure factors are associated with the outcome of CL and formally assess the quality of evidence related to each reported risk.

Materials and methods

Overview

Studies which investigated relationships between exposure variables and the outcome of lymphoma in dogs were reviewed. Systematic searches of computerized bibliographic databases were conducted and two relevance screenings were utilized in order to identify relevant research articles. Data abstraction was performed and data were grouped based upon similarity of exposure variable studied. Calculations of incidence of canine lymphoma, when reported, were also abstracted. Quality of individual studies as well as the strength of the body of evidence for individual exposure variables was assessed.

Literature search

Electronic literature searches in PubMed (1950 to present), Web of Science (1900 to present), ProQuest (1982 to present), Medline (1950 to present), CAB Abstracts (1973 to present), Biological abstracts archives (1969 to 2009), and AGRICOLA (1970 to present) were conducted in April 2012. Terms that described the population, outcome, and risk factors of interest were identified in the Medical Subject Headings (MeSH) database.[10]

The search strings used were as follows: [lymphoma OR lymphosarcoma] AND [dog* OR canine] AND [risk OR "risk factor*"]. Retrieved citations were stored in reference management software (Endnote Web, version 3.5). Duplicate citations were removed by electronic and hand scanning of the database. When multiple instances of the same citation were identified, the most complete citation was retained. Hand searching of the reference lists of relevant papers was conducted as the review progressed. Reviewers evaluated the reference list and identified potentially relevant citations. If the electronic search had not captured the citation, it was added to the reference management software.

Relevance screenings

A relevance screening was conducted to rapidly remove citations not relevant to the review, as the literature search process was highly sensitive, with low specificity. Eligible studies were primary research papers (experimental or observational) that reported associations between exposure variables and the outcome of lymphoma in dogs. Two levels of relevance screening were used.

For level 1 relevance screening, each abstract was reviewed independently by two reviewers (Page and Ruple-Czerniak). Citations advanced to the second relevance screening if both reviewers agreed the citation described primary research pertaining to the effects of risk factors on the development of lymphoma or if the citation did not include enough information to determine eligibility. In cases where the two reviewers did not agree about a citation a third reviewer (Morley) was involved for adjudication. The second relevance screening was conducted by the same reviewers (Page and Ruple-Czerniak) using the full manuscript. Manuscripts advanced to the next level of the review if the manuscript met all inclusion criteria

determined for the level 1 relevance screening and the study was published in English. Again, a third reviewer (Morley) adjudicated disagreements between the primary reviewers.

Data abstraction and quality assessment

Data were independently abstracted by a single reviewer (Ruple-Czerniak). All abstracted data were reviewed by a second reviewer (Morley) for accuracy and completeness. Abstracted data from individual studies included the author list, years the study was performed and reported, study design, study population, sample size, study location, number of subjects with outcome of lymphoma included in the study, method utilized to diagnose lymphoma, and exposure variables analyzed. Data were grouped by exposure variable investigated and study-specific risk estimates, covariates that were used for adjustment of estimates, and confidence intervals were abstracted when available. If multiple risk estimates were available the estimate that adjusted for the most covariates was abstracted. If no adjusted risk estimates were presented, we abstracted the crude estimate.

The quality of individual studies was determined based on the following criteria: (i) representativeness of study sample; (ii) selection of study participants; (iii) data collection methods utilized; and (iv) statistical and analytic methods used. The quality of the body of evidence for each exposure variable was assessed using guidelines developed by The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Working Group and summarized as high (true effect likely close to the estimate of effect), moderate (true effect likely close to the estimate of effect, but may be substantially different), low (limited confidence in the effect estimate, true effect may be substantially different), or very low (very little confidence in the effect estimate, true effect likely to be substantially different).[9] GRADE quality levels are influenced by a number of factors including the magnitude of effect size, with large effects

positively influencing the rating, and risk of bias, with serious risk negatively influencing ratings (Table 1.1).

Table 1.1 Factors affecting quality of evidence according to the scale developed by GRADE.[9]

Randomized trials, Initial Quality Rating High (+4)	Large effect (+1 Large, +2 Very large), Dose response (+1 Evidence of a gradient), All plausible residual confounding would reduce a demonstrated effect (+1), All plausible residual confounding would suggest a spurious effect if no effect observed (+1)	Factors Increasing Quality Score
Observational studies, Initial Quality Rating Low (+2)	Risk of bias, Inconsistency, Indirectness, Imprecision, Publication bias For all factors: -1 if Serious, -2 if Very serious	Factors Decreasing Quality Score

Results

Study Characteristics

In total, 380 unique articles were identified of which 38 articles met the inclusion criteria for both relevance screenings (Fig. 1.1). These articles, published between 1967 and 2012,

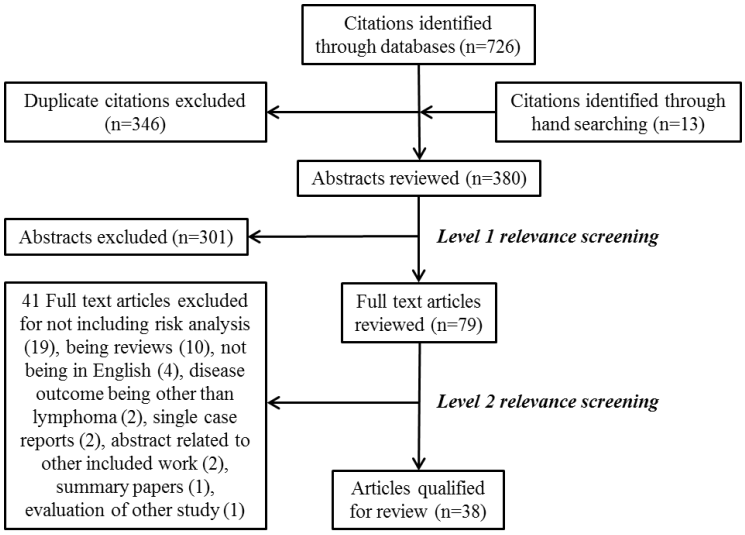

Figure 1.1. Screening and selection process of studies investigating exposure variables associated with the outcome of CL

represent 28,569 dogs diagnosed with CL and nearly 2.5 million non-case animals (Table 1.2).

Table 1.2. Studies evaluating exposure variables associated with the outcome of lymphoma in dogs.

First Author	Year	Country	Study Design	Control group	Study Population	Sample Size	Number of Events	Risk factors analyzed
Dorn	1967	US	Descriptive	None	Population based	93	83	Age, sex, breed
Dorn	1968	US	Descriptive	None	Population based	4,842	92	Sex
Dorn	1968	US	Descriptive	None	Population based	915	52	Age, sex
Lobetti	2009	South Africa	Descriptive	None	Related individuals of a single breed	3	3	Breed
Lurie	2004	US	Descriptive	None	Hospital based	102	43	Breed
Modiano	2005	US	Descriptive	None	Laboratory based	1,263	1,263	Breed
Mukaratirwa	2005	Zimbabwe	Descriptive	None	Hospital based	900	27	Age, sex
O'Brien	1999	US	Descriptive	None	Hospital based	528	227	Proximity to metropolitan center
Onions	1984	US	Descriptive	None	Related individuals of a single breed	59	9	Breed
Pastor	2009	France	Descriptive	None	Population based	608	608	Age, breed, waste, pollution
Sueiro	2004	Brazil	Descriptive	None	Laboratory based	55	55	p53
Teske	1994	Netherlands	Descriptive	None	Related individuals of a single breed	6	5	Breed
Thomas	2003	UK	Descriptive	None	Laboratory based	25	25	Chromosome aberrations
Bryan	2009	US	Case-Control	Dogs free of lymphoma	Hospital based	28	21	DLC1 hypermethylation
Cohen	1974	US	Case-Control	Dogs with any diagnosis other than lymphoma	Hospital based	60,000	193	Age, sex, breed
Duncan	2008	US	Case-Control	Matched, healthy animals	Population based	84	28	Vector-borne infections
Edwards	2003	UK	Case-Control	Healthy dogs and dogs with other cancer types	Population based	130,684	103	Age, Breed
Gavazza	2001	Italy	Case-Control	Matched, dogs with any diagnosis other than cancer	Hospital based	292	101	Chemicals, industrial areas, pesticides
Hayes	1991	US	Case-Control	Dogs with other cancer types and Dogs with non-cancer diagnoses	Hospital based	1,436	491	2,4-Dichlorophenoxyacetic acid herbicides
Hayes	1995	US	Case-Control	Dogs with other cancer types and Dogs with non-cancer diagnoses	Hospital based	1,436	491	2,4-Dichlorophenoxyacetic acid herbicides
Jagielski	2002	Poland	Case-Control	Dogs with any diagnosis other than lymphoma	Hospital based	7,730	63	Age, sex, breed

Keller	1992	US	Case-Control	Dogs with same cancer type and of older age	Hospital based	69	12	Age, sex
Keller	1992	US	Case-Control	Dogs with any diagnosis other than lymphoma	Hospital based	1,036,428	8,412	Immune-mediated disease
Marconato	2009	Italy	Case-Control	Dogs with other cancer types	Hospital based	353	67	Waste, pollution, tobacco smoke, age, sex
Merlo	2008	Italy	Case-Control	Dogs with other cancer types	Population based	3,303	411	Age, sex
Modiano	2007	US	Case-Control	Dogs with other cancer types	Laboratory based	48	48	Breed
Priester	1967	US	Case-Control	Dogs licensed within specific geographical regions	Hospital based	240	240	Sex, breed
Reif	1995	US	Case-Control	Dogs with other cancer types	Hospital based	230	93	Electromagnetic fields
Takashima-Uebelhoer	2012	US	Case-Control	2 control groups: Dogs with other cancer types and Dogs with non-cancer diagnoses	Hospital based	733	263	Herbicides, pesticides, flea/tick control products, insect growth regulators
Villamil	2009	US	Case-Control	Dogs with any diagnosis other than lymphoma	Hospital based	1,171,915	14,573	Age, sex, breed
Wyatt	1998	Australia	Case-Control	Dogs with any diagnosis other than lymphoma	Hospital based	Not described	85	Age, sex, breed
Benjamin	1986	US	Control Trial	Non-treated animals	Research colony, beagles	1,680	1	Ionizing radiation
Benjamin	1998	US	Control Trial	Non-treated animals	Research colony, beagles	1,680	95	Ionizing radiation
Deeg	1980	US	Control Trial	Non-treated animals	Research colony, various breeds	108	3	Ionizing radiation
Deeg	1983	US	Control Trial	Non-treated animals	Research colony, various breeds	153	6	Ionizing radiation
Hansen	1971	US	Control Trial	Non-treated animals	Research colony, beagles	30	0	2,4-Dichlorophenoxyacetic acid herbicides
Lloyd	2004	US	Control Trial	Non-treated animals	Research colony, beagles	470	2	Plutonium
Lloyd	1995	US	Control Trial	Non-treated animals	Research colony, beagles	117	12	Americium

Study designs consisted of 18 case-control studies,[11-28] 13 descriptive studies,[4,29-40] and 7 controlled trials.[41-47] The majority of the research was conducted in the US (26/38),[4,11,12,15,16,18,19,22-26,28-30,32,33,40-48] but studies from Europe (8/38),[13,14,17,20,21,36,38,39] Africa (2/38),[31,34] Australia (1/38),[27] and South America (1/38)[37] were also included (Table 1.2). Most study subjects were drawn from hospital populations (17/38),[11,14-20,23-28,32,34,48] but 7 studies were population based.[4,12,13,21,29,30,36]

Exposure variables analyzed in the selected studies included (i) breed (n= 14);[4,11,13,17,22,23,26,27,31-33,36,38,40] (ii) age (n= 12);[4,11,13,17,19-21,26,27,30,34,36] (iii) sex status (n= 12);[4,11,17,19-21,23,26,27,29,30,34] (iv) exposure to environmental chemicals and pollutants (n= 9);[14-16,20,24,25,36,45,48] (v) exposure to radiation (n= 6);[41-44,46,47] (vi) the presence of specific genetic mutations (n= 3),[28,37,39] and (vii) diagnosis with other diseases or infections (n = 2).[12,18]

Studies investigating breed and the outcome of CL

Study characteristics and findings

Fourteen studies, published between 1967 and 2009, investigated dog breed as a risk factor for development of lymphoma (Table 1.2).[4,11,13,17,22,23,26,27,31-33,36,38,40] All of these studies utilized one of four different observational study designs and different breeds were investigated in each study. In total, 38 individual breeds were reported to have an association with the outcome of CL in at least one study (Table 1.3) and 11 breeds were reported to be at increased

Table 1.3. Dog breeds associated with occurrence of lymphoma reported in only one study included in review.

Breeds reported at increased risk	*Breeds reported at decreased risk*
Collie dogs	Samoyed
Fox terriers	Brittany Spaniel
Bull terriers	Maltese
Deerhound	Pug
Bernese mountain dog	Yorkshire terrier
Scottish terrier	Miniature poodle
Vizsla	Pomeranian
Bouvier des Flandres	Chihuahua
Old English sheepdog	Toy poodle
Pembroke Welsh corgi	Dachshund
Standard poodle	Pekingese
Boston terriers	Mixed breed
Bulldog	
Otterhound	
Setters	

risk of developing CL in more than one study (Table 1.4). These findings are suggestive of a heritable predisposition for development of lymphoma that varies between different breeds of dogs. However, use of different study populations, reference groups, and methods utilized to calculate measures of risk makes it difficult to compare specific calculated risks between these different studies.

Table 1.4. Dog breeds associated with occurrence of lymphoma reported in more than one study included in review.

Breeds reported at increased risk	*Number of reports*
Boxer	9
Rottweiler	6
Bullmastiff	3
Golden retriever	3
Irish wolfhound	3
Gordon Setter	2
Saint Bernard	2
Airedale terrier	2
Basset hound	2
Cocker spaniels	2
German shepherds	2

Summary

Despite the inconsistency in study design and the variability in specific breeds examined, large measures of effect were often reported and there was no inconsistency in directionality of risk reported for individual breeds. Therefore, there is a high quality level of evidence present in the published literature suggestive of a heritable predisposition towards development of CL in dogs, but only low quality published evidence for associations regarding the risk of CL in specific dog breeds.

Studies investigating age and the outcome of CL

Study characteristics and findings

Twelve studies, published between 1967 and 2009, investigated dog age as a risk factor for development of lymphoma (Table 1.2). [4,11,13,17,19-21,26,27,30,34,36] All of these studies utilized observational study designs and the statistical methods utilized were not consistent between studies. However, evidence of an increased risk of CL as age increased was reported in several studies[4,13,21,30] and large effect sizes were reported in more than one study.[26,27]

Summary

There is moderate quality of evidence in the published literature suggestive of an increased risk of CL as age increases. This evidence did not rise to the level of high quality evidence primarily due to the inconsistent use of study designs and utilization of non-standardized control groups.

Studies investigating sex and neutering and the outcome of CL

Study characteristics and findings

Twelve studies, published between 1967 and 2009, investigated sex and neutering status as a risk factors for development of CL (Table 1.2).[4,11,17,19-21,23,26,27,29,30,34] All of these studies utilized observational study designs and the statistical methods utilized varied between studies. There was marked inconsistency between study results and only a few reports detected associations between sex and neuter status and the outcome of CL,[17,20,26,34] but most studies reporting negative findings (i.e. no significant associations) were conducted using small sample sizes and a biological difference may be present though not statistical detected.

Summary

There is low quality of evidence in the published literature that suggests a weak association between sex and neuter status of dogs and the occurrence of CL.

Studies investigating exposure to environmental pollutants, pesticides, tobacco smoke, or electromagnetic fields and the outcome of CL

Study characteristics and findings

Nine studies, published between 1971 and 2012, have investigated risks associated with exposure to different chemicals and pollutants found in the environment and include a combined total study population of more than 5,600 dogs and more than 2,300 cases of CL (Table 1.2).[14-16,20,24,25,35,36,45] Specific risk factors investigated include exposure to pesticides,[14-16,25,45] environmental pollutants,[14,20,35,36] tobacco smoke,[20,25] and electromagnetic fields (EMF).[24] Most of these studies utilized observational study designs, but one study used a controlled trial design in which randomization was not reported.[45] Exposures were measured indirectly in the majority of studies, with only one study design utilizing direct measurement of the environmental exposure.[24] Study design and statistical methods utilized were inconsistent between studies and results pertaining to associations between individual environmental exposures and the outcome of CL were not consistent. However, there is strong evidence supporting the biologic plausibility of the association between exposure to environmental pollutants and the outcome of CL.[49-51]

Summary

There is moderate quality of evidence to support the relationship between exposure to environmental pollution and an increased risk for CL occurrence. However, there is only low quality or very low quality levels of evidence to support the relationship between individual environmental exposures (tobacco, EMF, and herbicides) and the occurrence of CL (Table 1.5).

Table 1.5. Quality of the body of evidence reported for risk factors associated with the outcome of CL.

High	Moderate	Low	Very low
Breed	Age	Sex status	Vector-borne infections
	Ionizing radiation	Herbicides	Immune-mediated diseases
	Environmental pollution	Electromagnetic fields	
		Environmental tobacco smoke	

Studies investigating radiation and the outcome of CL

Study characteristics and findings

Six studies, published between 1980 and 2004, investigated the risk associated with exposure to gamma radiation[41-44] or radioactive isotopes found near areas of nuclear weapons testing or sites of nuclear incidents[46,47] and the outcome of CL (Table 1.2). All of these studies were controlled trials involving experimental exposures, but none of them described randomized allocation to study groups. In all studies, exposure to radiation was measured directly and research results were consistent between studies.

Summary

There is a moderate quality of evidence to suggest there is an increased risk of CL in association with exposure to ionizing radiation and the outcome of CL, and also for the lack of association between exposure to radioactive isotopes and the occurrence of CL at the doses investigated.

Studies investigating genetic mutations and the outcome of CL

Study characteristics and findings

Three observational studies included in this review investigated associations between genetic abnormalities and the development of CL (Table 1.2).[20,37,39] The methods utilized to investigate this association were not consistent and each study focused on a separate genetic

abnormality. However, the results of the studies all concluded there was a positive association between the presence of specific genetic abnormalities and the outcome of CL.

Summary

There is a low quality level of evidence to support the reported associations between various genomic aberrations and the outcome of CL. This is mainly due to the lack of replicated findings in the literature.

Studies investigating diagnosis with other diseases or infections and the outcome of CL

Study characteristics and findings

Two observational studies investigated associations between diagnosis with immune-mediated disease (lupus disorders, pemphigus disorders, autoimmune polyarthritis, immune-mediated hemolytic anemia, and immune-mediated thrombocytopenia)[18] and vector-borne infections (*Bartonella* spp., *Anaplasma*, and *Ehrlichia*)[12] as potential risk factors for development of CL (Table 1.2). Of the 8 specific disorders studied only immune-mediated thrombocytopenia was found to have a statistically significant positive association with the outcome of CL, but the temporal relationship between the exposure and outcome was not established and it is therefore possible thrombocytopenia was diagnosed after the outcome of CL had occurred.

Summary

There is a very low quality level of evidence to support associations between either immune-mediated disease or infection with a vector-borne infection and the outcome of CL.

Discussion

One of the primary objectives for this review was to determine the overall quality of evidence published in the veterinary literature relating to associations between exposure variables and the outcome of CL based on GRADE criteria.[9] Though the current body of evidence for one exposure variable, dog breed, was found to be of high quality, the majority of exposure variables examined belong to a body of evidence that is of moderate or low quality. This was due in large part to inconsistent use of both study design and statistical methodology utilized to examine associations and was complicated by incomplete reporting. For example, there is an abundance of evidence in the published literature that suggests an association between dog breed and the outcome of CL exists, but the lack of consistency between studies in regards to use of study design, comparison group, and statistical analyses does not allow for direct comparison of specific risk estimates for individual dog breeds calculated in different studies. Thus, the specific exposure variables examined in the studies included in this review may in fact be strongly associated with the outcome of CL, but the heterogeneity of the study designs does not allow for synthesis of the individual results.

Another complication we discovered when attempting to synthesize information regarding specific exposure variables and the outcome of CL was the limited quantity of primary research on this topic available in the veterinary literature. In fact, only the intrinsic exposure variables analyzed (breed, age, sex, and neuter status) had >10 separate analyses reported in the literature. Each of the extrinsic exposure variables included in this review (environmental chemicals and pollutants, radiation, and diagnosis with other diseases or infections) had <10 individual analyses reported in the literature. The lack of replication of study results is, in

general, a limitation to the interpretation of and confidence in the effect estimates included in a body of evidence.

A potential limitation of the search methodology that may have contributed to the overall quantity or quality of research collected in this review was the broad search terminology utilized. Though this search method did capture an extensive list of studies investigating a variety of risk factors reported in the literature it is possible that relevant literature was not included in the initial screening due to the paucity of terms utilized. The hierarchical structure of MeSH terminology used by the National Library of Medicine to index articles allows for a greater catchment of articles by using broader terms (terms located towards the top of the hierarchy), but studies indexed using terminology specific to a particular study design or research area may be missed when only broad terms are utilized for the literature search. For instance, if examining only one risk factor for CL at a time one might include terminology related to the types of methods currently utilized to investigate the specific association (e.g., searching for immunophenotyping when investigating breed as a risk) which would not be included when searching for all risks associated with the outcome of disease. In addition, no differentiation was made between different subtypes of CL in the literature search and different human lymphoma subtypes have been shown to have differing etiologies.[52-54] In future investigations it may prove helpful to limit reviews to only one specific risk factor for CL rather than reviewing all risks factors simultaneously. It may also be of benefit to limit the outcome of interest to only one subtype of CL (e.g., B-cell lymphoma).

Based upon the results of this review it is clear that more epidemiologic research surrounding the etiology of CL is necessary in order to produce more precise measures of effect for exposure variables related to the outcome of CL. Methodological aspects which will be

important for future studies to address include use of adequate sample size to detect associations, use of comparison groups that are unlikely to lead to biased estimates of effect, and appropriate use of statistical methods that account for potential confounders and effect modifiers in the analysis. Furthermore, adherence to reporting guidelines such as STROBE[55] or CONSORT[56] will ensure reporting transparency and completeness, which is necessary in order to allow for further synthesis of results to occur through ongoing use of systematic reviews.

Supplementary **Table 1.** List of the 41 excluded full-text manuscripts and reason for exclusion

Reference	Reason for exclusion
Benjamin SA *et al.* 1981	Abstract related to published work included in review
Zagarins *et al.* 2008	Abstract related to published work included in review
Blackwood L *et al.* 2004	Case report
Brunker JD *et al.2007*	Case report
Richards HG *et al.* 2001	Disease outcome not lymphoma
Sinibaldi KS *et al.* 1976	Disease outcome not lymphoma
Carlo GL *et al.* 1992	Evaluation of another study
Bronden LB *et al.* 2010	Did not include risk factor analysis
Dobson JM *et al.* 2002	Did not include risk factor analysis
Edwards MD *et al.* 1993	Did not include risk factor analysis
Fosmire SP *et al.* 2007	Did not include risk factor analysis
Gentilini F *et al.* 2009	Did not include risk factor analysis
Greenlee PG *et al.* 1990	Did not include risk factor analysis
Grindem CB *et al.* 1994	Did not include risk factor analysis
Hipple AK *et al.* 2003	Did not include risk factor analysis
Hofer J *et al.* 2011	Did not include risk factor analysis
Kaneko N *et al.* 2009	Did not include risk factor analysis
Keller RL *et al*. 2004	Did not include risk factor analysis
Knottenbelt C 1992	Did not include risk factor analysis
Kristensen A 1994	Did not include risk factor analysis
Lee JJ *et al.* 1996	Did not include risk factor analysis
Liao AT *et al.* 2006	Did not include risk factor analysis
MacVean DW *et al.* 1978	Did not include risk factor analysis
Mortarino M *et al.* 2010	Did not include risk factor analysis
Reynolds PM *et al*. 1994	Did not include risk factor analysis
Teske E *et al.* 1994	Did not include risk factor analysis
Arnesen K *et al.* 2000	Not in English

Enriquez BA *et al.* 2009	Not in English
Lecoindre P *et al.* 1997	Not in English
Walter JH *et al.* 1997	Not in English
Bronden LB *et al.* 2007	Review
Bukowski JA *et al.* 1997	Review
Gandhi R *et al.* 2000	Review
Jowa L *et al.* 2011	Review
Kelsey JL *et al.*1998	Review
Modiano JF *et al.* 2008	Review
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van der Schalie WH *et al.* 1999	Summary paper

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Chapter 2 - Multicenter case-control investigation of risks associated with development of canine lymphoma in North America: 18,826 cases (1990-2009)[2]

Overview

Background: Canine lymphoma (CL) is considered to be a valid model for the study of non-Hodgkin's lymphoma (NHL) in humans. The incidence of NHL in humans has increased worldwide since the 1960s, yet the causal factors for this disease remain largely unknown in both species. Determining an appropriate dog breed model for use in risk factor analyses may prove helpful in gaining a greater understanding of causal factors associated with the outcome of lymphoma in both species.

Objective: To investigate associations between breed and other intrinsic risk factors and the occurrence of CL in order to determine the most appropriate dog breed model for use in NHL research.

Animals: Dogs (n=67,712) admitted to one of 23 veterinary teaching hospitals in North America.

Methods: This retrospective case-control study included dogs diagnosed with CL at veterinary teaching hospitals (VTH) in the United States and Canada between 1990 and 2009, as identified using the Veterinary Medical Database (VMDB). A comparison group was randomly selected from a restricted group of dogs diagnosed with any musculoskeletal condition in the same population of dogs. The association between intrinsic factors and the diagnosis of CL was estimated using logistic regression models.

Results: There is inherent risk associated with dog breed and the outcome of CL with some breeds, such as Scottish terriers, being at increased risk of developing disease and other breeds,

[2]A. Ruple-Czerniak, P. S. Morley

such as Dachshunds, being at low risk of developing disease. Neutered dogs are at increased risk of developing CL as compared to intact female dogs.

Conclusions and clinical importance: The proportion of cases of CL diagnosed in dogs at VTHs has increased, which is similar to trends seen in the human population. Identification of individual dog breeds with an increased risk of developing CL offers the opportunity to localize genetic abnormalities that have been difficult to identify in human populations.

Introduction

Canine lymphoma (CL) is one of the most common life-threatening cancers diagnosed in dogs, accounting for up to 24% of all malignancies and 80% of all hematopoietic cancers.[1-4] The incidence of the analogous disease in humans, non-Hodgkin's lymphoma (NHL), has increased worldwide since the 1970s, and yet the etiology of the disease remains largely unknown in both species despite the frequency of occurence.[2,5-14] Dogs have been proposed as a model for NHL because it has been demonstrated that the pathogenetic basis for development of CL and NHL are similar and may in fact be ancestrally retained (i.e., genetically predisposed) in both species.[1,2,7,15,16] In addition, dogs share many environmental risk factors with humans and the accelerated aging process in dogs results in more rapid tumor progression than is seen in humans.[1-4,15,17] While there may be a generally high risk for this disease among dogs, several investigations have been conducted utilizing both breed-specific phenotype association analysis and molecular genetics and have indicated there is a difference in risk of developing CL amongst different breeds of dogs.[11-14,18-22] In order to further characterize appropriate dog models for use in research designed to determine specific genetic aberrations and environmental factors associated with the outcome of NHL in humans we must evaluate which breeds of dogs have an increased incidence of CL.[2]

However, estimation of cancer incidence and prevalence among different dog breeds is particularly difficult to accomplish due to the lack of availability of complete demographic and health registries for pets.[23] The most comprehensive source of information regarding frequency of disease diagnoses in companion animals in North America is the Veterinary Medical Database (VMDB), which includes medically coded data contributed by more than 20 veterinary schools over varying periods since 1964.[24,25] Individual veterinary schools participate by transcribing their medical records into standardized medical nomenclature which are then compiled in the VMDB.[25] In spite of the number and diversity of sources from which data are collected, the population of animals included in the VMDB may not be fully representative of the entire population of animals within North America.[25] Consequently, use of VMDB data to calculate incidence or prevalence would result in biased estimates due to the incomplete detection of all cancer cases in dogs in the US, admission rate biases, and lack of demographic information about the entire population at risk.[25] However it is still possible to consider risk factors for CL, including breed, by comparing different breeds against an internal reference category. As such, choosing an appropriate reference group is critical to the external validity and generalizability of the results.[26] The purpose of this study was to investigate associations between the host factors breed and sex and the occurrence of CL.

Materials and Methods

Overview

A retrospective case-control study design was utilized to examine associations between exposure variables and the outcome of CL. The VMDB was used to identify dogs diagnosed with CL at veterinary teaching hospitals in the United States and Canada. A comparison group

was selected from the same population of dogs. The association between risk factors and the diagnosis of CL was estimated using logistic regression models.

Study population

Information was obtained from VMDB[24] regarding all dogs visiting participating veterinary teaching hospitals between January 1, 1990 and December 31, 2009. These data were categorized by the managers of VMDB based upon demographic information (breed, sex status, age, year of admission, and admitting institution) and tabulated on the basis of the presence or absence of a diagnosis of CL. Standardized diagnostic coding for CL includes all subtypes of the disease, but does not include other lymphoproliferative disorders. Individual dogs were included in the dataset only once.

Case selection

Dogs diagnosed with CL at any of the 23 veterinary teaching hospitals that participated in the VMDB between January 1, 1990 and December 31, 2009 were included as cases in this study. A dog was considered to have a diagnosis of CL if the diagnosis was coded and entered into the VMDB according to the Standard Nomenclature of Veterinary Diseases and Operations (SNVDO) or the Systematized Nomenclature of Medicine (SNOMED), the two medical coding systems utilized by VMDB during the study time period.

Control selection

A comparison group was comprised of dogs with a recorded diagnosis of any orthopedic or musculoskeletal condition and the absence of the diagnosis of any neoplastic disorders. Within the list of eligible dogs identified at each institution, up to 4 dogs were randomly selected for each case dog identified, restricted on the basis of admitting institution, age (either within the

same SNVDO age category or older), and year of admission (within 2 years either before or after the case dog). Each control dog was included only once in the dataset.

Data extraction

Information regarding breed, weight, age, sex status, year of admission, admitting institution, and discharge status was collected for each case and control animal included in this study. Individual dog breeds were included in the database if there were ≥50 dogs of that breed within the study population. Breeds with <50 animals in the study population were combined based upon the American Kennel Club (AKC) breed grouping system (Herding, Hound, Non-Sporting, Sporting, Terrier, Toy, Working, and Foundation Stock Service). Dogs belonging to breeds not recognized by the AKC were combined into a single category (Other purebred dogs not recognized by the AKC) to facilitate analysis.

Records entered in VMDB using SNOMED, the current medical coding nomenclature utilized in both human and veterinary healthcare systems, included exact numerical values for weight (in kilograms) and age (in years). However, records entered in VMDB using SNVDO, the historic medical coding system, include categorical ranges for weight (in pounds) and animal age (in years) rather than including an exact numerical value for these variables. For the purpose of consistency within the dataset, individual entries for weight and age collected using SNOMED were categorized using the coding ranges established within SNVDO. Weight categories consisting of ranges less than 15 pounds (6.8 kg) were collapsed into a single variable to facilitate analysis. Likewise, age categories consisting of ranges less than 1 year were collapsed into a single variable. Discharge status was divided into two categories (alive and died/subjected to euthanasia).

Data analysis

Information retrieved from the VMDB was summarized by calculating descriptive statistics and frequency distributions were evaluated. Stratum-specific proportions of affected dogs were calculated using the frequency data collected from all dogs admitted to participating institutions. Multivariable logistic regression was used to examine associations between two exposure variables (breed and sex/neuter status) and the outcome of CL. Two separate unconditional logistic regression models were constructed using standard statistical software.[a] This modeling method allows for the greatest gain in power and efficiency for frequency-matched case-control studies.[27] One model used Labrador retrievers as the referent breed for analysis and the other model used mixed breed dogs as the reference group. The critical α for retention for both models was ≤0.05. The variables considered during the restricted selection of control dogs (age, admitting institution, and year of admission) were included in the models regardless of *P*-values. Two-way interactions between breed and sex status were evaluated. Odds ratios and 95% confidence intervals were calculated using the results of the logistic regression models. A third multivariable logistic regression model was constructed to examine the relationship between diagnosis with CL and death or euthanasia in the study population. The critical α for retention for this model was ≤0.05. The variables considered during the restricted selection of control dogs (age, admitting institution, and year of admission) were included in the model regardless of *P*-values. Odds ratios and 95% confidence intervals were calculated using the results of the logistic regression model.

Results

Characteristics of all dogs

A total of 23 institutions contributed data to VMDB for the 20 year period between January 1, 1990 and December 31, 2009, but only 7 (30.4%) of those hospitals contributed

information throughout the entire study period. Six (26.1%) hospitals contributed data for 10-15 years of the study period, 6 (26.1) hospitals contributed data for 5-10 years, and 4 (17.4%) hospitals contributed data for <5 years. A total of 714,036 individual dogs were admitted to participating hospitals at least once during the study period. Of those dogs, 18,826 (2.6%) were diagnosed with lymphoma. Each of the participating institutions reported dogs diagnosed with lymphoma, but the hospital-specific proportion of dogs affected varied from <1% up to 5% of the total number of dogs admitted to individual hospitals (Table 2.1). The SNVDO age category with the highest proportion of affected dogs (4.90%) was 7 to 10 years and the SNVDO weight category with the highest proportion of affected dogs (4.33%) was 75 to 100 pounds (Table 2.1).

Table 2.1. Characteristics of dogs admitted to VMDB participating institutions between 1990 and 2009.

Variable	Category	Total number of dogs admitted to VTH (n=714,036)		Dogs diagnosed with lymphoma (n=18,826)		Proportion of dogs affected (%)
Institution	Auburn University	25,162	(3.5)	451	(2.4)	1.79
	Colorado State University	92,904	(13.0)	3,001	(15.9)	3.23
	Cornell University	6,707	(0.9)	128	(0.7)	1.91
	Iowa State University	24,582	(3.4)	374	(2.0)	1.52
	Kansas State University	31,169	(4.4)	512	(2.7)	1.64
	Louisiana State University	10,638	(1.5)	123	(0.7)	1.16
	Michigan State University	100,210	(14.0)	2,008	(10.7)	2.00
	Mississippi State University	1,608	(0.2)	6	(0.03)	0.37
	North Carolina State University	1,526	(0.2)	48	(0.3)	3.15
	Ontario Veterinary College	5,646	(0.8)	184	(1.0)	3.26
	Purdue University	48,915	(6.9)	2,442	(13.0)	4.99
	Texas A&M University	40,948	(5.7)	661	(3.5)	1.61
	The Ohio State University	43,923	(6.2)	2,036	(10.8)	4.64
	Tuskegee University	2,166	(0.3)	3	(0.02)	0.14
	University of Florida	44,244	(6.2)	751	(4.0)	1.70
	University of Georgia	40,051	(5.6)	901	(4.8)	2.25
	University of Illinois	60,353	(8.5)	2,171	(11.5)	3.60

	University of Minnesota	35,257	(4.9)	1,390	(7.4)	3.94
	University of Missouri	18,845	(2.6)	175	(0.9)	0.93
	University of Pennsylvania	31,362	(4.4)	403	(2.1)	1.28
	University of Tennessee	16,611	(2.3)	246	(1.3)	1.48
	University of Wisconsin	23,683	(3.3)	597	(3.2)	2.52
	Virginia-Maryland Regional	7,525	(1.1)	215	(1.1)	2.86
Year	1990-1994	283,605	(39.7)	5,170	(27.5)	1.82
	1995-1999	176,602	(24.7)	4,875	(25.9)	2.76
	2000-2004	141,073	(19.8)	4,901	(26.0)	3.47
	2005-2009	112,756	(15.8)	3,880	(20.6)	3.44
Age	Less than 1 year	142,652	(20.0)	116	(0.6)	0.08
	1 to 2 years	72,944	(10.2)	281	(1.5)	0.39
	2 to 4 years	114,136	(16.0)	1,412	(7.5)	1.24
	4 to 7 years	139,392	(19.5)	5,434	(28.9)	3.90
	7 to 10 years	123,805	(17.3)	6,070	(32.2)	4.90
	10 to 15 years	111,437	(15.6)	5,403	(28.7)	4.85
	Greater than 15 years	8,739	(1.2)	110	(0.6)	1.26
Weight	Less than 15 pounds	117,366	(16.4)	846	(4.5)	0.72
	15 to 30 pounds	95,750	(13.4)	2,065	(11.0)	2.16
	30 to 50 pounds	94,062	(13.2)	2,793	(14.8)	2.97
	50 to 75 pounds	115,648	(16.2)	4,398	(23.4)	3.80
	75 to 100 pounds	70,891	(9.9)	3,073	(16.3)	4.33
	Greater than 100 pounds	30,968	(4.3)	1,258	(6.7)	4.06
	Unknown	170,525	(23.9)	4,393	(23.3)	2.58

The proportions of affected dogs estimated for each individual year during the study period varied from approximately 1.5% to about 4%, with generally increasing proportions of affected dogs being reported in later years (Fig. 2.1). Individual-level data were not obtained for the entire population of dogs admitted to the VMDB during the study time period and therefore these proportions could not be adjusted for breed, age, and sex. Thus, this trend was not tested statistically.

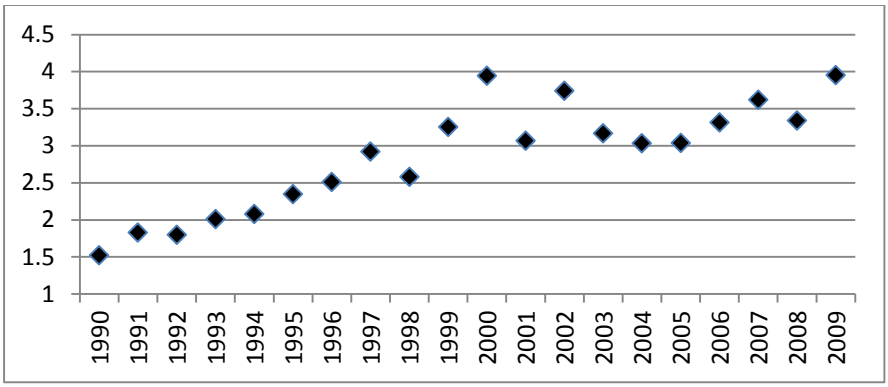

Figure 2.1. Unadjusted proportion of lymphoma cases diagnosed in all dogs seen in VMDB participating institutions from 1990 to 2009.

Characteristics of case-control study population

All 18,826 dogs diagnosed with lymphoma during the study period were enrolled in the case-control study as were 48,886 dogs diagnosed with orthopedic conditions. The majority of the population was identified in computer records as purebred dogs (79.1%) with more than 350 distinct breeds represented; 90 of those breeds had ≥50 individual dogs in the study population. Crude proportions of CL within individual breeds calculated from the entire VMDB population varied from less than 1% to nearly 7% (Table 2.2). Purebred dogs within the AKC Toy Group had the lowest average proportion of CL (0.51%) while the average proportion of

Table 2.2. Breeds of all dogs included in the case control study [n, (%)].

Breed	Total CC Study Population (n=67,712)	Dogs with lymphoma (n=18,826)	Comparison group (n=48,886)	Proportion (%) of dogs affected*	Average Proportion (%)
Purebred dogs, AKC Sporting group					1.93
Labrador retriever	6,969 (10.3)	1,420 (7.5)	5,549 (11.4)	1.36	
Golden retriever	4,887 (7.2)	2,432 (12.9)	2,455 (5.0)	3.94	
American Cocker Spaniel	2,304 (3.4)	738 (3.9)	1,566 (3.2)	1.83	
English Springer Spaniel	645 (1.0)	199 (1.1)	446 (0.9)	1.68	
Brittany	374 (0.6)	47 (0.2)	327 (0.7)	0.73	
German shorthaired pointer	360 (0.5)	55 (0.3)	305 (0.6)	0.90	
Weimaraner	293 (0.4)	72 (0.4)	221 (0.5)	1.50	

Chesapeake Bay retriever	264 (0.4)	56 (0.3)	208 (0.4)	1.85	
English setter	238 (0.4)	67 (0.4)	171 (0.3)	1.65	
Irish setter	217 (0.3)	55 (0.3)	162 (0.3)	1.51	
Pointer	158 (0.2)	51 (0.3)	107 (0.2)	1.79	
Gordon setter	146 (0.2)	68 (0.4)	78 (0.2)	2.82	
Vizsla	110 (0.2)	51 (0.3)	59 (0.1)	3.21	
German wirehaired pointer	103 (0.2)	19 (0.1)	84 (0.2)	1.98	
English Cocker spaniel	92 (0.1)	22 (0.1)	70 (0.1)	1.43	
Flat-coated retriever	90 (0.1)	33 (0.2)	57 (0.1)	3.21	
Other purebred dogs, AKC Sporting group	180 (0.3)	47 (0.2)	133 (0.3)	1.40	
Purebred dogs, AKC Working group					1.95
Rottweiler	2,318 (3.4)	759 (4.0)	1,559 (3.2)	2.32	
Doberman Pinscher	1,768 (2.6)	353 (1.9)	1,415 (2.9)	1.95	
Boxer	1,519 (2.2)	866 (4.6)	653 (1.3)	4.46	
Great Dane	560 (0.8)	109 (0.6)	451 (0.9)	1.10	
Siberian Husky	551 (0.8)	144 (0.8)	407 (0.8)	1.20	
Newfoundland	345 (0.5)	54 (0.3)	291 (0.6)	0.72	
Saint Bernard	324 (0.5)	90 (0.5)	234 (0.5)	1.90	
Samoyed	293 (0.4)	45 (0.2)	248 (0.5)	0.72	
Alaskan Malamute	292 (0.4)	73 (0.4)	219 (0.4)	1.90	
Bernese mountain dog	251 (0.4)	97 (0.5)	154 (0.3)	3.33	
Mastiff	239 (0.4)	47 (0.2)	192 (0.4)	1.43	
Akita	224 (0.3)	18 (0.1)	206 (0.4)	0.54	
Great Pyrenese	218 (0.3)	25 (0.1)	193 (0.4)	0.74	
Bullmastiff	160 (0.2)	90 (0.5)	70 (0.1)	3.55	
Standard Schnauzer	126 (0.2)	46 (0.2)	80 (0.2)	1.78	
Giant Schnauzer	91 (0.1)	44 (0.2)	47 (0.1)	4.46	
Portuguese water dog	59 (0.1)	25 (0.1)	34 (0.1)	1.96	
Other purebred dogs, AKC Working group	120 (0.2)	24 (0.1)	96 (0.2)	1.08	
Purebred dogs, AKC Herding group					1.96
German Shepherd dog	3,147 (4.6)	559 (3.0)	2,588 (5.3)	1.31	
Shetland sheepdog	1,075 (1.6)	444 (2.4)	631 (1.3)	2.29	
Australian shepherd	649 (1.0)	211 (1.1)	438 (0.9)	1.84	
Collie	544 (0.8)	217 (1.2)	327 (0.7)	2.12	
Border Collie	522 (0.8)	157 (0.8)	365 (0.7)	2.05	
Australian Heeler	290 (0.4)	55 (0.3)	235 (0.5)	1.07	
Pembroke Welsh Corgi	285 (0.4)	89 (0.5)	196 (0.4)	2.85	
Old English Sheepdog	250 (0.4)	78 (0.4)	172 (0.4)	2.21	

Bouvier des Flandres	209 (0.3)	99 (0.5)	110 (0.2)	3.59	
Cardigan Welsh Corgi	135 (0.2)	40 (0.2)	95 (0.2)	1.88	
Belgian Malinois	56 (0.1)	5 (<0.1)	51 (0.1)	0.31	
Other purebred dogs, AKC Herding group	173 (0.3)	64 (0.3)	109 (0.2)	2.00	
Purebred dogs, AKC Hound group					1.74
Standard Dachshund	2,619 (3.9)	95 (0.5)	2,524 (5.2)	0.41	
Beagle	1,012 (1.5)	318 (1.7)	694 (1.4)	2.01	
Miniature Dachshund	802 (1.2)	23 (0.1)	779 (1.6)	0.31	
Basset hound	787 (1.2)	405 (2.2)	382 (0.8)	4.40	
Greyhound	568 (0.8)	100 (0.5)	468 (1.0)	1.28	
Irish Wolfhound	149 (0.2)	42 (0.2)	107 (0.2)	2.39	
Rhodesian ridgeback	146 (0.2)	69 (0.4)	77 (0.2)	2.54	
Norwegian Elkhound	95 (0.1)	25 (0.1)	70 (0.1)	1.67	
Afghan hound	76 (0.1)	23 (0.1)	53 (0.1)	1.82	
Treeing Walker coonhound	74 (0.1)	22 (0.1)	52 (0.1)	1.81	
Whippet	72 (0.1)	15 (0.1)	57 (0.1)	1.04	
Other purebred dogs, AKC Hound group	322 (0.5)	87 (0.5)	235 (0.5)	1.15	
Purebred dogs, AKC Toy group					0.51
Shih tzu	852 (1.3)	187 (1.0)	665 (1.4)	1.08	
Yorkshire terrier	844 (1.2)	106 (0.6)	738 (1.5)	0.64	
Miniature Poodle	769 (1.1)	88 (0.5)	681 (1.4)	0.65	
Toy Poodle	548 (0.8)	31 (0.2)	517 (1.1)	0.27	
Chihuahua	473 (0.7)	48 (0.3)	425 (0.9)	0.01	
Pomeranian	446 (0.7)	43 (0.2)	403 (0.8)	0.51	
Pekingese	398 (0.6)	16 (0.1)	382 (0.8)	0.27	
Maltese	342 (0.5)	63 (0.3)	279 (0.6)	0.77	
Pug	279 (0.4)	46 (0.2)	233 (0.5)	0.68	
Miniature Pinscher	164 (0.2)	18 (0.1)	146 (0.3)	0.45	
Cavalier King Charles	63 (0.1)	5 (<0.1)	58 (0.1)	0.18	
Italian greyhound	63 (0.1)	18 (0.1)	45 (0.1)	0.74	
Papillon	54 (0.1)	10 (0.1)	44 (0.1)	0.40	
Other purebred dogs, AKC Toy group	165 (0.2)	28 (0.1)	137 (0.3)	0.50	
Purebred dogs, AKC Non-sporting group					1.27
Dalmatian	519 (0.8)	99 (0.5)	420 (0.9)	0.77	
Lhasa apso	484 (0.7)	86 (0.5)	398 (0.8)	1.05	
Standard Poodle	468 (0.7)	138 (0.7)	330 (0.7)	1.72	
Bulldog	406 (0.6)	154 (0.8)	252 (0.5)	1.45	
Bichon frise	382 (0.6)	49 (0.3)	333 (0.7)	0.66	

Chow Chow	340 (0.5)	58 (0.3)	282 (0.6)	0.73	
Chinese Shar-Pei	331 (0.5)	194 (1.0)	137 (0.3)	2.86	
Boston terrier	243 (0.4)	43 (0.2)	200 (0.4)	0.62	
Keeshond	156 (0.2)	36 (0.2)	120 (0.2)	1.33	
American Eskimo dog	122 (0.2)	38 (0.2)	84 (0.2)	1.11	
Schipperke	80 (0.1)	22 (0.1)	58 (0.1)	1.87	
Other purebred dogs, AKC Non-sporting group	122 (0.2)	24 (0.1)	98 (0.2)	1.10	
Purebred dogs, AKC Terrier group					1.98
Miniature Schnauzer	780 (1.2)	353 (1.9)	427 (0.9)	1.53	
Scottish terrier	443 (0.7)	353 (1.9)	90 (0.2)	6.69	
Airedale terrier	388 (0.6)	164 (0.9)	224 (0.5)	3.01	
American Staffordshire terrier	378 (0.6)	129 (0.7)	249 (0.5)	1.63	
West Highland white terrier	333 (0.5)	135 (0.7)	198 (0.4)	1.91	
Russell terrier	240 (0.4)	50 (0.3)	190 (0.4)	0.65	
Cairn terrier	183 (0.3)	70 (0.4)	113 (0.2)	1.78	
Wire Fox terrier	143 (0.2)	48 (0.3)	95 (0.2)	1.72	
Bull terrier	108 (0.2)	53 (0.3)	55 (0.1)	1.83	
Rat terrier	70 (0.1)	14 (0.1)	56 (0.1)	0.85	
Soft-coated wheaten terrier	64 (0.1)	10 (0.1)	54 (0.1)	0.75	
Other purebred dogs, AKC Terrier group	232 (0.3)	83 (0.4)	149 (0.3)	1.42	
Other dog groups					1.76
Other purebred dogs, AKC FSS group	58 (0.1)	18 (0.1)	40 (0.1)	1.87	
Other purebred dogs, not recognized by the AKC	79 (0.1)	11 (0.1)	68 (0.1)	1.78	
Mixed breed dogs	14,155 (20.9)	4,104 (21.8)	10,051 (20.6)	1.63	

*Calculated from data collected from all 714,036 dogs seen at VMDB participating hospitals between 1990 and 2009

affected dogs in other AKC groups was typically closer to 2% (Table 2.2). The case-control population was evenly distributed among male (50.9%) and female (48.7%) dogs and the majority of the population, regardless of sex, was neutered (75.1%). When data from all dogs seen at VMDB

participating institutions was grouped by sex status, intact female dogs had the lowest proportion of dogs with CL (0.95%) and neutered male dogs had the highest proportion of dogs with CL (3.77%) (Table 2.3). Dogs diagnosed with CL were nearly 3 times more likely (OR: 2.78; 95% CI: 2.64, 2.94; *P*- value<0.001) to die or be subjected to euthanasia than were the animals in the control group when controlling for the effects of breed, sex status, age, hospital and year of admission.

Table 2.3. Sex status of all dogs included in the case control study [n, (%)].

Category	Total Study Population (n=67,712)	Dogs with lymphoma (n=18,826)	Comparison group (n=48,886)	Proportion of dogs affected* (%)
Female, intact	5,039 (7.4)	1,199 (6.4)	3,840 (7.9)	0.95
Female, neutered	27,918 (41.2)	7,644 (40.6)	20,274 (41.5)	3.22
Male, intact	11,570 (17.1)	3,178 (16.9)	8,392 (17.2)	1.90
Male, neutered	22,911 (33.8)	6,753 (35.9)	16,158 (33.1)	3.77
Unknown	274 (0.4)	52 (0.3)	222 (0.5)	0.24

*Calculated using data collected from all 714,036 dogs seen at participating hospitals between 1990 and 2009

Risk factors for canine lymphoma

Variables included in each multivariable model were breed, sex status, age of the animal, year and hospital of admittance. Interaction terms for main effects were not significant when included in the final models. When controlling for the effects of sex, age, hospital and year of admission, 46 breeds or breed groups were found to be at increased risk of developing CL with 28 of those breeds being significantly different from both referent groups (Fig. 2.2). The breed with the highest odds of developing CL was the Scottish terrier with an OR of 14.45 (95% CI: 11.37, 18.37) as compared to Labrador retrievers and 9.46 (95% CI: 7.48, 11.96) as compared to mixed breed dogs. Thirty-nine breeds or breed groups were found to be at decreased risk of developing CL with 18 of those breeds being significantly different from both referent groups

(Fig. 2.2) when controlling for the effects of other factors included in the model. The breed with the lowest odds of developing CL was the Miniature Dachshund with a greater than 8-fold

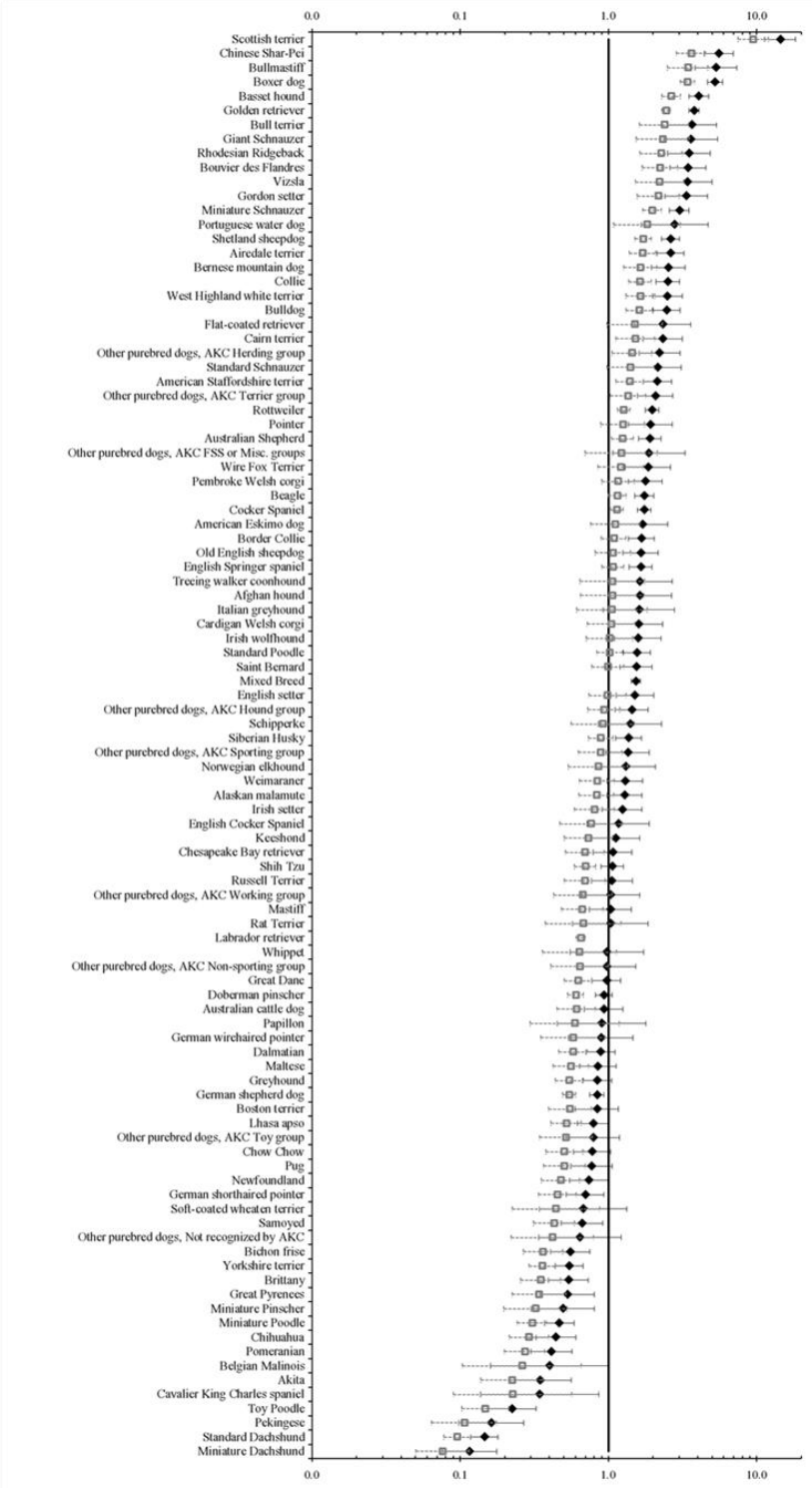

Figure 2.2. Odds (OR) of developing CL as associated with breed when controlling for the effects of age and sex of the animal, institution of record, and year of diagnosis as compared to Labrador retrievers (•) and mixed breed dogs (□).

decreased risk of developing CL (OR: 0.12; 95% CI: 0.08, 0.18) as compared to Labrador retrievers and a greater than 12-fold decreased risk of developing CL (OR: 0.08; 95% CI: 0.05, 0.12) as compared to mixed breed dogs. Fifteen breeds and breed groups were found to have no statistical difference in risk from either reference group (Fig. 2.2). Neutered dogs, regardless of sex, and intact male dogs were found to be at increased risk of developing CL as compared to intact female dogs (Table 2.4) when controlling for other risk factors.

Table 2.4. Association between sex status and risk of developing lymphoma when controlling for the effects of age of the animal, admitting institution, and year of diagnosis. *P*-value < 0.001

	Odds Ratio	95% CI	
Female, intact	Reference		
Female, neutered	1.15	1.07,	1.24
Male, intact	1.12	1.04,	1.22
Male, neutered	1.27	1.18,	1.37
Unknown	0.71	0.52,	0.98

Discussion

The results of this study show there are differences in risk associated with developing CL amongst both specific breeds and AKC breed groups. These data were compared to two distinct reference groups in order to obtain specific point estimates of effect and more than half of the breeds found to have a significantly different risk of developing CL as compared to one or both of the referent groups in this study (52/85) had not been previously reported in the veterinary literature. However, the risk of developing any subtype of CL in different breeds is comparative

regardless of which breed is chosen as a reference group during modeling. For example, the risk of developing CL is higher in Boxer dogs than in Chihuahuas irrespective of which specific reference group is chosen for analysis. It is important to note that there was a significant difference detected between odds of having CL amongst our two reference groups, resulting in more conservative point estimates being calculated from the model utilizing mixed breed dogs as the referent. This illustrates the importance of careful consideration of which breed is chosen as referent when interpreting results of analyses in the published literature.

Knowledge of specific breed predispositions towards developing CL is beneficial because the architecture of the canine genome has remained fairly constant across breeds despite the continued use of selective breeding practices.[2,16,28] In fact, there is evidence that suggests there are areas within the mammalian genome in which recurrent chromosomal aberrations related to the occurrence of blood cancers are conserved between humans and dogs.[16] Despite the many advances in technology related to the study of genetics, these aberrations remain difficult to identify using human study populations due to the high level of genetic heterogeneity.[22,29] The reduced genetic heterogeneity within a single dog breed, allows for more straightforward genetic studies and, in fact, linkage disequilibrium has been shown to be up to 100-fold greater in dogs than humans.[28,30] Therefore, identification of individual dog breeds with an increased risk of developing CL offers the opportunity to localize genetic abnormalities that have been difficult to identify in human populations.[31] We were not able to analyze associations between breed risk and outcomes of different subtypes of CL with these data. It is therefore possible the difference in risk detected with this study for a dog breed is actually a risk associated with development of only one subtype of CL. Further analysis characterizing breed-specific phenotype associations

would likely be helpful in determining which breeds of dogs should be utilized for genomic analysis in the future.

When examining the proportion of affected dogs over time in this study population it appears as though there is an increase in the burden of disease within the dog population in North America. Were this to be accurate, it would mirror the increased incidence of NHL diagnosed in humans in the United States during the same timeframe.[7-9] However, caution should be taken in interpreting these results as the reported proportions are not adjusted for the effects of breed, age of the animal, year of diagnosis, or other potential confounders. Indeed, these findings may reflect improvement in veterinary diagnostic practices, a difference in the medical specialties being offered in VTHs during the study time period, or a change in the number of dogs in the population that belong to breeds at higher risk of developing CL.

In humans, NHL is diagnosed more commonly in men than in women with 23.9 new cases per 100,000 men and 16.3 new cases per 100,000 women being diagnosed each year in the United States.[32] This suggests that sex hormones may be associated with the outcome of NHL in humans. In this study, the association detected between neutering status and the outcome of CL also suggests an association between sex hormones and development of disease. A reported association between sex hormones and the outcome of CL in dogs has been previously published,[33] but the methods used here adjusted for the effects of potential confounders. Other research studies have shown associations between neutering status and increased risk of other types of cancers in dogs, including osteosarcoma, transitional call carcinoma, and prostate cancer.[34-37]

It is important to recognize the possibility that referral bias may exist in the data collected through the VMDB as all patient information collected comes from large, multispecialty referral

and teaching hospitals.[25] For this reason, extrapolation of the results of this study to dog populations other than those seen in referral centers within North America may not be possible as we are unable to determine how representative our study population is to other populations of dogs. Inference of these results is further complicated by the fact that not all hospitals contributed data during the entire study period, which may have resulted in a study population that is unequally represented geographically. Furthermore, risk factor analysis was limited to the variables recorded consistently within the VMDB and other potential risk factors for CL, such as diet and environmental factors, were not accounted for in the analysis. This may have resulted in biased estimates of effect.

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Chapter 3 - Differences in the geographic distribution of lymphoma subtypes in Golden retrievers in the United States[3]

Overview

Background: Malignant lymphomas in both dogs and humans are a heterogeneous group of diseases believed to be associated with both environmental and genetic etiologic components. In humans, distribution of lymphoma subtypes have been shown to vary based upon geographic locations. This suggests there are differences in environmental etiologic factors in different geographic locations.

Objective: The purpose of this study was to examine differences in the geographic distribution of two distinct subtypes of CL, B-cell lymphoma (BCL) and T-zone lymphoma (TZL), in the United States while accounting for heritable risks associated with the outcome of disease.

Animals: A total of 454 Golden retrievers diagnosed with either BCL or TZL using flow cytometry at the Clinical Immunology Laboratory at Colorado State University (CSU-CI) were included in this study.

Methods: Cross sectional study. Samples submitted to the CSU-CI between January 1, 2007 and April 30, 2014 were analyzed using a Coulter XL flow cytometer. Subjects were categorized according to the zip code location of the veterinary hospital which submitted the sample, using the US Census Divisions. Associations between geographic areas of the US and the phenotypic variant of lymphoma diagnosed were examined using multivariable logistic regression.

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Results: There is a difference in the geographic distribution of BCL and TZL subtypes of CL diagnosed in Golden retrievers in the United States with dogs in the northeast (OR =3.4, 95%CI =1.6-7.0) and East North Central regions (OR =12.1, 95%CI =3.6-40.5) being more likely to be diagnosed with TZL as compared to dogs in the Mountain region of the US.

Conclusions: Future work in veterinary medicine related to etiologic investigations of CL should differentiate between specific subtypes of the disease as each subtype may have different etiologies.

Introduction

Canine lymphoma (CL) is the third most commonly diagnosed tumor in dogs[1,2] and is considered to be a valid model for the study of lymphoma in humans.[3-7] Malignant lymphomas are a heterogeneous group of diseases with more than 50 differentiated subtypes of human lymphoma included in the World Health Organization (WHO) revised system of classification for hematopoietic and lymphoid tumors[8] many of which have analogous tumors in dogs.[9,10] Classification of CL subtypes using the World Health Organization (WHO) criteria has been shown to be an accurate tool for use in veterinary medicine, especially when used to identify the most common subtypes of CL, which includes diffuse large B-cell lymphoma (BCL) and T-zone lymphoma (TZL).[9] Differences in clinical presentation of disease, response to treatment, mortality rates, and survival times have been shown to exist between different CL subtypes.[10-16] In addition, it has been shown that some breeds of dogs develop B-cell or T-cell derived lymphoma in disproportionate frequency, suggesting there is heritable risk for developing specific subtypes of CL.[17-20]

Though the complete etiology of CL and human non-Hodgkin's lymphoma (NHL) remains unclear, it is suspected that both genetic and environmental factors contribute to the development of disease.[21-23] In dogs, exposure to specific environmental factors including pollution[20,24,25], tobacco smoke[25], pesticides[26-30], electromagnetic fields,[31] and infection with tick-borne disease[32,33], have been previously investigated. However, most of the published investigations regarding environmental factors as risks for development of CL do not differentiate between risks of developing different subtypes of lymphoma.[34] In humans, however, specific environmental factors have been shown to be associated with the occurrence of different subtypes of lymphoma and the geographic distributions of lymphoma subtypes have been shown to vary widely.[23,35-40] Given the many similarities between CL and NHL, further investigation into the possibility of heterogeneous etiologic factors being associated with the occurrence of different subtypes of CL in dogs is warranted. In order to investigate differing etiologies without measuring specific environmental exposures it is possible to use geographic distribution as a proxy for all environmental exposures in a particular area. However, in order to differentiate between genetic and environmental etiologies, investigations pertaining to this question must account for the possibility of genetic influence in the development of CL either through study design (e.g., through restricted sampling) or in the statistical analysis (e.g., stratification). Considering this, the purpose of this study was to examine differences in the geographic distribution of two distinct subtypes of CL, BCL and TZL, in the United States in Golden retrievers.

Materials and methods

Overview

Golden retrievers diagnosed with either BCL or TZL using flow cytometry were selected for inclusion in this cross-sectional study. Subjects were classified according to the geographic region of the United States from which the sample was submitted, using the zip code data from the referring hospital. Associations between geographic areas of the US and the phenotypic variant of lymphoma diagnosed were examined using multivariable logistic regression.

Study Population

The study population was selected from the database maintained by the Clinical Immunology Laboratory at Colorado State University (CSU-CI). This database includes information regarding signalment, clinical findings and histology reports provided by referring veterinarians on more than 5,000 dogs with a suspected diagnosis of lymphoproliferative disorder. Information regarding sample type (blood or lymph node aspirate) and results of immunophenotyping are also maintained in the database. In order to be eligible for this cross-sectional study, samples must have been collected from a Golden retriever and submitted to the CSU-CI between January 1, 2007 and April 30, 2014. Furthermore, only dogs with diagnoses of BCL or TZL (based upon results of immunophenotyping at the CSU-CI) were eligible for this study. Subjects were categorized according to the zip code location of the veterinary hospital which submitted the sample, using the US Census Divisions (Figure 3.1).[41] Because submissions were sparse for eligible subjects from some regions, to facilitate statistical analyses, adjacent census divisions were grouped for West North and West South Central regions, South Atlantic and East South Central regions, and New England and Middle Atlantic regions. Other data collected from submission forms and recorded in the laboratory database included sex (male / female) and age ($\leq$ 6 yrs, 7-8 yrs, 9-10 yrs, $\geq$ 11 years).

Figure 3.1. US Census Divisions and the 35 states from which samples from study subjects were submitted (gray).

Flow Cytometry

Samples were submitted directly to the CSU-CI or shipped overnight on ice. Samples were refrigerated upon receipt and were analyzed within 72 hours of being collected from the dog. Samples were prepared as described previously.[42,43] Briefly, material from lymph node aspirates arrived at the laboratory in a solution consisting of 10% serum and saline. These samples were centrifuged and suspended in 1mL of lysis buffer (0.15 M NH_4CL, 1 M KHO_3, 0.1 mM Na_2EDTA, 1 N HCL at a pH of 7.2–7.4) for 5 minutes at room temperature. Blood samples were lysed by combining 400 μL of blood with 1mL of lysis buffer for 5 minutes at room

temperature. All samples were centrifuged and lysed a second time and suspended in 200 μL of phosphate buffered saline and 2% fetal bovine serum (PBS-2% FBS). Twenty-five μL of cell suspension and 25 μL of antibody cocktail were added to individual wells in a 96-well plate. Samples remained at room temperature for 15 minutes, were washed twice, and were resuspended in 10 μg/mL of propidium iodide and PBS-2% FBS for dead cell exclusion, and analyzed within an hour using a Coulter XL flow cytometer.

Data Analysis

Demographic information retrieved from the CSU-CI database regarding the case population for this study was summarized by calculating descriptive statistics and frequency distributions. Associations between the geographic location categories of subjects and the phenotypic variant of lymphoma diagnosed were evaluated using multivariable logistic regression, with the outcome of the model being TZL vs BCL (i.e., T-zone y/n).[a] Sex and age of the animal were forced into the model as potential confounders, regardless of *P*-value, as these have been previously associated with the occurrence of lymphoma in dogs.[44-47] The *a priori* critical alpha for statistical testing of the geographic variable was 0.05. Two-way interactions between all three variables included in the model were evaluated. Odds ratios and 95% confidence intervals were estimated using the results of the logistic regression model.

Results

Characteristics of study population

A total of 454 Golden retrievers met inclusion criteria and were enrolled in the study. Samples submitted for immunophenotyping from >50% of the subjects examined were collected from lymph nodes (255, 56.2%) and the lymphoproliferative disease was characterized in the

remaining subjects (196, 43.2%) using blood samples. The majority (89.9%) of B-cell samples were based on lymph node aspirates while the majority (76.7%) of T-zone samples were immunophenotyped using blood (Table 3.1). Most dogs included in the study were >7 yrs-old,

Table 3.1. Characteristics of the 454 Golden retrievers in the study population [n (%)].

Variable	Category	B cell (n=227)	T zone (n=227)	Total (n=454)
Year of diagnosis	2007	28 (12.3)	22 (9.7)	50 (11.0)
	2008	22 (9.7)	17 (7.5)	39 (8.6)
	2009	25 (11.0)	20 (8.8)	45 (9.9)
	2010	34 (15.0)	40 (17.6)	74 (16.3)
	2011	33 (14.5)	29 (12.8)	62 (13.7)
	2012	31 (13.7)	24 (10.6)	55 (12.1)
	2013	38 (16.7)	41 (18.1)	79 (17.4)
	Jan - April 2014	16 (7.0)	34 (15.0)	50 (11.0)
Sample site	Lymph node	204 (89.9)	51 (22.5)	255 (56.2)
	Blood	22 (9.7)	174 (76.7)	196 (43.2)
	Both	1 (0.4)	2 (0.9)	3 (0.7)
Age (in years)	6 or younger	57 (25.1)	9 (4.0)	66 (14.5)
	7 to 8	48 (21.1)	31 (13.7)	79 (17.4)
	9 to 10	48 (21.1)	66 (29.1)	114 (25.1)
	11 and older	30 (13.2)	66 (29.1)	96 (21.1)
	Unknown	44 (19.4)	55 (24.2)	99 (21.8)
Sex	Male, intact	10 (4.4)	16 (7.1)	26 (5.7)
	Male, neutered	109 (48.0)	103 (45.4)	212 (46.7)
	Female, intact	2 (0.9)	1 (0.4)	3 (1.3)
	Female, neutered	103 (45.4)	103 (45.4)	206 (45.4)
	Unknown	3 (1.3)	4 (1.8)	7 (1.5)
US Census Region	Pacific	25 (11.0)	34 (15.0)	59 (13.0)
	Mountain	67 (29.5)	42 (18.5)	109 (24.0)
	West North Central	8 (3.5)	5 (2.2)	13 (2.9)
	West South Central	19 (8.4)	18 (7.9)	37 (8.1)
	East North Central	8 (3.5)	31 (13.7)	39 (8.6)
	East South Central	1 (0.4)	2 (0.9)	3 (1.3)
	New England	13 (5.7)	24 (10.6)	37 (8.1)
	Mid Atlantic	13 (5.7)	30 (13.2)	43 (9.5)

	South Atlantic	73 (32.2)	41 (18.1)	114 (25.1)

with 46.3% (210) of the population being ≥9 yrs-old (Table 3.1). The population was roughly equally divided between male and female dogs and the majority of the population (92.1%) was neutered. Veterinarians submitting samples for study subjects were located in 35 states (Figure 3.1). Nearly half of the study population originated from only 2 of the geographic regions: Mountain (24.0%) and South Atlantic (25.1%) (Table 3.1).

Results of logistic regression modeling

The variables for geographic region and age were statistically significant in the multivariable logistic regression model. Interaction terms for main effects were not significant when included in the model. Controlling for effects of age and sex, the distribution of the two subtypes of lymphoma were not uniformly distributed among the different regions of the United States, with TZL being more common in the Northeastern US and BCL being more common in the Southeast and Western regions (Table 3.2). When compared to dogs in the Mountain region,

Table 3.2. Odds of developing T-zone lymphoma as compared to developing B-cell lymphoma in Golden retrievers

Variable	Category	Odds Ratio	95% CI	*P*-value
Region	New England and Middle Atlantic	3.37	1.61, 7.04	<0.001
	South Atlantic and East South Central	1.10	0.57, 2.13	
	East North Central	12.08	3.60, 40.53	
	West North and South Central	1.12	0.49, 2.56	
	Pacific	1.70	0.73, 3.95	
	Mountain	Reference		
Age	≥ 11 years	16.04	6.70, 38.40	<0.001
	9 to 10 years	8.08	3.50, 18.65	
	7 to 8 years	3.78	1.57, 9.12	
	≤ 6 years	Reference		
Sex	Female	1.25	0.77, 2.03	0.36

Male	Reference

Golden retrievers in the Middle Atlantic and New England areas were >3 times more likely to be diagnosed with TZL (OR =3.4, 95%CI =1.6-7.0) and those in the in the East North Central region were >12 times (OR =12.1, 95%CI =3.6-40.5) more likely to be diagnosed with TZL as compared to Golden retrievers in the Mountain region of the US. Distributions of TZL vs. BCL in the other regions were not significantly different from the Mountain region. Controlling for the effects of geographic distribution and sex, age was positively associated with the occurrence of TZL with dogs ≥11 years being more than 16 times (OR =16.04, 95%CI =6.7-38.4) more likely to develop TZL compared to BCL.

Discussion

This study detected marked differences in the geographic distribution of B-cell and T-zone subtypes of CL diagnosed in Golden retrievers in the United States, based upon patterns of submission to the CSU-CI laboratory. These differences could be related to genetic differences in the dogs, patterns of environmental exposure, or could be related to patterns of submission to the laboratory. It is quite possible that dogs from specific lineages predominate within different geographic regions, influencing the genetic makeup of the majority of dogs within a geographic region and resulting in varying genetic predispositions towards development of one subtype of lymphoma over another. However, studies investigating genetic variation within different dog breeds have shown individual breeds to be genetically isolated,[48-51] likely due to the advent of breed clubs and their imposition of the pedigree barrier, wherein registration of a dog requires both parents be registered members of the same breed.[48,50] The genetic homogeneity found within an individual breed makes this possibility less likely to have occurred.

An alternative hypothesis is the difference in occurrence of CL subtype in different regions of the US is influenced by differing exposures to environmental factors that influence the occurrence of different subtypes of lymphoproliferative disease. These factors could be natural, (e.g., radon) or could be related to anthropogenic factors (e.g., industrial pollution) that differ across geographic regions in the US. One way to differentiate between these two hypotheses in future research would be to include more than one breed of dog in the study population. If the difference between distributions of CL subtypes were similar in multiple dog breeds, it would suggest this difference is more likely due to environmental factors rather than due to regional differences in genetic predispositions amongst members of one breed of dog.

A potential source of bias in this study was that dogs diagnosed with B-cell lymphoma were more likely to be diagnosed based upon a lymph node aspirate rather than a blood sample submission. This indicates there was a clinical presentation in these dogs (e.g., enlarged lymph nodes) that may not have been present in dogs diagnosed with T-zone lymphoma, which is considered an indolent disease.[14,52,53] Lack of a clinical presentation indicative of lymphoproliferative disorder may have resulted in fewer cases of T-zone lymphoma being diagnosed than were actually present within the overall population of Golden retrievers in the US. However, there is no indication there would be a geographic component to the potential under diagnosis of this subtype of disease, so even if under diagnosis did occur, it is unlikely to have affected results of this study.

We arbitrarily chose to use geographic boundaries based on census divisions, as an efficient state-based method for classifying larger contiguous regions. However, this may not be the most appropriate method for classifying geographic region if geographic differences are related to differences in causal exposures that can be summarized geographically. For example,

an alternative way to describe regional differences may be to use ecologically and geographically defined areas, such as the ecoregions defined by the US Environmental Protection Agency (EPA)[51] rather than state-line boundaries. Another approach that could be used to evaluate spatially related disease determinants would be to use geographic information systems (GIS) analysis to test specific exposure hypotheses, such as presence or absence of a particular type of industry (e.g., waste incinerators) or amount of a particular chemical (e.g., 2,4-dichlorophenoxyacetic acid) measured in groundwater within a region.

Another limitation of this study is the lack of complete patient history – including historical address information – resulted in the need for classification of location based upon location of the veterinary clinic, not the subject's home address. This could have resulted in misclassification of geographic exposure information. However, the summarizing geographical regions as large contiguous blocks would make geographic misclassification less likely, as owners are likely to have lived within the same analysis region where their veterinarian's hospital is located.

In humans, differences in distributions of different subtypes of lymphoma have been well established.[23,35-39,54-56] This has resulted in descriptive and analytic investigations into risks associated with the outcome of lymphoma being restricted to specific subtypes of the disease. The findings of this study suggest that future work in canine populations related to etiologic investigations of CL should differentiate between the specific subtypes of the disease.

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[a]Stata 11, StataCorp, College Station, Texas, USA

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Chapter 4 - Risk factors associated with development of histiocytic sarcoma in Bernese mountain dogs[4]

Overview

Histiocytic sarcoma (HS) is a rare but aggressive malignancy in humans that is poorly responsive to existing therapies. HS, though rare in most breeds of dogs, is a common malignancy in Bernese mountain dogs (BMD). The purpose of this study was to determine risk factors associated with development of histiocytic sarcoma in BMD. This was accomplished through use of an internet-based cross-sectional survey. Data were collected for a total of 216 BMD registered with the Berner-Garde Foundation, a non-profit organization maintaining a database consisting of submissions related to health outcomes in BMD. Owners of BMD diagnosed with HS and owners of disease-free littermates of dogs with HS were invited to participate in the study. Mixed effects logistic regression (MELR) and conditional logistic regression (CLR) were used in parallel to examine associations between potential risk factors and the occurrence of histiocytic sarcoma. When controlling for litter as a marker of relatedness, dogs diagnosed with orthopedic conditions were found to be more likely to develop HS (MELR, OR: 2.5, 95% CI: 1.5, 5.2; CLR, OR: 2.81, 95% CI: 1.1, 7.3), while dogs receiving anti-inflammatory medications were found to be at a considerably lower risk of developing this disease (MELR, OR: 0.42, 95% CI: 0.2, 0.8; CLR, OR: 0.32, 95% CI: 0.1, 0.8). These data suggest inflammation may be a modifiable risk factor for the development of histiocytic sarcoma in BMD.

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Introduction

Histiocytic sarcoma (HS) is a rare but aggressive cancer associated with high mortality in both dogs and humans.[1-11] Tumors are comprised of mononuclear phagocytic cells; the pathology, histology, and immunodiagnostic features of histiocytic disorders of dogs have recently been reviewed.[12] In human populations, the rarity of HS diagnoses coupled with a high case fatality rate results in few opportunities to research this disease.[2,13] Though uncommonly diagnosed in the general dog population, there is a strong predisposition for this type of neoplasia to occur in a few breeds of dogs.[3-5,8, 11] One breed in particular, the Bernese mountain dog (BMD), has been shown to be considerably more likely to be affected than dogs of other breeds, and reports have suggested that the lifetime risk may be as great as 25% of the breed population.[11, 13] It has been estimated that BMD are 225 times more likely to develop HS than other breeds of dogs[8] and are 17 times more likely to die due to tumor than are other breeds of dogs.[15] The heritable predisposition towards development of HS in this breed was originally thought to be due to polygenic effects,[16] but has more recently been linked to aberrations associated with the CDKN2A/B gene region.[11, 13] Abnormalities in the same gene region in humans encoding for p16 have been associated with several cancers, including HS.[17, 18]

The Berner-Garde Foundation (BGF) was established with the goal of reducing the burden of HS and other genetic diseases within the BMD population. To aid in these efforts, the BGF maintains a breed-specific database comprised of data submitted by BMD owners or collected through public sources of information such as the Canine Eye Registration Foundation and the Orthopedic Foundation for Animals.[19] The BGF database tracks lineage information, and all dogs included in this database are assigned a litter number which is used to identify

siblings. In addition, the BGF database is used to collect information on the health of individual dogs. However, health information reported by owners must be verified through submission of supporting documentation from veterinarians and other health experts, such as histopathology reports, before the information is included as a diagnosis in the database.[20] The database is freely accessible to the public and BMD breeders are encouraged to investigate health status of dogs and their ancestors when making breeding decisions. Despite high awareness among owners and breeders and the availability of data regarding the occurrence of HS, the number of HS cases diagnosed in BMDs appears to have increased steadily during the past two decades.[11] Over this same period of time almost all research regarding the occurrence of HS in BMD has focused on the heritability of the disease and the search for the genes which might be responsible for the breed predisposition. This information is obviously valuable for owners of BMDs and may be used to reduce the risk through genetic selection, but it is immutable and cannot be used to alter the risk of HS in existing dogs. There is no research available regarding the influence of environmental or health-related factors on the outcome of HS occurrence in BMDs. Therefore, it was the purpose of this study to investigate risk factors for the development of HS in BMD while accounting for the familial (genetic) effects within the study population.

Materials and Methods

Overview

Owners of BMDs were recruited using the BGF breed registry. Participating owners were asked to provide information using a web-based survey regarding signalment, diet and other exposures, and medical history, including the occurrence of HS. Logistic regression was used to investigate potential risk factors for HS using 2 different statistical methods.

Study population

Owners of BMD were invited to participate in this study indirectly through articles and advertisements placed in breed-specific newsletters or directly (via phone or email) when contact information was available within the BGF database. Study participation was voluntary and no incentives for participation were offered. In order to be considered eligible for study participation dogs were required to be registered with the BGF database. Cases were defined as dogs with a histologically confirmed diagnosis of HS (supporting documentation was submitted to BGF) that had occurred within five years prior to completion of the survey. The comparison group consisted of disease-free littermates of cases. The BGF litter number was used as a unique identifier of siblings born in the same litter.

Owner Survey

A survey instrument was designed to elicit data necessary to evaluate associations between potential risk factors and the outcome of HS. The survey was divided into three sections: demographics, health history, and environmental exposures. Demographic questions collected information on sex/neuter status, geographic location, date of birth, and BGF litter number. Health history questions collected information on diagnoses of medical conditions (orthopedic conditions, tick-borne diseases, and chronic conditions), prior surgeries, use of preventive treatments for fleas, ticks, or heartworm, vaccination status, body condition score, and use of medications (prescribed by a veterinarian), nutritional supplements (non-prescription supplements given by the owner with the intent to prevent illness or injury), or homeopathic remedies (non-prescription supplements given by the owner with the intent to alleviate an illness or injury) for a period of more than six months. Questions about environmental exposures collected information on the type of food most often consumed by the dog (e.g., commercial dry food, commercial wet food, or homemade diet), the feeding frequency utilized by the owner,

exposure to lawn or other chemicals, exposure to cigarette smoke, rural environments, and frequency of exposure to outdoor sources of water (irrigation ditches or canals, lakes, or streams). The final survey instrument[a] comprised 35 questions and was administered by use of online survey software.[b] The recruitment period was open for 18 consecutive months during which time the survey was accessible to owners interested in participating.

Data Analysis

The survey responses were transferred into an electronic database and incomplete or duplicate responses were removed. Verification of study eligibility was completed by ensuring documentation of histopathologic diagnosis of disease in cases or in at least one littermate for the controls was present within the BGF database. Study results were summarized by calculating descriptive statistics. Frequency distributions of categorical variables were evaluated. Continuous variables were analyzed by calculating means, medians, SD, and ranges and were categorized to facilitate regression analysis.

Mixed effects logistic regression and conditional logistic regression were used in parallel to examine associations between potential risk factors (exposure variables) and the occurrence of histiocytic sarcoma using standard statistical software.[c] The mixed effects logistic model used data from all dogs for which owners had submitted a survey (data were not necessarily submitted for HS cases and non-cases from all litters); the BGF litter number was modeled as a random effect to account for potential clustering among litters. The conditional logistic regression model only included dogs from litters which had data from both HS case and non-case within the same litter (cases and non-cases were siblings).

Exposure variables that were included in both of the modeling approaches (Table 4.1)

Table 4.1. Characteristics of the 216 Bernese Mountain Dogs enrolled in the study [n (%)]

Characteristic	Category	All dogs (n=216)	Dogs with HS (n=135)	Dogs without HS (n=81)
Demographics				
Age	<5	7 (3.2)	3 (2.2)	4 (4.9)
	5 to 7	86 (39.8)	60 (44.4)	26 (32.1)
	8 to 10	97 (44.9)	58 (43.0)	39 (48.1)
	>10	19 (4.6)	10 (7.4)	9 (11.1)
Gender	Male	28 (13.0)	21 (15.6)	7 (8.6)
	Male castrated	77 (35.7)	44 (32.6)	33 (40.7)
	Female	25 (11.6)	14 (10.4)	11 (13.6)
	Female spayed	84 (38.9)	55 (40.7)	29 (35.8)
Geographic region	Northeast US	50 (23.2)	30 (22.2)	20 (24.7)
	Midwestern US	30 (13.9)	21 (15.6)	9 (11.1)
	Southern US	25 (11.6)	16 (11.9)	9 (11.1)
	Western US	77 (35.7)	48 (35.6)	29 (35.8)
	Canada	30 (13.9)	18 (13.3)	12 (14.8)
	Europe	4 (1.9)	2 (1.5)	2 (2.5)
Medical history				
Orthopedic condition	Yes	57 (26.4)	43 (31.9)	14 (17.3)
	No	158 (73.2)	92 (68.2)	66 (81.5)
Any surgical procedure	Yes	126 (58.3)	79 (58.5)	47 (58.0)
	No	88 (40.7)	56 (41.5)	32 (39.5)
Tick-borne disease	Yes	26 (12.0)	15 (11.1)	11 (13.6)
	No	189 (87.5)	119 (88.2)	70 (86.4)
Flea preventative	Yes	143 (66.2)	93 (68.9)	50 (61.7)
	No	65 (30.1)	39 (28.9)	26 (32.1)
Tick preventative	Yes	129 (59.7)	83 (61.5)	46 (56.8)
	No	83 (38.4)	50 (37.0)	33 (40.7)
Heartworm preventative	Yes	157 (72.7)	105 (77.8)	52 (64.2)
	No	57 (26.4)	29 (21.5)	28 (34.6)
Vaccinated	Yes	213 (98.6)	134 (99.3)	79 (97.5)
	No	3 (1.4)	1 (0.7)	2 (2.5)
Other serious illness	Yes	72 (33.3)	40 (29.6)	32 (39.5)
	No	142 (65.7)	93 (68.9)	49 (60.5)
Long-term medications	Yes	73 (33.8)	40 (29.6)	33 (40.7)
	No	143 (66.2)	95 (70.2)	48 (59.3)
Homeopathic treatments	Yes	67 (31.0)	48 (35.6)	19 (23.5)
	No	148 (68.5)	86 (63.7)	62 (76.5)
Weight within normal range	Yes	190 (88.0)	114 (84.4)	76 (93.8)
	No	26 (12.0)	21 (15.6)	5 (6.2)

Environmental exposures				
Type of food	Commercial Dry	160 (74.1)	101 (74.8)	59 (72.8)
	Cooked meat	9 (4.2)	6 (4.4)	3 (3.7)
	Raw meat	36 (16.7)	24 (17.8)	12 (14.8)
	Other	11 (5.1)	4 (3.0)	7 (8.6)
Feeding frequency	Once a day	11 (5.1)	5 (3.7)	6 (7.4)
	Twice a day	189 (87.5)	122 (90.4)	67 (82.7)
	Three times a day	8 (3.7)	5 (3.7)	3 (3.7)
	Free choice	7 (3.2)	2 (1.5)	5 (6.2)
Lawn chemicals	Yes	122 (56.5)	57 (42.2)	37 (45.7)
	No	94 (43.5)	78 (57.8)	44 (54.3)
Other chemicals	Yes	74 (34.3)	41 (30.4)	33 (40.7)
	No	137 (63.4)	92 (68.2)	45 (55.6)
Rural	Yes	188 (87.0)	114 (84.4)	74 (91.4)
	No	27 (12.5)	20 (14.8)	7 (8.6)
Cigarette smoke	Yes	44 (20.4)	26 (19.3)	18 (22.2)
	No	172 (79.6)	109 (80.7)	63 (77.8))
Irrigation ditches/canals	Yes	11 (5.1)	5 (3.7)	6 (7.4)
	No	204 (94.4)	130 (96.3)	74 (91.4)
Lakes or streams	Yes	99 (45.8)	62 (45.9)	37 (45.7)
	No	116 (53.7)	73 (54.1)	43 (53.1)

were age, sex, sex status, geographic location, diagnosis of any orthopedic condition (y/n), any reported surgical procedure other than spay or neuter (y/n), reported diagnosis of a tick-borne disease (y/n), long-term (≥6 months) use of veterinary-prescribed medications , long-term (≥6 months) use of homeopathic medications given by the owner as a treatment for a disease or condition, long-term (≥6 months) use of nutritional supplements given by the owner to prevent occurrence of a disease or condition, diagnosis of any diseases other than HS, use of flea, tick, or heartworm preventive treatments (frequency was recorded if exposure was reported), primary type of food (commercially prepared wet or dry, or home prepared), feeding frequency (free choice, once, twice, three or more times per day), exposure to lawn chemicals used either to prevent weed growth or exposure to other chemicals (paints, solvents, lubricants, or other),

amount of time spent in rural environments, exposure to cigarette smoke (frequency was recorded if exposure was reported), and exposure to irrigation ditches or canals (y/n), or lakes or streams (y/n). For each modeling method, univariable models were used to screen individual exposures. Variables that were statistically associated with the outcomes in initial screening ($P \leq 0.25$) were included in multivariable model building. Final multivariable models were identified by use of a backward selection procedure with a critical α for retention of ≤ 0.05. Previously excluded variables were reintroduced to the final model to ensure that the exclusion was appropriate and to evaluate confounding effects (identified by ≥20% change in parameter estimates). First order interaction terms for main effects variables included in final models were evaluated. Subject-specific odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using the results of the logistic regression models. Stratified analyses were conducted based upon the presence or absence of diagnosis with an orthopedic condition and Pearson's Chi-squared tests were utilized to better understand the relationship between long term use of medications and the outcome of HS. In addition, a mixed-effects logistic regression model (outcome = HS) was constructed using data only from dogs within the study population that were free from diagnosis with an orthopedic disease. The independent variable included in this model was long-term use of medications and the BGF litter number was modeled as a random effect to account for potential clustering among litters. A subject-specific odds ratio (OR) and 95% confidence interval (95% CI) was calculated using the results of this logistic regression model.

Results

Characteristics of study population

The majority of owners who elected to participate were contacted directly (via phone or email) rather than indirectly (via articles and advertisements placed in breed-specific

newsletters). Four hundred ninety surveys were initiated using the online survey software, but 274 (55.9%) of those surveys were excluded from the analyses (Fig. 4.1). Data were collected

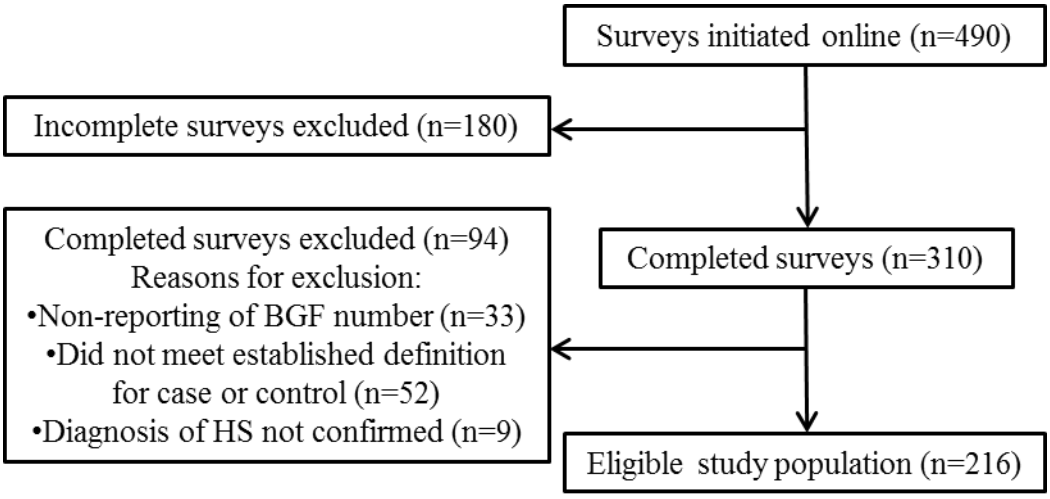

Figure 4.1. Survey responses from time of initiation online to determination of eligibility.

for a total of 216 eligible BMD representing 140 different litters (Table 4.1). Mean ± SD age of dogs was 7.7 ± 2.0 years (median, 8 years; range, 2 to 13 years). The population was evenly distributed between males and females and the majority of the population, regardless of sex, was neutered (76%). Owners classified most dogs enrolled in the study as being of normal weight (88.0%) and were without significant illness (65.7%). However, more than half of the dogs (58.3%) included in the study had undergone some type of surgical procedure other than neutering. One-third (73/216) of the study population used medications for ≥6 months, and the most frequently reported (81.6%) types were anti-inflammatory drugs (prednisone, carprofen, and meloxicam) and supplements given for treatment and prevention of joint disease (polysulfated glycosaminoglycan and glucosamine/chondroitin combination). The environmental exposure variables that were reported with the highest frequency were exposure to rural environments (87.0%) and exposure to lawn chemicals (56.5%).

Risk factors for histiocytic sarcoma – Mixed effects logistic model

Exposure variables that were included in multivariable modeling in mixed effects models were the diagnosis of an orthopedic condition, whether heartworm preventative had been used, vaccination for rabies, history of other significant illnesses, long-term use of prescribed medications, use of nutritional supplements, use of homeopathic medications, weight being outside of the normal range (high or low), exposure to rural environments, exposure to chemicals other than lawn chemicals, and exposure to irrigation ditches or canals. Variables retained in the final multivariable model were the diagnosis of an orthopedic condition, treatment with heartworm preventatives, long-term use of medications, and use of homeopathic medications (Table 4.2). Interaction terms for main effects were not significant when included in the model.

Table 4.2. Results of logistic regression models examining risk factors associated with development of histiocytic sarcoma in Bernese mountain dogs.

		All Dogs, n=216 (Mixed effects model)			Matched siblings, n=118 (Conditional logistic model)		
Risk factor	**Category**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Orthopedic condition	Yes	2.49	1.21, 5.14	0.014	2.81	1.08, 7.26	0.034
	No	Reference			Reference		
Homeopathic medications	Yes	2.10	1.05, 4.18	0.036	2.88	1.04, 7.90	0.041
	No	Reference			Reference		
Use of heartworm preventative	Yes	2.11	1.10, 4.05	0.024	Not Significant		
	No	Reference					
Long-term medication use	Yes	0.43	0.22, 0.81	0.010	0.32	0.12, 0.83	0.020
	No	Reference			Reference		
Diagnosis with another illness	Yes	Not Significant			0.38	0.15, 0.93	0.035
	No				Reference		

Individual dogs were 2.5 times more likely (OR: 2.49; 95% CI: 1.21, 5.14) to develop HS if they were diagnosed with an orthopedic condition than if they had not received such a diagnosis. Dogs were also at a greater than two times increased risk of developing HS if they were given homeopathic medications (OR:2.10; 95% CI: 1.05, 4.18) or heartworm preventatives (OR:2.11; 95% CI: 1.10, 4.05). However, dogs also had a greater than twofold decrease (OR: 0.43; 95% CI: 0.22, 0.81) in risk of developing HS when they were given medications long-term (≥6 months) as compared to their risk had they not received medications.

Risk factors for histiocytic sarcoma – Conditional logistic model

Exposure variables included in multivariable modeling using conditional logistic regression were the diagnosis of an orthopedic condition, history of other significant illnesses, long-term use of prescribed medications, use of nutritional supplements, use of homeopathic medications, weight being outside of the normal range (high or low), age of the dog, and the frequency with which the dog was fed. Variables retained in the final multivariable model were the diagnosis of an orthopedic condition, history of other significant illnesses, long-term use of medications, and use of homeopathic medications (Table 4.2). Interaction terms for main effects were not significant when included in the model. In this model, dogs diagnosed with an orthopedic condition were nearly three times more likely (OR: 2.81; 95% CI: 1.08, 7.26) to develop HS than they would have been had they not developed an orthopedic condition. Use of homeopathic medications was also associated with an increased risk of developing HS (OR: 2.88; 95% CI: 1.04, 7.90) in individual dogs. However, dogs receiving long-term (≥6 months) medications had a greater than threefold reduction in risk (OR: 0.32; 95% CI: 0.12, 0.83) and dogs diagnosed with an illness other than HS had a greater than twofold reduction in risk (OR:

0.38; 95% CI: 0.15, 0.93) associated with diagnosis of HS as compared to their risk had they not received medications.

Risk factors for histiocytic sarcoma – Stratified analyses

When the study population was stratified based upon presence or absence of diagnosis with an orthopedic disease, long-term use of medications only remained significantly associated (*P*-value =0.038) with the outcome of HS in the population of dogs not diagnosed with an orthopedic condition. Amongst dogs without an orthopedic condition, dogs receiving long-term (≥6 months) medications had a greater than twofold reduction in risk (OR: 0.48; 95% CI: 0.24, 0.96) associated with diagnosis of HS as compared to their risk had they not received medications.

Discussion

The concurrent findings of an increased risk of developing HS in association with the occurrence of an orthopedic disease, and decreased risk of HS in dogs that had a history of long-term treatments which were predominantly anti-inflammatory and joint support medications suggests there may be an association between chronic inflammatory conditions and the occurrence of HS in BMDs. Both modeling approaches identified similar relationships with the outcome of HS in BMDs, which strengthens conclusions that might be drawn from this study. The relationship between orthopedic conditions and HS has been examined previously and it was reported that BMD were greater than 5 times more likely to develop peri-articular HS in a previously diseased joint than a dog with no prior diagnosis of joint disease.[21] To the authors' knowledge however, associations between use of anti-inflammatory medications and a reduced risk of developing HS have not been previously reported in either dogs or humans.

The association between inflammation and cancer is well documented in humans and it is estimated that approximately 1 in 4 human cancers worldwide are associated with chronic inflammation.[22-27] Associations have also been made between inflammation and the occurrence of multiple cancers in dogs including osteosarcoma, lymphoma, transitional cell carcinoma, mesothelioma, squamous cell carcinoma, and myxosarcoma.[26-31] Both exposure variables reported here, diagnosis with an orthopedic condition and use of anti-inflammatory medications, could be considered surrogate indicators for inflammation, but direct measurement of inflammatory processes would not have been possible given the study design and retrospective nature of data collection. However, the findings of this study are consistent with associations that have been reported between inflammatory disorders and cancer in both humans and dogs,[20-29] and thus support the hypothesis that chronic inflammation is associated with the occurrence of HS in BMDs.

The use of a cross-sectional survey instrument was beneficial in that we were able to examine several exposure variables simultaneously, but this type of study design is not useful for detecting associations between rare exposures and the outcome of disease. It is therefore possible an exposure variable included in this study that was not statistically associated with the outcome of HS in BMD is biologically related to disease occurrence. Also, because information pertaining to both exposure and disease status was collected simultaneously, a temporal sequence was not established and it is possible that exposures associated with the outcome of HS may have actually occurred after the disease was detected. Another potential concern is that data collected in this study were reported by individual dog owners and interpretation regarding exposures could be biased (e.g. recall bias, misclassification of frequency of exposures, etc.). There is no way to assess the effects of these potential biases within this study and, if present, this may have

resulted in biased study results. However, we are confident that dogs classified as cases were accurately diagnosed due to the pathologic confirmation of all HS diagnoses through BGF. Though it is possible misclassification of non-cases could have occurred, we feel this is unlikely due to the severity of the disease when present and the fact that death due to other causes was also confirmed through pathologic reports submitted to BGF.

According to the Oxford Centre for Evidence Based Medicine, results from studies such as this one are considered mid-level in the hierarchy of the likely best evidence produced by different study designs.[32] This indicates further research is needed in order to substantiate the findings contained in this report. Ideally, a large-scale prospective study design similar to the design utilized by the Morris Animal Foundation's Golden Retriever Lifetime Study[33] would be employed in order to examine a large population of BMDs over time while collecting data on both exposures and outcomes throughout the study period. This would allow for a temporal sequence to be established and, if the study population were large enough, may allow for distinctions to be made between different types and dosages of anti-inflammatory and joint support medications.

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[a] Copies of the survey are available from the corresponding author on request.

[b] Surveygizmo 2.6, Widgix, LLC, Boulder, CO

[c] STATA, release 11, College Station, TX, USA

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CONCLUSIONS

The primary aims of my dissertation were to examine the current body of evidence produced through canine cancer epidemiology research, produce new research using study designs similar to those used in human cancer epidemiology research, and show how we can advance knowledge of cancer risk and pathogenesis in both fields using the canine spontaneous tumor model. Each chapter contributes to these aims in the following ways:

CHAPTER 1

- Through use of systematic review methodology I was also able to identify a lack of consistency in design of research studies, varied approaches to statistical methods used in similar studies, and a lack of use of standardized reporting guidelines in veterinary research.
- Most studies included in the review were observational studies with great potential for bias in the methods used to select the study population, measure exposures, and identify the outcome of canine lymphoma.
- The quality of the overall body of evidence related to the identification of risk factors for lymphoma in dogs was low to moderate, with only one body of evidence, which related to the association between breed and lymphoma outcome, being of high quality.
- Despite a general lack of confidence in estimates of specific effect size, several risk factors that are likely positively associated with the outcome of lymphoma in dogs were identified
- Strengths of this chapter include the systematic approach utilized in collection of research studies to be included in the review and the use broad search terminology to capture a

wide range of potential risks for developing disease.

- Limitations of this chapter include the quality assessment of both individual studies and bodies of evidence were conducted by one researcher and the broad terminology utilized may have resulted in missing research investigating specific risk factors for development of lymphoma.

CHAPTER 2

- Through use of multivariable logistic regression modeling I was able to identify differences in risk of developing lymphoma based upon dog breeds, suggesting a heritable predisposition towards development of lymphoma in some breeds of dogs, and based upon sex and neuter status of the dogs, suggestive of hormonal influence on development of lymphoma.
- The proportion of dogs diagnosed with lymphoma at referral centers in the US did increase over time, which is similar to what is reported in regards to the incidence rate of human NHL, but this proportion is unadjusted and does not account for differences in diagnostic techniques available at different periods of time, the age of animals in the population, sex and neuter status, or breed.
- Strengths of this chapter include the very large study population and the use of frequency matching to gain statistical power and efficiency.
- Limitations of this chapter include the lack of control for environmental risk factors due to the limited amount of information recorded in the VMDB, the potential for referral bias, the possibility this population is not representative of the entire canine population in the US, and the fact that all diagnoses were coded as lymphoma and different subtypes of the disease were not analyzed separately.

CHAPTER 3

- The differences detected in the geographic distribution of B-cell and T-zone subtypes of CL diagnosed in Golden retrievers in the US may be influenced by differing exposures to environmental factors that influence the occurrence of different subtypes of lymphoproliferative disease.
- This finding is similar to reports of environmental exposures being associated with outcome of a particular subtype of NHL.
- Strengths of this study include the level of confidence we have in the diagnosis of subtypes and the large geographical regions utilized in the analysis.
- Limitations of this study include the use of only one breed of dog and the possibility dogs with different subtypes of lymphoma may not be given the same level of diagnostics, resulting in a potential under diagnosis of one subtype of disease, and the lack of patient history including residential address.

CHAPTER 4

- In this chapter I was able to identify a positive association between diagnosis with an orthopedic condition and a negative association with long-term use of medications and the outcome of malignant histiocytosis, a very rare cancer in humans. This is suggestive of chronic inflammation being a modifiable risk factor associated with development of disease.
- Strengths of this study include the use of two separate modeling techniques to estimate odds ratios and the use of a study design that allowed for assessment of multiple exposures.
- Limitations of this study include the relatively small sample size, the lack of

establishment of a temporal sequence, and the potential for recall bias.

In conclusion, veterinary cancer epidemiologists can positively contribute to research that occurs at the interface of human and animal medicine by increasing the rigor with which we are conducting studies, reporting research in a transparent manner by conforming to accepted reporting guidelines, and ensuring we are investigating appropriate research questions. By continuing to add high quality research to the field of cancer epidemiology we are able to contribute to a greater body of work that will be beneficial to both species.