

## STANDARD ARTICLE

# Demographic risk factors for lymphoma in Australian dogs: 6201 cases

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**Background:** Lymphoma is common in the dog. Studies of population risk factors primarily have been derived from referral institution or insurance data.

**Objective:** To identify and quantify the host risk factors for lymphoma in a broad population of Australian dogs.

**Animals:** Data on 6201 client owned dogs were retrieved from a commercial veterinary laboratory, a general practice group and 2 referral hospitals.

**Methods:** Data collected included breed, sex, and neuter status. A reference population of 640 105 dogs was generated from the referral hospitals and from council registration data. The risk of lymphoma by sex and neuter status was calculated as odds ratios (OR).

**Results:** The study identified 30 breeds at increased risk of lymphoma, 15 that have not been reported previously, and 26 breeds at decreased risk, 18 that have not been reported previously. Males were over represented compared to females with an OR of 1.1 (95% CI, 1.1–1.2;  $P < .001$ ). Neutered animals were at higher risk compared to intact animals with an OR of 3.2 (95% CI, 2.9–3.5) which was found in both males (OR, 2.8; 95% CI; 2.5–3.2) and females (OR, 4.4; 95% CI, 3.5–5.1).

**Conclusions and Clinical Importance:** Breed, sex, and neuter status alter the risk of lymphoma in dogs. These 3 factors must be considered when evaluating lymphoma risk as potential markers of underlying differences in disease etiology. Comparison of breeds at increased and decreased risk could be advantageous when evaluating specific etiological factors.

**KEYWORDS**

breed, canine, epidemiology, gender, lymphoma, lymphosarcoma, neuter status

## 1 | INTRODUCTION

Lymphoid neoplasia in dogs is dominated by lymphoma and is used as a model for human disease, having a comparable incidence.<sup>1–5</sup> The genetic restrictions that arose from the development of modern dog breeds offer unique opportunities to study the etiology of many diseases<sup>5</sup> and incorporate genetic strategies into disease management. Previous studies have identified breeds at increased or decreased risk of lymphoma, with studies adopting different approaches to assessing

risk relative to a general or hospital population.<sup>4,6–15</sup> Most of these studies have been based on referral hospital or insurance data. Approximately, 77% of dogs are registered with a veterinary practice with the number attending referral centers is likely to be much lower. Approximately, 42% of dogs are insured. Thus, the use of referral hospital and insurance data might not be a true reflection of the general population.<sup>12,14,15</sup> All of the identified studies are summarized in Table 1.

A limited amount of data on sex risk for lymphoma in dogs shows male dogs are at increased risk,<sup>3,8,14</sup> although 1 study did not identify sex as a risk factor.<sup>18</sup> Variation in hormonal exposure in dogs is generated by neutering, usually performed at an early age. Neutering has

**Abbreviations:** CI, confidence interval; MVSC, Melbourne Veterinary Specialist Centre; NSDTR, Nova Scotia Duck Tolling Retriever; OR, odds ratio; UVTHS, University Teaching Hospital Sydney; VMDB, Veterinary Medical Database

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**TABLE 1** Previous studies of lymphoma risk in dogs

Reference	Data source	Breed(s)	Case number	Reference population
Bremer et al <sup>16</sup>	Insured dogs	NSDTR <sup>a</sup>	NS <sup>b</sup>	442 446
de la Riva et al <sup>17</sup>	University Hospital	Golden Retriever	35	759
Dorn et al <sup>6</sup>	Population survey in California	All	52	1031
Edwards et al <sup>7</sup>	Insured dogs	All	103	130 684
Gruntzig et al <sup>8</sup>	Swiss Canine Cancer Registry	All	2955	121 963
Jagielski et al <sup>9</sup>	University Hospital	All	63	7730
Jankowska et al <sup>10</sup>	University Hospital	All—T cell only	118	14 460
Pastor et al <sup>4</sup>	Veterinary Laboratories	All	702	Estimated <sup>c</sup>
Priester et al <sup>15</sup>	VMDB <sup>d</sup>	All	1452	NS
Teske <sup>11</sup>	University Hospital	All	254	35 633
Villamil et al <sup>12</sup>	VMDB	All	14 573	1 157 342
Wyatt et al <sup>13</sup>	University Hospital	All	85	NS
Yau et al <sup>14</sup>	University Hospital	All	134	13 575
Zink et al <sup>18</sup>	Dog owner survey	Hungarian Vizsla	46	2505

<sup>a</sup>Nova Scotia Duck Tolling Retriever.

<sup>b</sup>Not stated.

<sup>c</sup>Reference population was estimated from surveys of the most popular breeds in France in 2000.

<sup>d</sup>Veterinary Medical Database.

been reported to increase the risk of lymphoma as well as other neoplastic diseases.<sup>17,19</sup> In humans, a decreased risk for non-Hodgkin's lymphoma has been reported for postmenopausal women who are on hormonal replacement treatment, although this finding has been inconsistent.<sup>20</sup> Low testosterone as a risk for cancers in men has not been reported but there are infrequent reports of decreased cancer mortality, not risk, with increases in testosterone blood concentrations.<sup>21</sup>

Our study was developed to evaluate a large patient population for breed risk for lymphoid neoplasia drawn from a broader population of Australian lymphoma cases in dogs and comparing to a reference population derived from a source more reflective of the general dog population than referral hospital data. The overall and within breed influence of sex and neuter status also was evaluated.

## 2 | MATERIALS AND METHODS

### 2.1 | Patient populations

A retrospective review of medical records from 2 referral hospitals, University Veterinary Teaching Hospital Sydney (UVTHS) and

Melbourne Veterinary Specialist Centre (MVSC), from January 2000 to June 2017 was performed with a search for the terms lymphoma or lymphosarcoma. These records were reviewed by a board-certified veterinary oncologist and patients found to have compatible clinical signs in addition to a cytological or histological diagnosis were included in the study. The patient's sex and neuter status as reported in the medical record were recorded.

A commercial veterinary diagnostic laboratory<sup>1</sup> performed a search of their laboratory reports from February 2012 to May 2015 for the word lymphoma. Copies of all reports including this term were provided and each record was reviewed as described above. If there was sufficient clinical information with supportive cytological or histological information or a confirmed pathological diagnosis of lymphoma, the case was included.

A corporate, predominately general, veterinary practice<sup>2</sup> searched medical records from January 2012 to December 2015 for the word lymphoma. The record entry was provided and reviewed as described above and if there was sufficient clinical information and reference to a cytological or histological diagnosis of lymphoma, the patient was included with the same data collected.

### 2.2 | Reference populations

The hospital databases of 2 referral hospitals, UVTHS and MVSC, were accessed and all dog data retrieved including breed, sex, and neuter status. Lymphoma cases were removed. The hospital population data from the University of Adelaide Veterinary Teaching Hospital was obtained. Because the hospital population for the corporate practice and the list of all submissions to the veterinary diagnostic laboratory were not available, local councils from across Australia were contacted for information on the dogs registered within their jurisdiction with breed, sex, and neuter status requested. Because the majority of the lymphoma cases came from general practices with no hospital population available, this data then was combined to generate the reference population.

### 2.3 | Statistical Analysis

Statistical analysis was performed by commercially available statistical software.<sup>3</sup> From the above populations, the most popular 50 breeds from the reference population and any breed with  $\geq 10$  cases from the lymphoma population were included in the analysis, as well as any breed previously reported to be at increased or decreased risk of lymphoma. Odds ratios for each breed, for sex, and neuter status were calculated with 95% confidence intervals (CI) calculated by the Baptista-Pike method, or the Woolf Logit for larger samples, with *P* values calculated by Fisher's exact test. A *P* value of  $< .05$  was considered significant.

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<sup>2</sup>Greencross Vets, Woolloongabba, QLD, Australia.

<sup>3</sup>GraphPad Prism version 7.02 for Windows, GraphPad Software, La Jolla, CA, USA.

### 3 | RESULTS

The searches of UVTHS and MVSC hospital data bases retrieved 340 and 925 cases of lymphoid neoplasia, respectively. The search of the diagnostic laboratory retrieved 11 677 reports with a mention of lymphoma. Of these 4040 were considered to be individual cases of lymphoma. The search of the corporate practices' medical records retrieved 5412 records including the word lymphoma. On review, 896 of these were considered cases of lymphoma. There were a total of 6201 cases of lymphoma. One hundred and thirty-four cases from UVTHS have been reported previously.<sup>14</sup>

The hospital populations from UVTHS and MVSC contained data on 34 495 and 50 255 dogs, respectively. The university of Adelaide veterinary teaching hospital had a hospital population of 6752. There was a low response rate to the requests to councils for information on dog registrations. The data that were obtained was for dogs registered in 2015. In South Australia, a central register provided information for all dogs registered in South Australia. Ten councils provided data on 255 244 dogs. The South Australian data included 293 359 dogs. The total reference population was 640 105 dogs.

Four hundred breeds or breed crosses identified in the data. All breed crosses were combined in 1 group and named "Cross Breed." Two-hundred and five pure breeds were identified and used for analysis. Some records did not include breed, and these were identified as unknown. In the veterinary diagnostic clinicopathologic data, not all breeds were included in the database, and some were identified as "other." These cases also were included as unknown.

In the lymphoma cases, the sex, and neuter status was known in 98.5% of dogs from the referral hospitals and the corporate veterinary practices. Sex was known in 97.8% of cases but the neutering status was not known in 1796 (44%) of the cases from the veterinary diagnostic laboratory. Sex and neuter status was known for 97.4% of the reference population from the referral hospitals and the university. Sex was identified in the registration data from only 5 councils (73 109 dogs) and neuter status from only 2 councils (39 158 dogs). Sex evaluations used a total reference population of 164 611 dogs and neuter status had a reference population of 130 660 dogs.

In total, 78 breeds were evaluated for lymphoma risk. Thirty breeds were at increased risk of lymphoma, 15 of which have not been reported previously, and 26 breeds, including Cross Breeds, were at decreased risk, 18 of which have not been previously reported. The breeds at increased risk are listed in Table 2 and those at decreased risk in Table 3. Twelve breeds had been reported previously to be at increased or decreased risk but were not found to have statistically significant results in our study. These 12 breeds were Irish Wolfhound, Bouvier des Flandres, Old English Sheepdog, Samoyed, Brittany Spaniel, Pekingese, Nova Scotia Duck Tolling Retriever, Briard, Bernese Mountain Dog, Doberman, Schnauzer, and German Shepherd.

Overall, males were found to be at increased risk of lymphoma compared to females (OR, 1.1; 95% CI, 1.12-1.2;  $P < .001$ ). When individual breeds were evaluated, this also was seen in Cross Breeds (OR, 1.1; 95% CI, 1.02-1.2;  $P = .02$ ), Boxers (OR 1.6; 95% CI, 1.2-2.2;  $P = .001$ ), and Cocker Spaniels (OR, 1.4; 95% CI, 1.02-2.0;  $P = .04$ ).

**TABLE 2** Breeds at increased risk of lymphoid neoplasia

Breed	OR	95% CI	P value
Airedale Terrier <sup>a</sup>	2.5	1.6-4.0	.001
Australian Cattle Dog <sup>a</sup>	1.7	1.5-2.0	<.001
Basset Hound <sup>a</sup>	2.4	1.3-4.5	.01
Beagle	1.7	1.4-2.0	<.001
Border Collie	1.8	1.6-2.0	<.001
Boxer <sup>a</sup>	3.2	2.8-3.7	<.001
Bull Terrier	2.2	1.7-2.9	<.001
Bulldog <sup>a</sup>	2.8	2.2-3.5	<.001
Bullmastiff <sup>a</sup>	4.8	3.8-6.0	<.001
Chesapeake Bay Retriever	9.8	2.3-42.0	.02
Cocker Spaniel	1.6	1.4-1.9	<.001
Dandie Dinmont Terrier	14.2	5.0-40.4	<.001
Dogue de Bordeaux <sup>a</sup>	4.3	2.9-6.4	<.001
Flat Coated Retriever	4.2	2.0-8.4	.001
Foxhound	10.7	4.6-24.8	<.001
Golden Retriever <sup>a,b</sup>	2.1	1.9-2.4	<.001
Gordon Setter <sup>a</sup>	4.9	2.5-9.7	<.001
Great Dane	2.4	1.8-3.3	<.001
Hungarian Vizsla <sup>a</sup>	1.9	1.2-2.9	.01
Labrador Retriever <sup>a,b</sup>	1.2	1.1-1.4	<.001
Mastiff	5.3	4.1-6.9	<.001
Neapolitan Mastiff	5.7	3.0-10.8	<.001
Rhodesian Ridgeback	1.8	1.3-2.3	<.001
Rottweiler <sup>a</sup>	2.0	1.7-2.4	<.001
Schnauzer (Giant)	4.1	1.5-11.1	.02
Scottish Terrier <sup>a</sup>	2.4	1.4-3.9	.003
Shetland Sheepdog	2.0	1.5-2.8	<.001
St Bernard <sup>a</sup>	2.7	1.4-5.2	.01
Welsh Corgi <sup>a</sup>	3.1	2.3-4.2	<.001
Whippet	1.4	1.0-2.0	.05

<sup>a</sup> Previously reported at increased risk.

<sup>b</sup> Previously reported at decreased risk.

Two breeds were identified with males at decreased risk of lymphoma compared to females, Bulldogs (OR, 0.6; 95% CI, 0.4-0.9;  $P = .04$ ) and Dogue de Bordeaux (OR, 0.3; 95% CI, 0.1-0.8;  $P = .02$ ).

Neutered animals showed an increased risk of lymphoma compared to intact animals overall, and in both males and females separately. In individual breeds, neutering was identified to increase lymphoma risk in 23 breeds, including Cross Breed, for males in 22 breeds and for females in 16 breeds. The results are listed in Table 4.

### 4 | DISCUSSION

In our study, increased risk of lymphoma was identified in 30 breeds, half of which previously have not been reported and decreased risk was found in 26 breeds, the majority having not been reported previously. Ours is the first large study to derive cases and controls from a dog population that we propose to be more representative of the general canine population. Males were identified to be at increased risk

**TABLE 3** Breeds at decreased risk of lymphoid neoplasia

Breed	OR	95% CI	P value
Akita	0.1	0.0–0.9	<.001
American Staffordshire Terrier	0.5	0.4–0.7	<.001
Australian Bulldog	0.3	0.1–1.0	.03
Australian Kelpie	0.6	0.5–0.7	<.001
Bichon Frise	0.4	0.3–0.7	<.001
Chihuahua <sup>a</sup>	0.3	0.2–0.4	<.001
Chinese Crested	0.1	0.0–1.5	.01
Chow Chow	0.1	0.0–2.1	.04
Cross Breed <sup>a</sup>	0.7	0.7–0.8	<.001
Dachshund <sup>a</sup>	0.6	0.3–0.9	.02
Dachshund (Miniature)	0.3	0.1–0.7	.001
French Bulldog	0.2	0.1–0.6	<.001
Greyhound	0.6	0.4–1.0	.04
Jack Russell Terrier	0.8	0.7–0.9	<.001
Japanese Spitz	0.2	0.0–0.7	.002
King Charles Spaniel	0.4	0.2–0.7	.002
Koolie	0.1	0.0–0.9	.01
Maltese <sup>a</sup>	0.5	0.4–0.6	<.001
Papillon	0.1	0.0–0.5	<.001
Pomeranian <sup>a</sup>	0.5	0.3–0.7	<.001
Poodle (Miniature) <sup>a</sup>	0.4	0.3–0.7	<.001
Poodle (Standard)	0.1	0.0–0.2	<.001
Poodle (Toy) <sup>a</sup>	0.1	0.1–0.3	<.001
Pug <sup>a</sup>	0.5	0.4–0.8	<.001
Siberian Husky	0.4	0.2–0.6	<.001
Tenterfield Terrier	0.1	0.0–0.3	<.001
Yorkshire Terrier <sup>a</sup>	0.2	0.0–1.1	.02

<sup>a</sup> Previously reported at decreased risk.

overall and in most breeds where sex was able to be evaluated. Neuter status was a significant risk factor for lymphoma overall and within both males and females, as well as within multiple breeds. Our results support previous findings where similar risks were identified in individual breeds or sexes.

In dogs, the majority of lymphoid neoplasia cases are lymphoma with diffuse large B-cell lymphoma being most common.<sup>11</sup> In our study, insufficient descriptive data was available to identify the various subtypes of lymphoma that can occur such as cutaneous epitheliotropic, peripheral T-cell, gastrointestinal lymphoma, and many other types. We found that the number of cases in which subtype was identified was inadequate to evaluate breed, sex, or neuter status risk related to individual subtypes.

A previous study based on the Swiss Tumor Registry data was the most representative of the general dog population to date, but breed risk was computed relative to Cross Breed dogs, not the entire registry population.<sup>9</sup> Two other larger studies used the Veterinary Medical Data Base for both case and reference populations, which collects data from North American veterinary schools. Other studies had <1000 cases or had poorly defined or restricted reference populations.<sup>4,6,7,10,11,14</sup> Only 2 small studies in dogs in Australia have been reported.<sup>13,14</sup>

Our study identified a number of breeds not previously recognized as being at increased or decreased risk of lymphoma, but also showed no increased risk in some previously reported breeds. The relative geographic isolation of Australia, strict quarantine regulations contributing to small genetic pools in some breeds, and breed preferences could account for some of these differences. These factors may contribute to a degree of genetically isolated subpopulation variability in the susceptibility of a breed to lymphoid neoplasia when compared to other subpopulations. The use of Cross Breed dogs as the reference point for calculating OR in some of the previous studies<sup>10,12,14</sup> also may have affected the results. Our study, as well as 2 previous studies,<sup>9,10</sup> showed a significantly decreased risk in Cross Breed dogs, which would alter the apparent risk if Cross Breed dogs are used as the reference point rather than the entire population as a reference point.

Genetic evaluation of risk for lymphoma or lymphoid neoplasia has been performed in dogs, both within a breed<sup>22</sup> and across breeds.<sup>23,24</sup> A difference in prevalence of lymphoma types among breeds<sup>25</sup> suggests different underlying genetic risk factors. Work at UVTHS has shown a genetic risk region in which bullmastiff dogs are at increased risk of lymphoma that does not appear to be present in Border Collies with lymphoma (unpublished data). It is not known if different breeds with an increased risk of the same type of lymphoma would share the same genetic risk factors.

Although some studies have evaluated genetic and molecular markers of prognosis in canine lymphoma, few have explored etiological factors. Clustering of lymphoma in related dogs has been reported, suggesting an inherited predisposition.<sup>26</sup> Genetic and epigenetic changes have been identified in canine lymphoma, but the studies generally have been across multiple breeds.<sup>27–29</sup> Studies within a breed have identified germline changes across tumor types, potentially supporting a breed-associated cancer risk that is not specific for lymphoma.<sup>22</sup>

One factor that might account for the decreased risk of lymphoid neoplasia in Cross Bred Dogs in our study is the common use of poodles, a breed at decreased risk in ours and other studies,<sup>4,6,8,12,15</sup> in what have been termed designer crosses, including Labradoodles, Cavoodles, and Moodles. However, this would not account for the finding in the earlier study that was performed at a time when designer crosses were likely less common.<sup>15</sup> The nature of crosses was not reliably recorded in both the lymphoma and reference population to investigate this possibility further. Further studies to investigate a basis for the decreased risk in Cross Breed Dogs and to identify markers of decreased risk might provide a genetic rationale for preventative measures in high risk populations.

Sex has been reported as a statistically significant risk factor in a previous study.<sup>14</sup> One other study did not report a *P* value, but the CI did not include 1.0, an indicator of potential significance, with males at increased risk.<sup>8</sup> The increased male risk was found in our study overall, and within Cross Bred Dogs, Boxers, and Cocker Spaniels. The increased risk for females in Bulldogs and Dogue de Bordeaux was unexpected and could indicate a different underlying genetic predisposition in these breeds compared to others. Some lymphoma subtypes in people have an increased risk in males compared to females.<sup>20</sup>

**TABLE 4** Sex and neutering risk for lymphoid neoplasia

Breed	Neutered versus intact			Males neutered versus intact			Females neutered versus intact		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
All cases	<b>3.2</b>	<b>2.9–3.5</b>	<b>&lt;.01</b>	<b>2.8</b>	<b>2.5–3.2</b>	<b>&lt;.001</b>	<b>4.2</b>	<b>3.5–5.1</b>	<b>&lt;.001</b>
Cross Breed	<b>2.9</b>	<b>1.2–3.6</b>	<b>&lt;.001</b>	<b>2.3</b>	<b>1.8–3.0</b>	<b>&lt;.001</b>	<b>4.6</b>	<b>3.1–6.9</b>	<b>&lt;.001</b>
American Staffordshire Bull Terrier	3.3	1.0–11.0	.07	2.4	0.5–11.9	.45	4.1	0.6–46.1	.27
Australian Cattle Dog	<b>3.5</b>	<b>1.9–6.5</b>	<b>&lt;.001</b>	<b>2.4</b>	<b>1.2–4.5</b>	<b>.01</b>	<b>16.7</b>	<b>3.1–169.9</b>	<b>&lt;.001</b>
Australian Kelpie	2.4	1.2–4.7	.02	1.8	0.7–4.4	.22	3.3	1.1–10.3	.04
Beagle	<b>4.6</b>	<b>2.2–9.9</b>	<b>&lt;.001</b>	<b>3.7</b>	<b>1.6–8.6</b>	<b>&lt;.01</b>	<b>6.6</b>	<b>1.8–28.0</b>	<b>&lt;.01</b>
Border Collie	<b>5.6</b>	<b>3.4–9.4</b>	<b>&lt;.001</b>	<b>4.9</b>	<b>2.6–9.7</b>	<b>&lt;.001</b>	<b>6.6</b>	<b>2.9–14.1</b>	<b>&lt;.001</b>
Boxer	<b>4.4</b>	<b>2.5–7.8</b>	<b>&lt;.001</b>	<b>4.8</b>	<b>2.6–9.0</b>	<b>&lt;.001</b>	<b>6.8</b>	<b>1.9–28.5</b>	<b>&lt;.01</b>
Bull Terrier	<b>4.9</b>	<b>2.0–10.7</b>	<b>&lt;.001</b>	<b>4.6</b>	<b>1.6–12.8</b>	<b>&lt;.01</b>	<b>5.3</b>	<b>1.4–23.4</b>	<b>.02</b>
Bulldog	<b>4.3</b>	<b>2.0–9.2</b>	<b>&lt;.001</b>	<b>2.9</b>	<b>1.1–7.4</b>	<b>.04</b>	<b>8.2</b>	<b>2.0–36.2</b>	<b>&lt;.001</b>
Bullmastiff	<b>3.7</b>	<b>1.9–8.5</b>	<b>&lt;.001</b>	<b>3.2</b>	<b>1.3–8.1</b>	<b>.02</b>	<b>4.0</b>	<b>1.3–11</b>	<b>.01</b>
Cavalier King Charles Spaniel	<b>4.0</b>	<b>1.5–10.4</b>	<b>&lt;.01</b>	<b>5.8</b>	<b>1.6–24.7</b>	<b>&lt;.01</b>	<b>2.4</b>	<b>0.6–10.3</b>	<b>.40</b>
Cocker Spaniel	<b>3.2</b>	<b>1.5–6.9</b>	<b>&lt;.01</b>	<b>4.0</b>	<b>1.5–8.3</b>	<b>&lt;.01</b>	<b>3.0</b>	<b>0.8–12.7</b>	<b>.15</b>
Dobermann	1.9	0.8–4.5	.22	0.5	0.1–2.0	.46	3.4	0.9–15.2	.12
Dogue de Bordeaux	<b>7.8</b>	<b>1.9–35.3</b>	<b>&lt;.01</b>	<b>5.1</b>	<b>0.7–67.0</b>	<b>.12</b>	<b>6.4</b>	<b>0.9–72.2</b>	<b>.09</b>
Fox Terrier	<b>7.0</b>	<b>2.3–21.6</b>	<b>&lt;.001</b>	<b>12.6</b>	<b>2.1–130.6</b>	<b>&lt;.001</b>	<b>4.4</b>	<b>1.2–19.1</b>	<b>.03</b>
German Shepherd Dog	<b>2.6</b>	<b>1.6–4.2</b>	<b>&lt;.001</b>	<b>4.3</b>	<b>2.2–8.9</b>	<b>&lt;.001</b>	<b>1.2</b>	<b>0.6–2.4</b>	<b>.63</b>
Golden Retriever	<b>4.5</b>	<b>2.9–7.2</b>	<b>&lt;.001</b>	<b>3.9</b>	<b>2.1–7.3</b>	<b>&lt;.001</b>	<b>5.5</b>	<b>2.6–12.0</b>	<b>&lt;.001</b>
Great Dane	<b>3.6</b>	<b>1.3–9.7</b>	<b>.01</b>	<b>5.2</b>	<b>1.3–23.5</b>	<b>.02</b>	<b>2.2</b>	<b>0.5–10.2</b>	<b>.50</b>
Jack Russell Terrier	<b>4.0</b>	<b>2.0–8.3</b>	<b>&lt;.001</b>	<b>6.7</b>	<b>2.3–20.6</b>	<b>&lt;.001</b>	<b>2.4</b>	<b>1.0–5.7</b>	<b>0.06</b>
Labrador Retriever	<b>3.6</b>	<b>2.3–5.7</b>	<b>&lt;.001</b>	<b>2.8</b>	<b>1.7–4.8</b>	<b>&lt;.001</b>	<b>8.3</b>	<b>2.8–25.1</b>	<b>&lt;.001</b>
Maltese	<b>4.2</b>	<b>1.9–9.0</b>	<b>&lt;.001</b>	<b>3.1</b>	<b>1.1–8.4</b>	<b>.03</b>	<b>6.0</b>	<b>1.6–25.4</b>	<b>&lt;.01</b>
Mastiff	<b>8.6</b>	<b>2.7–27.5</b>	<b>&lt;.001</b>	<b>7.1</b>	<b>1.7–32.3</b>	<b>&lt;.01</b>	<b>8.5</b>	<b>1.3–91.7</b>	<b>.02</b>
Rhodesian Ridgeback	1.8	0.8–4.2	.24	3.1	1.0–10.1	.06	0.9	0.3–3.2	>.99
Rottweiler	<b>2.0</b>	<b>1.3–3.2</b>	<b>&lt;.01</b>	<b>2.0</b>	<b>1.1–3.5</b>	<b>.02</b>	<b>2.1</b>	<b>1.0–4.3</b>	<b>.07</b>
Schnauzer (Miniature)	1.5	0.6–4.2	.63	1.6	0.4–7.2	.74	1.4	0.4–6.5	>.99
Shetland Sheepdog	2.5	0.9–6.8	.09	3.8	0.9–17.0	.07	1.5	0.3–7.0	>.99
Shih Tzu	<b>4.9</b>	<b>1.3–20.8</b>	<b>.01</b>	<b>4.7</b>	<b>0.8–50.0</b>	<b>.13</b>	<b>4.8</b>	<b>0.9–50.3</b>	<b>.15</b>
Staffordshire Bull Terrier	<b>2.9</b>	<b>1.9–4.5</b>	<b>&lt;.001</b>	<b>2.3</b>	<b>1.4–3.7</b>	<b>&lt;.001</b>	<b>5.5</b>	<b>2.3–12.7</b>	<b>&lt;.001</b>
Welsh Corgi	<b>5.1</b>	<b>0.4–22.3</b>	<b>.01</b>	<b>6.8</b>	<b>1.0–74.2</b>	<b>.04</b>	<b>3.2</b>	<b>0.6–35.4</b>	<b>.47</b>
West Highland White Terrier	2.7	0.9–8.6	.13	2.0	0.6–6.5	.32	∞	0.6–∞	.23
Whippet	5.6	1.0–59.2	.09	3.1	0.5–34.7	.46	∞	0.7–∞	.21

Significant results ( $P < .05$ ) are highlighted in bold.

An increased risk for lymphoma in neutered dogs has been reported in 1 study of all breeds of dogs<sup>14</sup> and 2 that evaluated individual breeds.<sup>16,17</sup> The increase in risk for lymphoma associated with neutering was reported in both males and females in 1 study, and although  $P$  values were not calculated, the lower limit of the 95% CI did not include 1.0<sup>8</sup>. It was reported only in females in 1 study of all breeds<sup>15</sup> and only in males in Golden Retrievers.<sup>17</sup> One study reported an increased risk of lymphoma in neutered females compared to intact females, whereas a decreased risk in neutered males was found.<sup>12</sup> Neutering was found to increase the risk of lymphoid neoplasia in our study overall and in Cross Breed Dogs. Of the 30 breeds with sufficient number for analysis, 23 showed an increased risk with neutering. In 20 of these breeds, neutering increased the risk of lymphoid neoplasia in males and, in 17 breeds, in females. No breeds showed a decreased risk of lymphoid neoplasia in neutered animals. This finding provides an opportunity for further investigation to evaluate the role of hormone exposure in the development of

lymphoid neoplasia, and potentially identify underlying mechanisms. The mechanism of increased risk of lymphoma in neutered dogs is not known, but studies in rodents offer potential insights. Estrogen receptors are found in normal lymphocytes, and expression of estrogen receptor beta is increased in neoplastic lymphocytes.<sup>30</sup> Estrogen agonists have been shown to inhibit cell growth and lead to apoptosis by regulation of many proteins, including cyclins, bcl-2, and kinases.<sup>30</sup> In a study in male rats, the use of an androgen receptor antagonist did not alter growth of xenografted lymphoma whereas inhibition of the conversion of androgen to estrogen led to faster lymphoma growth.<sup>31</sup>

Our study had limitations as do most retrospective studies. The use of data from general practice and use of council registration data to increase the reference population generated a population that should more closely approximate the general canine population, which could be different to both insured and referral hospital populations. Because the health status of the control population was not known and was deidentified, lymphoma cases could have been included in

the reference population. This would lower the observed risk of lymphoma but, when we made estimates of impact, the change in the results was minimal. Insufficient information was available in many of the cases to fully classify the disease that was present in an individual animal including immunophenotype. The process of review by a single board-certified oncologist of all the case data was used to minimize the chance of including cases that were not lymphoma, and although cases might have been excluded, it was more likely than inclusion of cases that were not lymphoma. The information was obtained from medical records and laboratory reports with no possibility of reviewing the cytology or histology upon which the diagnosis was made, or to perform a full review of medical records other than in the referral hospitals. Whereas duplicate entries from 1 source were removed, it is possible that some cases were duplicated if they presented to a general practice and were referred, or the sample was sent to the commercial laboratory from which the reports were obtained. Lymphoma cases could have been included in the control population without knowledge because much of the material had been deidentified. Inclusion of cases can occur with any control population for which complete knowledge of the animal's clinical history is not available. There was some overlap of postcodes between the control population and lymphoma cases, with this overlap accounting for 0.14% of the control population. Removing the possible lymphoma cases involved or removing 4 times the number of potential cases made no difference in the results other than minor differences in breeds with small number of cases and controls. For example, the OR for Golden Retrievers (285 cases, 14 383 controls, and 39 overlap) remained 2.1 if the potential overlapping animals were removed, or 4 times the overlapping number were removed. In foxhounds (6 cases, 58 controls, and overlap 2), the OR increased from 10.7 up to 12.3 when 4 times the overlap was removed for the calculations. There was a discrepancy in the time periods of data collection and there could have been changes in breed preference over this time. The council registration data was from 1 year, and the hospital data was from over 17 years. Despite the relatively large number of cases overall, the presence of 205 breeds led to small groups of <10 individuals when looking at changes within many of the individual breeds.

It would have been ideal to include all confirmed cases from a population where all individuals were recorded. Demographic data in dogs in the general population in theory should be known because registration with the local council and use of microchips are required in Australia. Access to this data was attempted, but could not be obtained. The referral hospitals' populations are representative of the cases seen at those hospitals, but not necessarily of the population from which cases presenting to general practices are seen or the general population of dogs. For that reason, the dog registration data that covered the areas from which most of the cases from the veterinary diagnostic laboratory and the general practices would have been collected was included. We believe that the resulting information is representative of the dog population in Australia, but this could not be confirmed. The percentage of Cross Breed Dogs appears quite different from most other studies. In our study, 28% of the lymphoma cases were Cross Breed Dogs, compared to a range of 10.7%-24.7% in previous studies.<sup>7,9-12,14,15</sup> There is a larger discrepancy in the reference populations with our study having 52.2% of Cross Breed Dogs,

whereas most other studies have had a range between 6.8% and 29.2%,<sup>7,8,10-12,14</sup> including a previous study from UVTHS with 26.3%.<sup>14</sup> One small study from Poland had a similar proportion of 49.8%.<sup>9</sup> This discrepancy might be an indication that the populations from referral institutions or insured dogs are not a true reflection of the general dog population. There might be an increased likelihood of owners of purebred dogs using referral institutions or having their dogs insured as compared to owners of Cross Breed Dogs.

In conclusion, we have identified that breed, sex, and neuter status alter the risk of lymphoma in a large Australian canine population. We identified a number of breeds at both increased and decreased risk of lymphoma that have not been previously identified. A male sex risk was identified overall and within most breeds. Neutering was identified to increase the risk of lymphoma overall and in both males and females, which was consistent across breeds. These 3 factors need to be considered when evaluating lymphoma risk and can be used to plan studies to identify the underlying etiology of these diseases.

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## CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

## OFF-LABEL ANTIMICROBIAL DECLARATION

There was no off-label use of antibiotics in this study.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was necessary.

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