TITLE: Geographic distribution and environmental risk factors of lymphoma in dogs under primary-care in the UK

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Geographic distribution and environmental risk factors of lymphoma in dogs under primary-care in the UK

Abstract

Objectives: To integrate external data sources with VetCompass postcode data to explore the spatial distribution and examine potential associations with environmental risk factors in dogs diagnosed with lymphoma at primary-care veterinary practices.

Methods: Cases of lymphoma were identified from electronic patient records of 455,553 dogs under primary veterinary care during 2013 in the UK. Cases were defined as either laboratory confirmed or non-laboratory confirmed. Disease maps at the postcode-district level were used to visualise the geographic distribution of lymphoma incidence and spatial clustering was explored. Environmental risk factors from external data sources were transferred to a compatible format and logistic regression modelling was used to examine associations between environmental herbicide, fungicide and radon concentrations with lymphoma.

Results: From the denominator population of 455,553 dogs, 279 lymphoma cases (187 with laboratory-confirmation and 93 without) were identified. Heterogeneous geographic variation was observed with weak evidence of clustering around London and the South-West of England ($p = 0.07$). Herbicide and fungicide exposures were weakly associated with a diagnosis of lymphoma in the univariable analysis. After accounting for the age at diagnosis and breed in the multivariable analysis, herbicide exposure was associated with a diagnosis of lymphoma.

Conclusions: Heterogeneous distribution of lymphoma in UK dogs was found providing further evidence for geographic variation of lymphoma. This distribution could in part be

due to underlying environmental risk factors.This study suggested an association with environmental herbicide and canine lymphoma once accounting for age and breed.

Keywords

VetCompass, electronic patient records, primary-care, canine lymphoma, spatial epidemiology.

Introduction

Lymphoma is a malignancy of the lymphatic system that is a commonly diagnosed neoplasia in UK dogs (Dobson *et al.* 2002). The aetiology of lymphoma is multifactorial with no specific definitive causes reported for dogs but with studies identifying a range of patient-based risk factors including certain larger breeds and increased age (Edwards *et al.* 2003, Teske *et al.* 1994, Vezzali *et al.* 2010). Studies examining dogs' geographic areas of residence found those living near radioactive sites, waste incinerators, polluted and industrial areas had an increased risk of lymphoma (Gavazza *et al.* 2001, Kimura *et al.* 2013, Pastor *et al.* 2009).

Due to pathological similarities between human non-Hodgkin lymphoma (NHL) and canine lymphoma, there is interest as dogs as sentinels for the disease in man (National Research Council 1991). Dogs are suggested to live in a certain localised area for their lifetime or remain mainly at the owner's residential address, travelling infrequently from a specific location. Additionally dogs make more practical models to people, due to their shorter life span and lymphoma latency. This could mean that dogs may be better subjects to investigate environmental risk factors than people (National Research Council 1991).

The human literature has shown increasing interest in exploring the role of environmental risk factors in cancer incidence in recent years (Boffetta and Nyberg 2003). *The Cancer Atlas of the UK and Ireland* described the geographic incidence of NHL to show higher rates in males in London, the South West of England and Northern Ireland compared to the national average after standardising for age, while Scotland and Northern Ireland had higher rates in females (Quinn *et al.* 2005). Lower standardised rates for both males and females were found in the midlands and north of England compared to the national average. The heterogeneous geographic distribution could be explained by differing diagnostic criteria across different health authorities and socio-economic deprivation but other unknown risk factors could also have a role. Immunosuppresive agents, genetic factors and chemical exposures such as herbicides and other agrichemicals have been hypothesised to explain differing geographic distributions (Baris *et al.* 1998, Beral *et al.* 1991, Blair *et al.* 1998, Hayes *et al.* 1997, Zahm and Blair 1992). Radon gas is recognised as an important risk factor for lung cancer and has also been suggested to be associated with leukaemia but again no associations have currently been reported for NHL (Forastiere *et al.* 1998, Schwartz and Klug 2016, WHO 2009).

This study aimed to explore the application of animal home location partial postcode data collected within the VetCompass™ programme for geographic studies, to model the spatial distribution and to examine potential associations of environmental risk factors with lymphoma in dogs in the UK.

Materials and Methods

A cross-sectional analysis of a retrospective cohort of dogs attending participating VetCompass practices during 2013 was undertaken. Anonymised electronic patient record

(EPR) data were uploaded to VetCompass from participating veterinary clinics in the UK (VetCompass 2018). The study included dogs with a final diagnosis of lymphoma recorded in the EPR between 1 January 2013 and 31 December 2013. These cases were sub-categorised as 'laboratory confirmed' or 'non-laboratory confirmed'. 'Laboratory confirmed' cases had evidence of at least one of: fine needle aspiration, histological biopsy, a 'Canine lymphoma blood test' (Avacta Animal Health 2018) or blood smear identification of neoplastic lymphocytes by a clinical pathologist. The remaining cases were classified as 'non-laboratory confirmed'. Case-finding from the VetCompass database firstly identified potential lymphoma cases by searching in the clinical notes (search terms: lympho*, lymphoma, lymphosarcoma, LSA, B-cell, T-cell and immunophenotype) and also for the disease specific treatments (search terms: vinc*, doxo*, cyclop* and lomust*). All potential lymphoma cases identified were examined in detail by reading the free text clinical notes to identify dogs meeting the case definition. Potential cases that did not meet the case definition were excluded from analysis. For the analyses, dogs diagnosed with lymphoma were compared to the non-cases that were not identified by the search terms. All data were exported to a spreadsheet, cleaned and duplicates removed in Excel[a]. Sample size calculations estimated that, to detect an odds ratio of 1.75 - 2.00 or greater assuming 10% of the population was exposed to the environmental risk factor of interest, and an incidence of lymphoma 0.06 - 0.08% (Edwards *et al.* 2003) then approximately 175 - 200 cases derived from a population of approximately 350,000 – 400,000 dogs would be required (80% power and 95% confidence) (OpenEpi 2018). Ethics approval was provided by XXXXXX Ethics and Welfare Committee (URN 2015 1369).

Data on potential environmental risk factors were collected for radon and pesticide exposure. Information on radon potential was provided by Public Health England (PHE) for England and Wales for the year 2007 at a resolution of 1km^2. Radon potential was categorised based on the percentage of households estimated to exceed the radon action level of 200Bq/m^3 as follows: < 1%, 1 to < 3%, 3 to < 5%, 5 to < 10%, 10 to < 30% and ≥ 30% (UKradon 2018). Data on pesticides (herbicide and fungicide) were provided by the UK Small Area Health Statistics Unit (SAHSU) for England in 2000 at 1998 census ward level. Pesticides data were originally modelled as part of the Integrated Assessment of Health Risks of Environmental Stressors in Europe (INTARESE) project (Vienneau 2010) and derived from DEFRA's June 2000 Agricultural Returns census, and the Pesticides Usage Survey carried out by the Food and Environment Research Agency (Fera 2018). Data on pesticide levels show the kilograms of pesticide applied per census ward on agricultural land as reported from a national survey of a sample of farms. The pesticides were grouped into categories: a baseline group with no known exposure and three groups split into roughly equal sizes to form 'low', 'moderate' and 'high' exposure groups. Categorisation was based on roughly equal distribution of exposure within the non-cases. Fungicide levels (usage per census ward, in kg): 0 ('no exposure'), 1 to < 89 ('low exposure'), 90 - 529 ('moderate exposure') and ≥ 530 ('high exposure'). Herbicide levels (usage per census ward, kg): 0 ('no exposure'), 1 to < 134 ('low exposure'), 135 – 753 ('moderate exposure') and ≥ 754 ('high exposure'). A combined pesticide variable was formed of four categories depending on the level of exposure to both fungicide and herbicide based around the median exposure of non-cases: low-low (fungicide < 135 kg and herbicide < 313), low-high (fungicide < 135 kg and herbicide ≥ 313), high-low (fungicide ≥ 135 kg and herbicide < 313) and high-high (fungicide ≥ 135 kg and herbicide ≥ 313) (Zahm *et al.* 1990).

Age, breed and maximum recorded bodyweight from the electronic patient records were included as potential confounders in risk factor analyses (Edwards *et al.* 2003, Teske *et al.* 1994). Ages for cases described age at first diagnosis for the condition during 2013. Ages for non-case dogs described age on 31st December 2013. Ages (years) were categorised into 3 groups, formulated around the previously reported median age of diagnosis (Edwards *et al.* 2003, Yau *et al.* 2017): < 8, 8 to < 12 and ≥ 12. Dogs were categorised into their individual breed if at least three cases were present for that particular breed. All other breeds were grouped into an 'other purebred' category. Maximum recorded bodyweight (kg) during 2013 was included categorically as < 10, 10 to < 20, 20 to < 30 and ≥ 30 with missing values included in an unknown group. Descriptive statistics were reported separately for 'laboratory confirmed' and 'non-laboratory confirmed' cases. Comparisons were made using the chi-squared test.

<u>Spatial analysis</u>

Postcode district of dog owners' addresses (i.e. the first part of the postcode, e.g. AL9) were used for analyses. The population at risk was calculated using standardised morbidity ratios (SMR) as observed over expected number of dogs per postcode-district. Choropleth disease maps of the UK were produced to show the spatial distribution of district-level SMRs of lymphoma cases and the corresponding standard errors (SEs) of the SMRs for each district. Global spatial autocorrelation of district-level SMRs was explored using Moran's *I* statistic with Monte-Carlo randomisation and 499 permutations. Postcode districts were considered to be adjacent if they shared a common border or corner (i.e. queen contiguity). The local indicators of spatial association (LISA) scatterplot was used to identify clusters of high-high

SMR districts and spatial outliers. Spatial analyses were performed separately for 'laboratory confirmed' cases and all identified cases. ArcGIS[b] 10.2 was used for spatial data manipulations and Moran's *I* and the LISA statistic were implemented in GeoDA (Anselin et al., 2006).

<u>Environmental risk factor analysis</u>

Environmental risk factor logistic regression models were generated separately for 'laboratory confirmed' cases and all identified cases. The analysis was restricted to dogs at least three years of age. The restricted sample was kept to a relatively young age to minimise losses of cases due to the cut-off, with dogs under three years of age rarely diagnosed with lymphoma (Teske *et al,* 1994).Explanatory variables loosely associated with a diagnosis of lymphoma in the univariable regression model (likelihood ratio test (LRT), $p < 0.20$) were carried forward to the multivariable model. The multivariable model was built using a manual backwards stepwise approach to identify the variables associated with a diagnosis of lymphoma (LRT $p < 0.05$) adjusting for identified confounding factors. Potential confounding variables of environmental risk factors were identified by observing a significant change in variable odds ratios when added to the model (Dohoo *et al.* 2003). Multicollinear variables were assessed by observing a change in the standard errors and confidence intervals when included together in a model (Katz 2011). Only one variable would be included in the situation of multicollinearity. Continuous variables were assessed for linearity using the likelihood ratio test for departure from trend and the likelihood ratio test for extra-linear effect. Analyses were performed in Stata 15[c] and a p-value of < 0.05 was considered significant.

Results

From a study population of 455,553 dogs, 19,791 were excluded from analysis due to lack of postcode location resulting in a study population of 435,762. The clinical records of 1,991 potential cases were identified in the lymphoma search strategy. All those identified were examined in detail against the case definition, retaining 279 cases (187 laboratory confirmed cases, 93 non-laboratory confirmed cases) for analysis. Median age of laboratory confirmed cases was 7.9 years (IQR 5.8 – 10.7) and 10.4 years (8.7 – 12.4) for non-laboratory confirmed cases (X^2: $p < 0.001$). Median age was 4.0 years (1.6 – 7.5) for the non-case dogs. Median maximum recorded bodyweight was 23.5 kg (14.1 – 34.3) for laboratory confirmed cases and 18.6 (8.2 – 31.8) for non-laboratory confirmed cases (X^2: $p = 0.002$). Median bodyweight was 12.0 kg (6.1 – 24.6) for non-cases. The most commonly represented breeds identified were the Staffordshire bull terrier (all cases, n=17), West Highland white terrier (17) boxer (15) and German shepherd (13). No differences in breeds were found between cases with or without laboratory confirmation (X^2: $p = 0.64$).

Spatial analysis

The study dogs were heterogeneously distributed across the UK with twenty (16.26%) of the 123 postcode districts containing < 100 dogs, and two (1.62%) districts containing no dogs. SMR of the laboratory confirmed cases (n=186) was highest in Dudley (West Midlands) with an incidence 4.9 times higher than expected (SE=1.75), with SMRs of Stevenage, Blackburn and North London ≥ 3 (Fig. 1). District level SMRs for laboratory confirmed cases were weakly positively autocorrelated (Moran's *I*: 0.07, p=0.10) and the LISA analysis identified clustering of high SMR districts around London.

When analysing all cases (with or without a laboratory confirmation, n=279), the highest SMR was Dudley with an incidence 3.3 times higher than expected (SE=1.16) and Stevenage, Blackburn, North London, Telford, Leicester and Bournemouth had SMRs ≥ 3 (Fig. 2). A group of four adjacent districts in the south-west of England (Bournemouth, Dorchester, Salisbury and Swindon) had SMRs ≥ 1.8 but also had correspondingly high SEs as a result of low populations as risk. Conversely, the SMRs of 1.2 – 1.8 observed in the south-east of England and around London had smaller corresponding SEs due to larger numbers within the denominator. Lower rates (SMRs < 1) were observed in the east-midlands of England including Peterborough, Northampton and Milton Keynes postcode districts. There was weak evidence of positive autocorrelation of district-level SMRs of all cases (Moran's *I*: 0.07, p=0.07) with clustering of high SMR districts around London as well as in the south-west of England (Fig. 3).

Environmental risk factor analysis

Dogs < 3 years of age were excluded from logistic regression analysis. This age restriction retained 276/279 (98.9%) cases and 270,736/453,562 (59.7%) of non-cases for further analysis. Of the cases excluded, one (0.5%) was laboratory confirmed and two (2.2%) were non-laboratory confirmed. After excluding those < 3 years, median age of laboratory confirmed cases was 8.2 years (IQR 6.0 – 10.7) and 10.5 years (9.0 – 12.4) for non-laboratory confirmed cases. Median age was 6.7 years (4.6 – 9.6) for the non-case dogs. Maximum radon potential was available for 269 cases (97.4%) and 257,102 (95.0%) non-cases. One hundred and twenty-one laboratory confirmed cases (67.2%) lived in an area with a maximum radon potential < 1% compared to 45 (50.0%) non-laboratory confirmed cases and 165,352 (64.3%) of non-cases. Pesticide concentrations were available for 256 cases

(92.8%) and 244,620 (90.4%) non-cases. Median herbicide concentration was 0 kg (0 – 205) in laboratory confirmed cases, 8 kg (0 – 252) in non-laboratory confirmed cases and 0 kg (0 – 218) in non-case dogs. Median fungicide concentration was 0 kg (0 – 113) in laboratory confirmed cases, 0 kg (0 – 122) for non-laboratory confirmed cases and 0 kg (0 – 120) in non-cases. Herbicide and fungicide were non-linearly associated with a diagnosis of lymphoma and were therefore categorised during analysis.

For laboratory confirmed cases, maximum radon potential and fungicide exposure were not associated at the univariable level (LRT p = 0.83 and 0.57 respectively) (Table 1). Herbicide exposure was associated with a lymphoma diagnosis at the univariable level (p = 0.09) but was not retained in the multivariable analysis (p = 0.10). When including dogs without laboratory confirmation, maximum radon potential was not associated with lymphoma diagnosis (p = 0.73), however fungicide and herbicide exposure were weakly associated with the outcome (p = 0.13 and 0.01 respectively) in the univariable analysis and were taken forward for consideration in the multivariable model (Table 1). After adjusting for potential confounding factors in the multivariable analysis, herbicide exposure was associated with a diagnosis of lymphoma (p = 0.02) (Table 2). Dogs with a 'moderate' herbicide exposure level (135 – 754 kg usage per census ward) were associated with an increased odds of having lymphoma compared to those with no herbicide exposure (OR 1.55 (95% CI 1.13 – 2.13)). During analysis, weight was not found to be statistically collinear with breed. However, it was not retained in the final model as it could be considered biologically related to breed and its inclusion had little confounding effect on the herbicide effect measures.

Discussion

This study has demonstrated the potential to link primary-care veterinary practice health records across the UK with environmental risk factor data from external sources via owner's residential partial postcodes. Results of this study highlighted variation in geographic distribution of lymphoma cases with weak evidence of clustering around London and the south-west coast of England. The spatial distribution described was similar to the distribution of non-Hodgkin lymphoma in humans which found higher rates in males in London, the south-west of England and Northern Ireland (Quinn *et al.* 2005). Quinn et al (2005) found lower rates in the midlands and the north of the country. A similar cluster of lower rates were observed in the current study in the East Midlands including Peterborough, Northampton and Milton Keynes postcode areas.

Environmental herbicide was found to be statistically associated with a diagnosis of lymphoma in the multivariable analysis including both laboratory and non-laboratory confirmed cases, however was not retained in the multivariable analysis when including only laboratory confirmed cases. This could suggest further analyses with a larger number of cases and greater statistical power is merited. The 'moderate' herbicide exposure group was associated with a diagnosis of lymphoma (OR 1.55 (95% CI 1.13 – 2.13) compared to dogs with no herbicide exposure. No associations were found between the 'low' (OR 1.13 (95% CI 0.79 – 1.62)) or 'high' (OR 0.78 (95% CI 0.52 – 1.19)) herbicide exposure groups when compared to those with no herbicide exposure therefore no demonstration of a dose-response of exposure was evident. Fungicide levels were weakly associated in the univariable analysis but were not retained in multivariable analyses. Evaluation of links with non-Hodgkin lymphoma and agrichemicals has been of interest in the human literature however no strong association has been reported (Baris *et al.* 1998, Blair *et al.* 1998, Hayes *et al.* 1997, Zahm and Blair 1992). In the veterinary literature, a previous study in the US found an increased risk of lymphoma in dogs with exposure to the herbicide 2,4-dichlorophenoxyacetic acid use by owners at home on their lawn (Hayes *et al.* 1991). A case-control study in Italy also investigated the owner's response to their dogs contact with herbicides in and around the home with no associations found (Gavazza *et al.* 2001). In the wider veterinary literature, an association with herbicides and the risk of transitional cell carcinoma of the urinary bladder in Scottish terriers has been suggested (Glickman *et al.* 2004). Few studies have examined fungicide exposure levels and lymphoma. One human study in the US looking at non-Hodgkin lymphoma examined pesticide use and found increased risk in those with combined herbicide and fungicide exposure (Zahm *et al.* 1990). In the current study, there was no indication to suggest a similar interaction however there were very few cases within the discordant categories.

Regression analyses were restricted to dogs three years of age. Limiting the analysis to a slightly older population increased the likelihood of temporal association with environmental exposures and a diagnosis of lymphoma. A restriction of three years of age was applied to minimise the number of cases excluded and therefore the potential bias introduced (Dohoo *et al.* 2003). The data available on pesticide levels across England reported the agricultural kilogram application per census ward. There are limitations to these data; they were collected from a survey of sample farms in 2000 though geographical exposure patterns after 2000 were likely to be similar assuming agricultural practice did not change (Hansell et al. 2014). Further, no information was included about pesticide application on other land (such as home or garden use). However farming pesticide use makes up the majority of UK usage (Hansell *et al.* 2014). The pesticide data resolution was at census ward level therefore the direct level of exposure to the dogs' geographical residence prior to their diagnosis in 2013 is unknown. The continuous pesticide data were not linearly associated with a diagnosis of lymphoma therefore required categorisation for analysis. With no standardised categorisation to the authors' knowledge, cut offs of numerically similar grouping sizes were used. The pesticide data described a variety of compounds used each with varying levels of evidence of their carcinogenicity (IARC 2015, Fera 2018). Therefore the level of association of certain compounds deemed more probably carcinogenic could have been diluted in analysis.

Radon exposure was not found to be associated with canine lymphoma in this analysis. However the available data only reflected one measure of exposure, maximum radon potential. The maximum radon potential is the percentage of households in that area where radon levels are above the action level of 200 Bq/m^3, which is the action level that it is advised by PHE. Homes with levels greater than this are advised to carry out remedial works to the property to reduce exposure. The areas with over 30% of properties estimated to be above this action level are termed 'higher risk' and those between 10 – 30% at 'intermediate risk' (UKradon 2018). Such categorisation may miss more subtle exposure associations and in the current analysis only small numbers of cases fell within the highest exposure categories limiting the ability to evaluate an association. Future work could explore other measures of radon exposure if these data became available.

The case definition in this study encompassed all forms of lymphoma. Environmental risk factors could have varying levels of effect depending on the form of lymphoma diagnosed or the route of environmental agent exposure, for example it could be hypothesised that direct contact with pesticides may increase the risk of epitheliotropic lymphoma incidence. Additionally dogs included with a non-laboratory diagnosis could have been incorrectly diagnosed with lymphoma within the EPR. Their inclusion required only a veterinary clinical diagnosis and increased the statistical power of the study. The observed association of herbicide found within the non-laboratory confirmed group could be explained by bias introduced due to misclassification because significance was not reached in the multivariable model for laboratory confirmed cases. The distribution of herbicide exposure was similar for cases with and without a laboratory diagnosis. Therefore the statistical significance may reflect the increased study power of including both case types and a true underlying association, though further work would be required to confirm this. There was a difference in the age at diagnosis and bodyweight in these two case groups with older dogs generally not obtaining a confirmed diagnosis ($p < 0.001$), suggesting difference in the population were more likely to relate to different diagnostic approaches in older animals. Bodyweight difference in these dogs could be related to the different forms of lymphoma and possibly a differing breed predisposition, with a veterinary diagnosis of multicentric lymphoma likely to be easier to identify than other forms due to its characteristic presentation (Edwards et al, 2003). Breed distributions across the two case groups appeared approximately comparable.

There were limitations to the study. Data were not collected specifically for research purposes, limiting the ability to evaluate the primary exposures of interest. The length of time the dogs resided at the recorded address was unknown and it was assumed that dogs spent the majority of their lives at that address. The environmental risk factor data available for analysis may only partially reflect the likely exposure of dogs in that geographical area and as any underlying association may be diluted by this, an increased number of cases would be required to increase power and detect more subtle effects. Though the current study reported a relatively large number of cases, future work would benefit from further cases to improve this statistical power. The current analysis could have missed areas with significant clusters due to its moderate statistical power and the resultant low numbers within each grouping after stratifying into the 123 postcode districts. Ideally smaller sized areas or point data would have been examined but this was not practical due to relatively low numbers of cases. Nonetheless the results of this study derive from the largest study to date on canine lymphoma geographical distribution and are likely generalisable and representative to the UK dog population as the data were derived from a large sample of dogs under veterinary care across a network of primary care veterinary practices.

Conclusion

In summary, this is the first study to examine the geographic distribution of lymphoma in UK dogs under primary veterinary care. The study successfully linked external data sources with VetCompass partial postcode data and related health data, demonstrating its application for future research. There were geographic differences in the incidence rates of lymphoma in UK dogs with higher frequencies in London and the south-west of England similar to results previously found with non-Hodgkin lymphoma in humans. The explanation for this distribution could in part be due to underlying environmental risk factors with this study suggesting an association with canine lymphoma and herbicide exposure, once accounting for the dogs' age and breed.

Abbreviations

CI; confidence interval, SD; standard deviation, LRT; likelihood ratio test, EPR; electronic patient record, IQR; interquartile range, PHE; Public Health England, ICL; Imperial College London.

Ethics approval

Ethical approval was granted by XXXXXXX Ethics and Welfare Committee (URN 2015 1369).

Authors' contributions

All authors made contributions to conception and design, acquisition and extraction of data, and to analysis and interpretation of the results. All authors were involved in drafting and revising the manuscript and gave final approval of the version to be published. Each author agrees to be accountable for all aspects of the accuracy or integrity of the work.

Competing interests

The authors have no conflicts of interest to declare.

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Figures

Figure 1: Choropleth maps displaying a) district-level standardised morbidity ratios (SMRs) and b) corresponding standard errors (SEs) of canine lymphoma cases with a laboratory confirmed diagnosis in primary-care practices across the UK in 2013 (n=186).

Figure 2: Choropleth maps displaying a) district-level standardised morbidity ratios (SMRs) and b) corresponding standard errors (SEs) of all canine lymphoma cases (with and without a laboratory confirmation in primary-care practices across the UK in 2013 (n=279).

Figure 3: A local indicator of spatial autocorrelation (LISA) choropleth map highlighting postcode-districts with high-high spatial autocorrelation of all canine lymphoma cases (with or without laboratory confirmation) in primary-care practices across the UK in 2013 (Moran's *I*: 0.07, p = 0.07) (n=279).

Tables

Table 1: Descriptive and univariable logistic regression analysis of environmental risk factors in dogs with a laboratory confirmation of lymphoma and all dogs with lymphoma (with and without laboratory-confirmation), attending UK primary-care veterinary practices in 2013.

Variable	Non-cases (%)	Laboratory confirmed cases (%)	Odds ratio (95% Confidence Intervals)	LRT p-value	All cases (%)	Odds ratio (95% Confidence Intervals)	LRT p-value
Age (years)				0.001			<0.001
< 8	168407 (62.20)	91 (48.92)	-		105 (38.32)	-	
8 - <12	69394 (25.63)	66 (35.48)	1.76 (1.28 - 2.42)		112 (40.88)	2.59 (1.98 - 3.38)	
≥12	32934 (12.16)	29 (15.59)	1.63 (1.07 - 2.48)		57 (20.80)	2.78 (2.01 - 3.83)	
Breed				<0.001			<0.001
Crossbreed	61050 (22.61)	47 (25.27)	-		75 (27.27)	-	
Other purebreed	81179 (30.07)	37 (19.89)	0.59 (0.38 - 0.91)		53 (19.27)	0.53 (0.37 - 0.76)	
Border collie	8855 (3.28)	5 (2.69)	0.73 (0.29 - 1.84)		8 (2.91)	0.74 (0.35 - 1.53)	

Boxer	4259 (1.58)	12 (6.45)	3.66 (1.94 - 6.90)		15 (5.45)	2.87 (1.65 - 5.00)	
Bull terrier	1197 (0.44)	4 (2.15)	4.34 (1.56 - 12.07)		5 (1.82)	3.40 (1.37 - 8.42)	
Cavalier King Charles spaniel	6901 (2.56)	6 (3.23)	1.13 (0.48 - 2.64)		7 (2.55)	0.83 (0.38 - 1.79)	
Cocker spaniel	10205 (3.78)	7 (3.76)	0.89 (0.40 - 1.97)		7 (2.55)	0.56 (0.26 - 1.21)	
Dogue de Bordeaux	782 (0.29)	2 (1.09)	3.32 (0.81 - 13.70)		4 (1.45)	4.16 (1.52 - 11.41)	
German shepherd dog	8047 (2.98)	8 (4.30)	1.29 (0.61 - 2.73)		12 (4.36)	1.21 (0.66 - 2.23)	
Golden retriever	3944 (1.46)	4 (2.15)	1.32 (0.47 - 3.66)		8 (2.91)	1.65 (0.80 - 3.43)	
Jack Russell terrier	19578 (7.25)	7 (3.76)	0.46 (0.21 - 1.03)		12 (4.36)	0.50 (0.27 - 0.92)	
Labrador retriever	22119 (8.19)	9 (4.84)	0.53 (0.26 - 1.08)		12 (4.36)	0.44 (0.24 - 0.81)	
Lurcher	2099 (0.78)	5 (2.69)	3.09 (1.23 - 7.79)		6 (2.18)	2.33 (1.01 - 5.35)	
Schnauzer	2861 (1.06)	5 (2.69)	2.27 (0.90 - 5.71)		5 (1.82)	1.42 (0.57 - 3.52)	
Scottish terrier	672 (0.25)	3 (1.61)	5.80 (1.90 - 18.68)		4 (1.45)	4.84 (1.77 - 13.29)	
Springer spaniel	7563 (2.80)	4 (2.15)	0.69 (0.25 - 1.91)		8 (2.91)	0.86 (042 - 1.79)	
Staffordshire bull terrier	19353 (7.17)	9 (4.84)	0.60 (0.30 - 1.23)		17 (6.18)	0.72 (0.42 - 1.21)	
West Highland white terrier	9308 (3.45)	12 (6.45)	1.67 (0.89 - 3.16)		17 (6.18)	1.49 (0.88 - 2.52)	
Weight (kg)				<0.001			<0.001
Unknown	27012 (9.98)	9 (4.84)	0.30 (0.15-0.61)		27 (9.78)	0.66 (0.43 - 1.02)	
<10	67158 (24.81)	16 (8.60)	0.22 (0.13 - 0.38)		23 (8.33)	0.23 (0.14 - 0.36)	
10 - <20	67516 (24.94)	49 (26.34)	0.66 (0.46 - 0.97)		75 (27.71)	0.73 (0.54 - 1.00)	
20 - <30	52299 (19.32)	50 (26.88)	0.88 (0.60 - 1.27)		65 (23.55)	0.82 (0.59 - 1.13)	
≥30	56750 (20.96)	62 (33.33)	-		86 (31.16)	-	
Maximum radon potential (% of homes >200 Bq/m³)							
<1	165352 (64.31)	121 (67.22)	-	0.83	165 (61.34)	-	0.73
1 - <3	53045 (20.63)	37 (20.56)	0.95 (0.66 - 1.38)		62 (23.05)	1.17 (0.87 - 1.57)	
3 - <5	16148 (6.28)	8 (4.44)	0.68 (0.33-1.38)		20 (7.43)	1.24 (0.78 - 1.97)	
5 - <10	13647 (5.31)	8 (4.44)	0.80 (0.39 - 1.64)		11 (4.09)	0.81 (0.44 - 1.49)	
10 - <30	6285 (2.44)	5 (2.78)	1.09 (0.44 - 2.66)		8 (2.97)	1.28 (0.63 - 2.59)	
≥30	2625 (1.02)	1 (0.56)	0.52 (0.07 - 3.73)		3 (1.12)	1.15 (0.37 - 3.59)	
Fungicide (kg usage per census ward)				0.57			0.13
No exposure	142784 (58.37)	98 (56.32)	-		141 (55.08)	-	
Low (1-89)	33940 (13.87)	28 (16.09)	1.20 (0.79 - 1.83)		42 (15.41)	1.25 (0.89 - 1.77)	

Moderate (90-530)	34023 (13.91)	28 (16.09)	1.20 (0.79 - 1.83)		45 (17.58)	1.34 (0.96 - 1.87)	
High (>530)	33873 (13.85)	20 (11.49)	0.86 (0.53 - 1.39)		28 (10.94)	0.84 (0.56 - 1.26)	
Herbicide (kg usage per census ward)				0.09			0.01
No exposure	137698 (56.29)	95 (54.60)	-		134 (52.34)	-	
Low (1-134)	35564 (14.54)	25 (14.37)	1.02 (0.66 - 1.58)		40 (15.63)	1.16 (0.81 - 1.65)	
Moderate (135-754)	35700 (14.59)	36 (20.69)	1.46 (1.00 - 2.15)		55 (21.48)	1.58 (1.16-2.18)	
High (>754)	35658 (14.58)	18 (10.34)	0.73 (0.44 - 1.21)		27 (10.55)	0.78 (0.51-1.18)	
Fungicide-herbicide				0.99			0.72
Low-Low	205526 (75.91)	142 (76.34)	-		209 (75.72)	-	
Low-High	7976 (2.95)	6 (3.23)	1.09 (0.48 – 2.47)		11 (3.99)	1.36 (0.74 – 2.49)	
High-Low	4846 (1.19)	3 (1.61)	0.90 (0.29 – 2.81)		6 (2.17)	1.22 (0.54 – 2.74)	
High-High	52388 (19.35)	35 (18.82)	0.97 (0.67 – 1.40)		50 (18.12)	0.94 (0.69 – 1.28)	

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Table 2: Multivariable logistic regression analysis of environmental risk factors for lymphoma in all dogs (with and without laboratory confirmation) attending UK primary-care veterinary practices in 2013, after accounting for the dog’s age and breed. N=265 (cases), N=404,076 (non-cases).

Variable	Odds Ratio	95% Confidence Interval	LRT p-value
Herbicide (kg usage per census ward)			
No exposure	-		0.02
Low (1-134)	1.13	0.79 – 1.62	
Moderate (135-754)	1.55	1.13 – 2.13	
High (>754)	0.78	0.52 – 1.19	
Age (years)			
<8	-		<0.001

8 - <12	2.41	1.83 – 3.19	
>=12	2.66	1.89 – 3.75	
Breed			
Crossbreed	-		<0.001
Other purebreed	0.57	0.39 – 0.83	
Border collie	0.68	0.31 – 1.47	
Boxer	3.08	1.73 – 5.49	
Bull terrier	3.15	1.15 – 8.67	
Cavalier King Charles spaniel	0.98	0.45 – 2.13	
Cocker spaniel	0.65	0.30 – 1.42	
Dogue de Bordeaux	6.33	2.29 – 17.52	
German shepherd dog	1.12	0.58 – 2.19	
Golden retriever	1.48	0.68 – 3.22	
Jack Russell terrier	0.55	0.30 – 1.02	
Labrador retriever	0.45	0.24 – 0.85	
Lurcher	2.47	1.07 – 5.71	
Schnauzer	1.72	0.69 – 4.27	
Scottish terrier	4.03	1.26 – 12.85	
Springer spaniel	0.71	0.31 – 1.64	
Staffordshire bull terrier	0.77	0.45 – 1.32	
West Highland white terrier	1.50	0.88 – 2.55	

Footnotes

[a] Excel, Microsoft Corporation, Redmond, WA

[b] ArcGIS, Environmental Systems Research Institute, CA

[c] Stata 15, StataCorp LP, College Station, TX

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