

Incidence and risk factors for feline lymphoma in UK primary-care practice

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OBJECTIVES: The study aimed to estimate the incidence and prevalence of feline lymphoma in cats attending primary-care practices across the UK and to identify patient-based and environmental (radon and pesticide exposure) risk factors.

MATERIALS AND METHODS: Case records from the VetCompass programme from primary-care veterinary practices in the UK were searched for a diagnosis of lymphoma in cats in 2016. Cases were required to have had an external laboratory confirmed diagnosis based on cytology and/or histopathology. A nested case-control study design was used to identify risk factors for lymphoma using multivariable logistic regression.

RESULTS: From a cohort of 562,446 cats under veterinary care at VetCompass participating practices in 2016, a total of 271 lymphoma cases were identified (prevalence: 48/100,000, 95% confidence interval (CI) 44 to 56/100,000; incidence 32/100,000, 95% CI 26 to 35/100,000). There were 180 incident lymphoma cases and 803 controls, all aged 2 years and older. Male (odds ratio (OR) 1.7, 95% CI 1.2 to 2.4), insured (OR 3.6, 95% CI 2.3 to 5.6) and older cats (compared to cats 2 to <5 years, OR 5.0, 95% CI 2.8 to 8.8) were associated with increased odds of lymphoma diagnosis. Vaccinated cats were associated with decreased odds (OR 0.7, 95% CI 0.5 to 1.0) compared to unvaccinated cats, although the type of vaccination received was not statistically significant. Breed and environmental factors studied were not associated with a diagnosis of lymphoma.

CLINICAL SIGNIFICANCE: This is the first study to estimate the frequency and report risk factors for lymphoma in cats attending UK primary-care practice.

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INTRODUCTION

Feline lymphoma is a neoplastic disorder of the haemopoietic system that has been reported as the most commonly diagnosed neoplasia in domestic cats (Dorn 1967, Dorn *et al.* 1968). Haematopoietic tumours have been estimated to account for one-third of all feline tumours with lymphoma comprising 50 to 90% of these (Hardy 1981, Haga *et al.* 1988). However, there is as yet no information regarding the incidence and prevalence of feline lymphoma in the UK.

Lymphoma in cats typically presents in older domestic cross-breed cats with a median age at diagnosis of 10 years (Gabor *et al.* 1998, Sato *et al.* 2014). Alimentary lymphoma was described as the most common anatomical location, representing 40 to 50% of feline lymphomas in previous studies, with the majority of cats testing negative on blood tests for feline leukaemia virus (FeLV) (Gabor *et al.* 1998, Sato *et al.* 2014). Previously reported risk factors for feline lymphoma include breed (in particular Siamese and Oriental breeds) (Gabor *et al.* 1998, Louwerens *et al.* 2005, Fabrizio *et al.* 2014), FeLV and feline immu-

odeficiency virus (FIV) positive status (Shelton *et al.* 1990, Louwerens *et al.* 2005). A male predisposition has been suggested in a few reports (Gabor *et al.* 1998, Vail *et al.* 1998, Meichner *et al.* 2012) although other studies have failed to demonstrate an association between lymphoma, sex and neutering status (Haga *et al.* 1988, Louwerens *et al.* 2005, Sato *et al.* 2014).

Domestic environmental influences as risk factors for feline lymphoma have become of interest since the publication of a USA study (Bertone *et al.* 2002), showing that cats exposed to tobacco smoke had increased risk of developing lymphoma compared to cats from non-smoking households. Other environmental risk factors for lymphoma in both human and domestic species include exposure to radon and agricultural chemicals (Zahm *et al.* 1990, Hayes *et al.* 1991, Zahm & Blair 1992, Baris *et al.* 1998, Gavazza *et al.* 2008, Ha *et al.* 2017). However, conflicting evidence in both the human and canine literature, in addition to the limited studies involving cats, warrants further investigation of the role of radon and pesticide exposure and their association with lymphoma (Hayes *et al.* 1991, Zahm & Blair 1992, Baris *et al.* 1998, Forastiere *et al.* 1998, Gavazza *et al.* 2008, Ha *et al.* 2017).

To date, there have been no studies exploring radon and exposure to agricultural chemicals as risk factors for feline lymphoma. Additionally, no study has evaluated incidence, prevalence or risk factors for feline lymphoma using nationwide primary-care practice data in the UK. Previous veterinary research has so far been restricted to studying cats from referral practices, which may have limited relevance to the wider UK cat population (Bartlett *et al.* 2010). The current study aimed to evaluate the frequency of feline lymphoma and its associated risk factors, including signalment, vaccination status, FeLV status, FIV status and environmental risk factors, in cats registered to UK primary-care veterinary practices during 2016. The hypotheses for this study were based on current literature that older cats and Siamese/Oriental breeds would have higher odds of lymphoma and cats with FeLV or FIV would be at increased odds. Environmental factors and vaccination status were explored as additional potential risk factors.

MATERIALS AND METHODS

Data were collected from the VetCompass programme, which collates electronic patient records (EPRs) from primary-care veterinary practices in the UK (VetCompass 2020). The denominator population included cats in the VetCompass cohort that were under veterinary care in 2016. Ethical approval was provided by the Royal Veterinary College Ethics and Welfare Committee (SR2018-1652).

Candidate cases of lymphoma from the VetCompass database were identified by searching within the clinical notes using the search terms: lymphoma-1, lymphosarc*, COP, LGAL, HGAL, cyclop*, vincr*, lomust*, chlorambucil-1, doxorubicin-1. Confirmed cases of lymphoma were defined as veterinary diagnosed cases within 1 January 2016 and 31 December 2016; either prevalent or incident. "Prevalent" cases were those first diag-

nosed before the study period and "incident" cases were those first diagnosed during the study period. Cases were required to have had an external laboratory confirmed diagnosis based on cytology and/or histopathology. In some cases, PCR for Antigen Receptor Rearrangements (PARR) or immunohistochemistry/immunocytochemistry was used as an additional confirmatory test. Cases with a diagnosis of leukaemia where lymphoid neoplasia appeared to primarily affect the bone marrow or blood, without obvious lymph node involvement/lymphoid masses, were excluded.

A case-control study design nested within the cohort of cats under veterinary care at VetCompass participating practices in 2016 was used to identify risk factor association with lymphoma diagnosis. Only cats aged 2 years and older in 2016 were evaluated in the risk factor analysis for both cases and controls. Cases without a diagnosis date and those that were "prevalent" were excluded from the risk factor analysis (incident cases only were used). Control cats were selected from the "non-cases" within the denominator population using the random generator (RAND) in Excel (Microsoft 2019). All cats that did not fit the case definition were identified as a "non-case" and were eligible for selection as controls.

Data that were routinely available included demographic (sex, date of birth, clinic ID veterinary group, owner partial postcode) and clinical data (free-text clinical notes, VeNom diagnostic terms). The EPRs of cats identified as lymphoma cases were then further examined to extract information on histological/cytological grade, cell size, anatomical location, FeLV status, FIV status and vaccination status. Grade and cell size were extracted from the pathology report in the clinical notes when provided. Grade classification represented the grade given by pathologist based either on cytology or histology results and was categorised as either "low" or "intermediate/high" and cell size was categorised as "small" or "intermediate/large." Information in the clinical notes regarding physical examination, ultrasound scan results, radiographs and exploratory surgical evaluation was used to classify the anatomical location of lymphoma cases and categorise them according to a modified version of a published anatomical definition (Gabor *et al.* 1998) (Table 1). FeLV status and FIV status were categorised separately as "Positive," "Negative" or "Not Tested." "Positive" diagnosis of FeLV or FIV was determined by an antigen- or antibody-based screening test, respectively, which could be performed "in-house" or at an external laboratory. FeLV and FIV status of the selected controls were manually assessed using the same method and definition criteria described for lymphoma cases.

Vaccination status of cases and controls was analysed statistically in two ways. The first method categorised cats as "vaccinated" or "no evidence of vaccination." Classification as "vaccinated" required evidence of vaccination in their EPR before confirmatory diagnosis of lymphoma for the cases or 31 December 2016 for the controls. The second method used to assess vaccination status grouped cats depending on the type of vaccinations received based on available treatment data for each cat. Cases and controls were categorised into four groups depending on the type of vaccinations present in their clinical records before

Table 1. Classification of feline lymphoma based on anatomical tissue involvement as described by Gabor *et al.* (1998)

Anatomical Location	Subcategory	Organ/tissue involvement
Mediastinal		Any structure within the mediastinal space
Abdominal	Alimentary	Gastrointestinal tract and associated lymph nodes excluding liver and pancreas
	Renal	Involving one or both kidneys
	Hepatic	Sole involvement of liver
	Splenic	Sole involvement of spleen
	Combination	Combination of two or more of the abdominal subcategories
Nodal	Solitary	One peripheral lymph node only
	Regional	A chain of adjacent lymph nodes involving lymph nodes on one side of the diaphragm
	Multinodal	Many or all peripheral lymph nodes involving lymph nodes on both sides of the diaphragm
Atypical	Respiratory	A structure involving any of the respiratory passages (e.g. nasal cavity, pharynx, larynx, trachea)
	Other	Other non-lymphoid tissues such as the CNS and skin
Mixed		Combination of two or more of the above categories

the confirmatory diagnosis of cases or December 31, 2016 for controls: (1) "Not vaccinated," (2) "Vaccinated Unknown," (3) "Vaccinated: no FeLV," (4) "Vaccinated: FeLV." "Vaccinated Unknown" referred to cats who had evidence of vaccination in their clinical notes but no information on the type of vaccinations given. "Vaccinated: no FeLV" were cats with known vaccinations which did not include FeLV vaccination. "Vaccinated: FeLV" were cats vaccinated against FeLV with or without other vaccinations. It was not recorded in this study whether vaccinations were "up to date."

The age (years) of cases was calculated using their date of birth and date at which a first laboratory-confirmed diagnosis of lymphoma was obtained. When an exact date for laboratory diagnosis was not available, date at which the pathology sample was sent plus 3 days was used to estimate age. Age of controls was calculated using their date of birth and the date midway through the study period, June 30, 2016. Age was restricted to include only those aged 2 years and above to make the age-distribution of the case and control populations more similar in order to help control for time-related confounding variables such as exposure to environmental factors. Age (years) was categorised into four groups for analysis: 2 to <5, 5 to <8, 8 to <11 and ≥ 11 . Cats aged 2 to <5 years were used as the baseline in the analysis. Cats were categorised into a "Breed" variable using VeNom standardised breed terms (VeNom coding group, 2017). Breeds with three or more cases were included individually as a breed category while all remaining purebreds were grouped as "Other Purebreds." All non-purebred cats were classified as crossbred. A binary purebred variable was also evaluated in the analysis and categorised cats as "purebred" or "crossbred." Sex and insurance status were examined as categorical variables. Neutering status was deemed poorly reliable after data checking and was therefore not included in the study.

Partial postcodes (postcode sector) of owner home location from cases and controls were used to gather data on potential environmental risk factors. Pesticide (fungicide and herbicide) levels equated to kilogrammes of pesticide applied per census ward on agricultural land as reported from a national survey of a sample of farms and were supplied by the UK Small Area Health Statistics Unit for England in 2000 at 1998 census ward level. Pesticide potential exposure levels were categorised into three groups: A group with no known exposure and two groups split into roughly equal sizes to form "low" and "high" exposure groups. Potential fungicide exposure levels were (usage per census ward, in kg): Zero ("no exposure"), 1 to <150 ("low exposure") and ≥ 150 ("high exposure"). Herbicide levels were (usage per census ward, kg): Zero ("no exposure"), 1 to <230 ("low exposure") and ≥ 230 ("high exposure") (Schofield *et al.* 2019). A combined pesticide variable was created with four groups describing the level of potential exposure to both fungicide and herbicide: Low-low (fungicide <150 kg and herbicide <230), low-high (fungicide <150 kg and herbicide >230), high-low (fungicide >150 kg and herbicide <230) and high-high (fungicide >150 kg and herbicide >230) (Schofield *et al.* 2019). Data for radon were provided by Public Health England for England and Wales for the year 2007 at a resolution of 1 km² (UK radon 2018). Potential radon exposure was classified based on the percentage of households estimated to exceed the recommended radon action level of 200 Bq/m³ (2018, Schofield *et al.* 2019). Radon exposure for cases and controls was categorised into three groups: $\leq 1\%$, >1 to <2% and $\geq 3.0\%$. (Zahm *et al.* 1990).

Statistical analysis

Annual incidence risk and prevalence risk with 95% confidence intervals were calculated using the proportion of incident cases, and incident and prevalent cases respectively within the study cohort population during 2016. Cats of all ages were included in both the incidence and prevalence risk estimation with 95% confidence intervals for these estimates calculated using standard methods (Kirkwood *et al.* 2003).

For the evaluation of risk factors, data checking and cleaning were performed in Microsoft Excel (2019) producing one record per cat which were then imported into IBM SPSS version 25 statistical software for analysis. Categorical data were summarised with count and percentage. Medians and interquartile range (IQRs) were calculated for continuous variables. Univariable analysis tested associations between risk factors and a diagnosis of lymphoma using univariable logistic regression. Variables which were broadly significant at the univariable analysis ($P \leq 0.2$) were carried forward for multivariable evaluation. Collinearity was assessed between all variables taken forward for multivariable consideration using either chi-square or Fishers exact tests. A manual backwards stepwise elimination method was used for development of the logistic regression model. Final variables were evaluated for pairwise interaction. Receiver operating characteristic (ROC) curve analysis was carried out to evaluate the area under the curve (AUC) for the final model using predicted probability. Mixed effect logistic regression with clinic ID as a random effect was assessed for in both the univariable and multivariable analysis.

RESULTS

Prevalence and incidence estimate

The study included 562,446 cats registered to 626 clinics across the UK between January 1, 2016 and December 31, 2016. The initial search revealed 10,260 possible candidate lymphoma cases. After manual assessment, 271 (2.6%) cats met the case definitions and inclusion criteria. Of these lymphoma cases, 86 (32%) were prevalent and 185 (68%) were incident cases. Therefore, the annual prevalence risk of feline lymphoma was estimated as 48/100,000 cats per year (95% CI 44.2 to 55.8/100,000) and the annual incidence risk as 32/100,000 cats per year (95% CI 25.5 to 34.5/100,000).

Descriptive statistics

Histological/cytological grade was reported in 88 (48%) incident cases with 73% reported as intermediate/high grade (64/88) and 27% reported as low grade (24/88). Cell size was reported in 92 (51%) incident cases with 71% reported as intermediate/large (65/92), 24% small (22/92) and 5% large granular (5/92) (Fig. 1). Abdominal lymphoma was the most common anatomical type (118 cases, 64%), followed by nodal (15, 8%) and respiratory (13, 7%) (Table 2 and Fig. 2). Ten cats had no information regarding their anatomical locations and were grouped as a separate category.

Thirty-two percent of the incident cases were tested for FeLV (59 cases), of which only three tested positive (5%) (two mediastinal, one not mentioned anatomical site). Thirty-one percent were tested for FIV (58), of which, seven tested positive

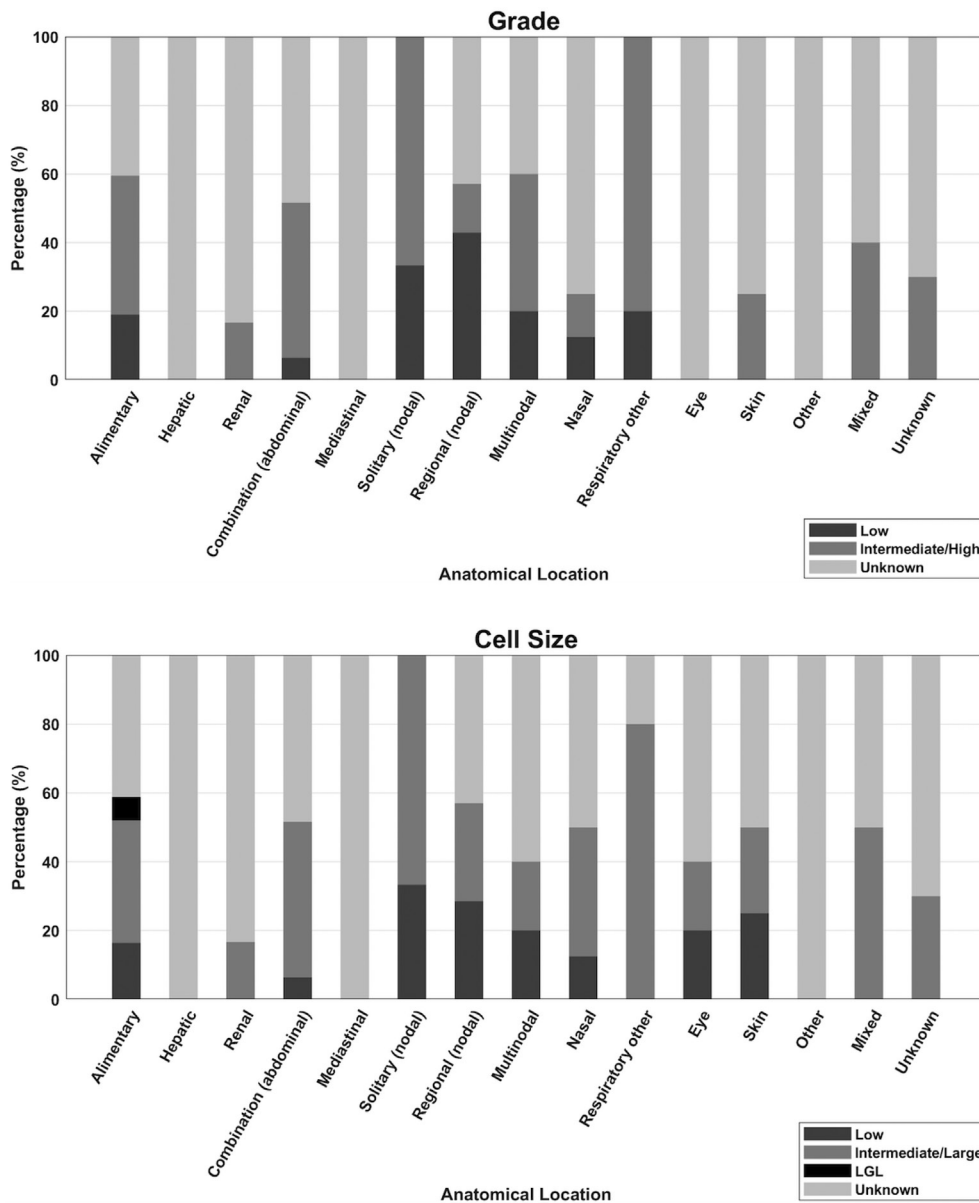


FIG 1. Anatomical locations of feline lymphoma cases according to grade (histological/cytological) and cell size in cats attending primary-care practices in the UK 2016. Percentages correspond to the percent of total number of cats with each grade/cell size within each anatomical location category. *"LGL" refers to large granular lymphoma

Table 2. Descriptive statistics for incident cases of feline lymphoma (n = 185) showing their anatomical locations/subcategories and patient characteristics from cats attending primary-care practices in the UK in 2016

Anatomical location	Case no. (%)	Age at diagnosis in years, median (range)	Breed distribution, n (%)	Subcategory	Case, n (%)	Age at diagnosis in years, median (range)			
Abdominal	118 (63.8)	10.9 (0.7 to 18.2)	102 (86.4) Domestic	Alimentary	73 (39.5)	10.7 (2.6 to 18.2)			
			3 (2.5) Persian	Hepatic	2 (1.1)	12.7 (10.1 to 15.3)			
			3 (2.5) British Short Hair	Renal	12 (6.5)	8.7 (0.7 to 15.4)			
			2 (1.7) British Blue	Combination	31 (16.8)	12.0 (0.94 to 17.4)			
			2 (1.7) Crossbred						
			1 (0.8) Siamese						
			1 (0.8) Oriental						
			1 (0.8) Bengal						
			1 (0.8) Ragdoll						
			1 (0.8) Russian blue						
			1 (0.8) Birman						
			Mediastinal	7 (3.8)	5.6 (0.7 to 12.1)	6 (85.7) Domestic		7 (3.8)	5.6 (0.7 to 12.1)
						1 (14.3) Crossbred			
Nodal	15 (8.1)	11.3 (2.0 to 18.7)	14 (93.3) Domestic	Solitary	3 (1.6)	7.9 (5.1 to 17.2)			
			1 (6.7) Siamese	Regional	7 (3.8)	13.0 (10.0 to 18.7)			
Respiratory	13 (7.0)	9.4 (5.7 to 16.8)	11 (84.6) Domestic	Multinodal	5 (2.7)	7.1 (2.0 to 11.7)			
			1 (7.7) Maine Coon	Nasal	8 (4.3)	10.7 (6.3 to 16.8)			
			1 (7.7) Burmilla	Other	5 (2.7)	9.0 (5.7 to 14.2)			
Atypical/Other	12 (6.5)	12.0 (5.5 to 15.9)	9 (75.0) Domestic	Eye	5 (2.7)	12.5 (8.1 to 14.6)			
			1 (8.3) Siamese	Skin	4 (2.2)	12.7 (5.5 to 15.9)			
			1 (8.3) British Short Hair	Other	3 (1.6)	11.6 (7.5 to 13.0)			
			1 (8.3) Norwegian Forest						
Mixed	10 (5.4)	10.7 (2.0 to 15.1)	7 (70.0) Domestic		10 (5.4)	10.7 (2.0 to 15.1)			
			1 (10.0) Exotic						
			1 (10.0) Russian Blue						
			1 (10.0) Siamese						
Unknown	10 (5.4)	13.1 (5.0 to 16.1)	8 (80.0) Domestic		10 (5.4)	13.1 (5.0 to 16.1)			
			1 (10.0) Birman						
			1 (10.0) British Short Hair						

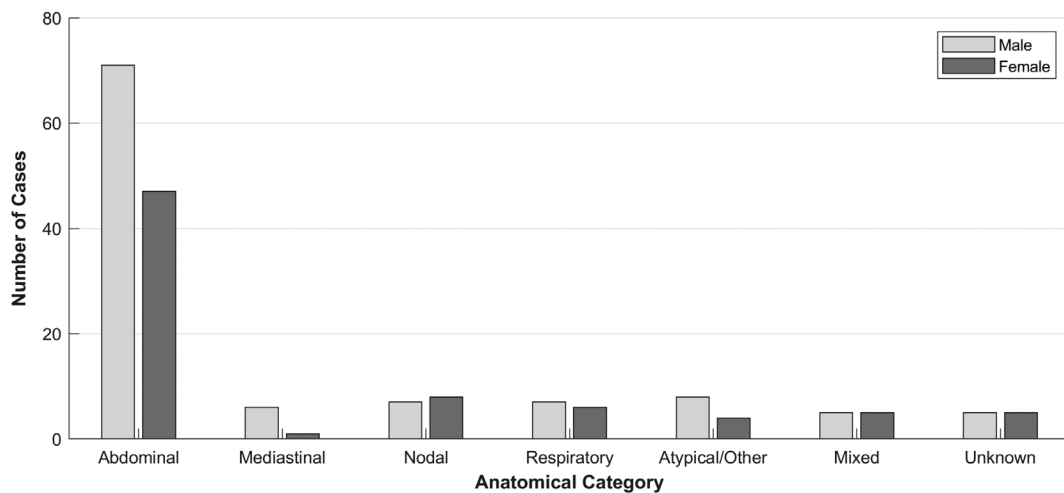


FIG 2. Anatomical locations of feline lymphoma cases according to sex in cats attending primary-care practices in the UK 2016

(12%) (three abdominal, two respiratory, one mixed, one atypical). No incident cases tested positive for both FeLV and FIV. Two percent of controls were tested for FeLV and FIV (n = 17) with only one control testing positive for FIV (6%, 1/17) and the remaining testing negative for both FIV and FeLV (94%, 16/17).

Case-control study

Out of the 185 incident cases, five were removed from the risk factor analysis to include only cats aged 2 years and above resulting

in 180 lymphoma cases taken forward to the case-control study. These were compared to 803 controls in the risk factor analysis, all aged 2 years and above. Descriptive statistics for cases included in the risk factor analysis were similar to those described for incident cases above and are summarised into Table 3. The distribution of incident cases and controls were very similar for crossbred and purebred cats with 86% (153) of incident cases being crossbred compared to controls where 87% (686) were crossbred cats. Of the incident cases, the most common breed types were

Domestic (83.8%, 151/180), Siamese (2.2%, 4/180) and British Blue (1.6%, 3/180). There was a greater distribution of males in the incident cases (59%, 109) compared to controls (46%, 366) whereas a slightly smaller proportion of cats were vaccinated in incident cases (59%, 106) compared to controls (66%, 527). Of the incident cases, 42% (75) were insured compared to only 13% (108) of cats in controls. The median age at diagnosis for incident cases was 11.0 years (IQR 8.1 to 14.0). Date of birth was not provided for one incident case. The median age reported for controls was 7.3 years (IQR 4.3 to 11.5).

Data on potential exposure to radon and pesticides for the risk factor analysis were available for 142 cases (78%) and 661 controls (82%). Exposure to radon and pesticide for cases and controls are summarised within Table 3.

Univariable analysis identified age ($P = <0.001$), sex ($P = <0.001$), insurance status ($P = <0.001$) and veterinary group ($P = <0.001$) as being associated with feline lymphoma (Table 3). Purebred status, breed, vaccination status, pesticide, fungicide, herbicide and radon were not significantly associated

with presence of feline lymphoma. FeLV and FIV status were not included in the univariable and multivariable analysis as the numbers where this information was recorded were considered to be insufficient for robust statistical analysis.

Final multivariable logistic regression model without clinic ID as a random effect (fixed effect) and with clinic ID as a random effect (mixed effect) was performed. Veterinary group was included in both final multivariable models as a fixed effect to account for clustering at a practice level as it was highly significant as a predictor variable in the univariable analysis. The final multivariable models identified age, sex, insurance status and vaccination status as significantly associated with a diagnosis of feline lymphoma (Table 4 and Fig. 4). In the mixed effect final model, cats over 11 years had 5.0 times the odds of being diagnosed compared to cats aged 2 to 5 years (95% CI 2.8 to 8.8). Insured cats were at 3.6 times the odds (95% CI 2.3 to 5.6) and male cats were at 1.7 times the odds (95% CI 1.2 to 2.4) of lymphoma compared to uninsured and female cats respectively. Vaccinated cats had decreased odds (OR 0.7, 95% CI 0.5 to 1.0)

Table 3. Descriptive and univariable logistic regression results for risk factors for diagnosis of lymphoma in cats attending UK primary-care practices in 2016. Only incident cases aged 2 years and older were included here

Variable	Category	Case, n (%)	Control, n (%)	Odds ratio	95% CI	Variable P-value
Purebred Status	Crossbred	153 (85.5)	686 (85.4)	Base		0.539
	Purebred	26 (14.4)	100 (12.5)	1.2	0.7 to 1.9	
	Not recorded	1 (0.1)	17 (2.1)			
Breed	Domestic	151 (83.8)	665 (82.8)	Base		0.116
	British Blue	3 (1.6)	2 (0.2)	6.6	1.1 to 39.9	
	Persian	3 (1.6)	9 (1.1)	1.5	0.4 to 5.9	
	Siamese	4 (2.2)	9 (1.1)	2.0	0.6 to 6.4	
	Cross breed	2 (1.1)	21 (2.6)	0.4	0.1 to 1.8	
	Other purebreds	16 (8.8)	80 (10.0)	0.9	0.5 to 1.5	
	Not recorded	1 (0.5)	17 (2.1)			
Age (years)	2.0 to 4.9	14 (7.8)	254 (31.6)	Base		<0.001
	5.0 to 7.9	26 (14.5)	186 (23.2)	2.5	1.3 to 5.0	
	8.0 to 10.9	47 (26.3)	146 (18.2)	5.8	3.1 to 11.0	
	≥11.0	92 (51.4)	217 (27.0)	7.7	4.3 to 13.9	
Sex	Female	74 (41.1)	435 (54.3)	Base		0.001
	Male	106 (58.9)	366 (45.7)	1.7	1.2 to 2.4	
Insurance status	Insured	75 (41.7)	108 (13.4)	4.5	3.2 to 6.6	<0.001
	No evidence/Non-insured	105 (58.3)	695 (86.6)	Base		
Vaccination status 1	Not vaccinated	74 (41.1)	276 (34.4)	Base		0.088
	Vaccinated	106 (58.9)	527 (65.6)	0.8	0.5 to 1.0	
Vaccination status 2	Not vaccinated	74 (41.1)	276 (34.4)	Base		0.258
	Vaccinated Unknown	26 (14.4)	111 (13.8)	1.4	0.9 to 2.0	
	Vaccinated: no FeLV	3 (1.7)	11 (1.4)	1.2	0.8 to 2.0	
	Vaccinated: FeLV	77 (42.8)	405 (50.4)	1.4	0.4 to 5.3	
Fungicide (kg usage per census ward)	"No exposure" (0)	99 (55.0)	437 (54.4)	Base		0.512
	1 to 150	16 (8.9)	97 (12.1)	0.7	0.4 to 1.3	
	≥150	27 (15.0)	127 (15.8)	0.9	0.6 to 1.5	
	Not available	38 (21.1)	142 (17.7)	1.1	0.8 to 1.8	
Herbicide (kg usage per census ward)	"No exposure" (0)	96 (53.3)	418 (52.1)	Base		0.548
	1 to 230	21 (11.7)	108 (13.4)	0.8	0.5 to 1.4	
	≥230	25 (13.9)	135 (16.8)	0.8	0.5 to 1.3	
	Not available	38 (21.1)	142 (17.7)	1.2	0.8 to 1.8	
Pesticide	Low-low	112 (62.2)	514 (64.0)	Base		0.502
	High-high	22 (12.2)	115 (14.3)	0.9	0.5 to 1.4	
	High-low	5 (2.8)	12 (1.5)	1.9	0.6 to 5.5	
	Low-high	3 (1.7)	20 (2.5)	0.7	0.2 to 2.4	
	Not available	38 (21.1)	142 (17.7)	1.2	0.8 to 1.9	
Vet Groups	Vet Group 1	82 (45.6)	206 (25.7)	Base		<0.001
	Vet Group 2	6 (3.3)	5 (0.6)	3.0	0.9 to 10.2	
	Vet Group 3	65 (36.1)	485 (60.4)	0.3	0.2 to 0.5	
	Vet Group 4	27 (15.0)	107 (13.3)	0.6	0.4 to 1.0	

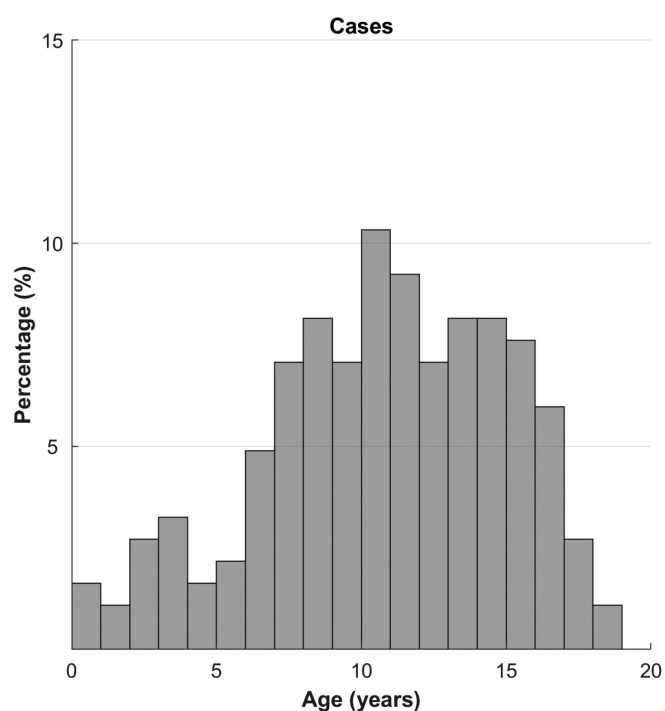


FIG 3. Ages of feline lymphoma cases (n = 185) in cats attending primary-care veterinary practices in England in 2016

of having lymphoma compared to unvaccinated cats, however this was significant in the fixed effect model ($P = 0.043$) and only weakly significant in the mixed effect model ($P = 0.054$), once clustering at a clinic level was adjusted for (Table 4). No major difference was found between the two final models and no significant interaction between final model variables was found. The AUC for the fixed effect multivariable model was 0.776 (95% CI 0.739 to 0.813) and 0.781 (95% CI 0.744 to 0.818) for the mixed effect multivariable model.

DISCUSSION

The current study is the first to report incidence and prevalence values and risk factors associated with lymphoma in cats registered to primary-care practices in the UK. Previous veterinary research is dominated by small populations of cats from referral practice with a focus on specific anatomical forms of lymphoma and their response to chemotherapy (Moore *et al.* 1996, Henderson *et al.* 2004, Sfiligoi *et al.* 2007). These studies may have limited relevance to the wider UK cat population (Bartlett *et al.* 2010). Instead, the current study explored cases diagnosed in primary-practice by evaluating the EPR of 562,446 cats and identified 185 lymphoma cases, of which 180, all aged 2 years and over, were taken forward to the risk factor analysis. The current study identified a greater risk of feline lymphoma with older aged, insured, unvaccinated and male cats in the multivariable analysis.

Alimentary lymphoma was the most common anatomical location followed by the abdominal combination classification and renal lymphoma. This is consistent with other studies

where alimentary lymphoma was reported in 40 to 50% of lymphoma cases (Gabor *et al.* 1998, Sato *et al.* 2014). The mediastinal anatomical subgroup had the youngest reported median age of 5.6 years compared to other anatomical subgroups. This is comparable with a relatively recent UK referral study where the median age of mediastinal lymphoma in cats was reported as 3 years (Fabrizio *et al.* 2014). This observation has also been frequently reported in older studies (Mooney *et al.* 1989, Gabor *et al.* 1998). In the current study, the “grade” classification represented the grade given by the pathologist based either on cytology or histology results, whereas most previous studies report histological grade exclusively. Nevertheless, 73% of lymphoma cases were classified as intermediate/high grade and 27% were low grade, which is comparable with other studies reporting 65 to 90% of feline lymphomas being intermediate/high grade (Gabor *et al.* 1998, Sato *et al.* 2014). Proportions described for lymphoma grade were similar to those reported for cell size in this study with 71% of lymphoma cases reported as intermediate/large and 24% small.

The annual prevalence risk of feline lymphoma in primary-care UK veterinary practices was 48/100,000 cats per year and the annual incidence risk was 32/100,000 cats per year in the current study. This incidence rate was slightly lower than that reported by a USA study (Dorn *et al.* 1968) over 50 years ago where 44/100,000 cats at risk per year was reported. Differences described between the two studies may be due to variations between the cat populations (*e.g.* geographical or genetic factors), study period and other methods of data collection (*e.g.* inclusion criteria).

Age was identified as a risk factor for feline lymphoma in the current study, with increased odds associated with increasing age. Previous studies have also found increased age associated with increased risk of feline lymphoma, with a reported median age at diagnosis of 10 years (Gabor *et al.* 1998, Sato *et al.* 2014). A bimodal age distribution for feline lymphoma has been described, with peaks seen at less than 2 years and greater than 8 years, with FeLV-associated lymphoma reported to be responsible for the peak seen at <2 years (Rezanka *et al.* 1992, Gabor *et al.* 1998, Fabrizio *et al.* 2014). This was not observed in the current study (Fig. 3), likely due to the overall low prevalence of FeLV-positive cats in the current study.

Overall, only 5% of lymphoma cases were reported as retrovirus-positive, similar to a multi-institutional referral UK study which reported 7% (10/149) of lymphoma cases being FeLV or FIV positive (Taylor *et al.* 2009). Interestingly, in the current study, only a minority of cases were tested for FeLV and FIV (32%) suggesting the prevalence of FeLV or FIV in cases could be higher than actually recorded. The low number of lymphoma cases tested for FeLV or FIV may reflect cost implications for owners but may also indicate a need for further awareness of the value of FeLV status for prognosis of lymphoma patients. Current literature highlights the importance of FeLV status on prognosis of lymphoma patients with FeLV positive patients being reported to have a poorer prognosis (Mooney *et al.* 1989, Vail *et al.* 1998). FeLV and FIV status were not included in statistical analysis due to the small numbers of cats tested for cases and

Table 4. Final multivariable logistic regression model without clinic ID as a random effect (fixed effect) and with clinic ID as a random effect (mixed effect) for risk factors for diagnosis of feline lymphoma in cats aged over 2 years attending UK primary-care practices in 2016

Variable	Category	Fixed effect			Mixed effect		
		Odds ratio	95% CI	P-value	Odds ratio	95% CI	P-value
Age	2.0 to 4.9	Base		<0.001	Base		<0.001
	5.0 to 7.9	2.3	1.1 to 4.5		1.8	1.0 to 3.5	
	8.0 to 10.9	5.1	2.7 to 9.8		4.0	2.2 to 7.5	
	≥11.0	6.3	3.4 to 11.6		5.0	2.8 to 8.8	
Sex	Female	Base		0.003	Base		0.004
	Male	1.7	1.2 to 2.5		1.7	1.2 to 2.4	
Insurance status	Non-insured	Base		<0.001	Base		<0.001
	Insured	3.7	2.4 to 5.8		3.6	2.3 to 5.6	
Evidence of vaccination	Not vaccinated	Base		0.043	Base		0.054
	Vaccinated	0.7	0.5 to 1.0		0.7	0.5 to 1.0	
Veterinary group	Vet Group 1	Base		0.129	Base		0.223
	Vet Group 2	0.3	0.8 to 1.1		0.3	0.1 to 1.1	
	Vet Group 3	0.3	0.1 to 1.4		0.3	0.1 to 1.3	
	Vet Group 4	0.5	0.1 to 1.7		0.4	0.1 to 1.6	
Random effect	Interclass correlation				Variance = 4.05×10^{-10}		

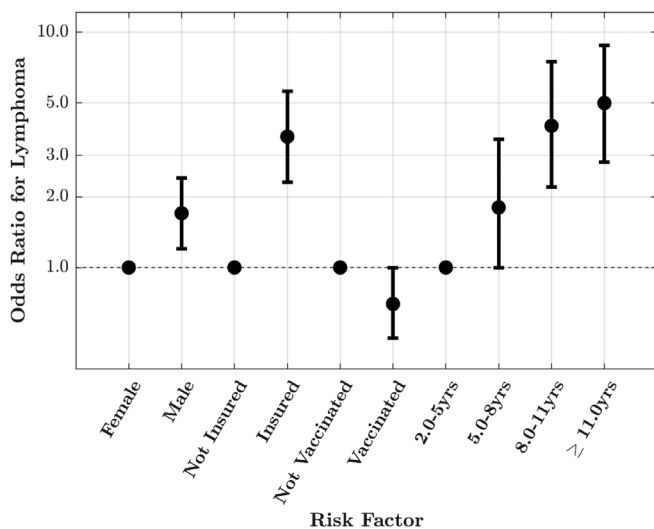


FIG 4. Risk factors for feline lymphoma from final multivariable binary logistic regression model with clinic ID included as a random effect (mixed effect). Veterinary group was included as a fixed effect. Odds ratio for lymphoma with corresponding 95% confidence intervals are reported. Baseline category reported first

controls, therefore no conclusions can be drawn in the current study on its role as a risk factor for feline lymphoma.

Vaccinated cats had decreased odds of lymphoma diagnosis compared to unvaccinated cats, once other variables were adjusted for in the final model. This is the first time this has been described in the literature and might suggest vaccination has a protective effect. The decreased risk of lymphoma in vaccinated cats in the current study is not easily explained. Several studies indicate that the introduction of routine FeLV vaccination programmes has decreased the frequency of lymphoma cases that test serum antigen positive suggesting FeLV vaccination has a protective effect against FeLV associated lymphoma (Louwerens *et al.* 2005, Graf *et al.* 2015). Recent studies suggest cats can be FeLV-antigenemia negative but still test positive for the FeLV pro-

virus DNA thereby increasing likelihood of lymphoma through insertional mutagenesis (Hofmann-Lehmann *et al.* 2006, Cattori & Hofmann-Lehmann 2008, Fujino *et al.* 2008). Interestingly, studies to date have reported that vaccination against FeLV does not provide a protective role against the presence of FeLV proviral DNA in feline tissues following FeLV exposure (Hofmann-Lehmann *et al.* 2006, Hofmann-Lehmann *et al.* 2007) and therefore does not reduce the risk of insertional mutagenesis and malignant transformation. This could explain why lymphoma cases are rising despite FeLV antigenemia-associated lymphoma cases decreasing (Louwerens *et al.* 2005). The type of vaccinations which cats received was not found to be statistically significant in the current study due to incomplete recording of the type of vaccination administered, limiting the interpretation of these findings. Further, it was not recorded whether vaccinations were up to date. Evidence of vaccination could additionally reflect other factors such as owners' dedication to the overall care of their pets which may influence the diet, environment and access to veterinary care which these cats receive. Future studies further exploring the role of FeLV vaccination and presence of FeLV provirus DNA in feline lymphoma are required.

Sex was significantly associated with a diagnosis of feline lymphoma in the current study with male cats at increased odds compared to females. Similar findings have been reported in a few studies where male cats have been reported with an increased risk of 2.3 in one study (Dorn *et al.* 1968) and a 1.5:1 male to female ratio was reported in other studies (Gabor *et al.* 1998, Vail *et al.* 1998, Meichner *et al.* 2012). Other studies have failed to report an association between lymphoma and sex (Meincke *et al.* 1972, Haga *et al.* 1988, Louwerens *et al.* 2005). The male predisposition in the current study appeared highest in the alimentary and atypical anatomical subgroups though anatomical subtypes were not evaluated in the risk factor analysis. The cause for a sex predisposition has not been determined; however, similar findings are reported in human lymphoma (Ghazawi *et al.* 2019) which might suggest a role for hormonal influence.

Insured cats were at increased odds of a lymphoma diagnosis compared to non-insured cats in this study. Insurance may be an indication of the “human-animal bond” where owners are more likely to undergo further diagnostic testing to confirm lymphoma and meet the inclusion criteria for the study (Egenvall *et al.* 2009).

Limitations in the present study are worth noting. Underestimation of feline lymphoma prevalence and incidence in this study is probable, due to the stated case definition as only some owners are able or willing to afford the diagnostic tests needed to reach a lymphoma diagnosis. In addition, it is difficult to apply the present study’s prevalence and incidence estimates to the wider cat population that do not attend VetCompass practices. No association between breed and lymphoma was observed in the current study despite several studies which report Siamese/Oriental breeds being over-represented amongst cats with lymphoma (Gabor *et al.* 1998, Louwerens *et al.* 2005, Fabrizio *et al.* 2014). It is possible that the small sample sizes of cases within any specific breed group limited the ability to detect an association. Similarly, no association between feline lymphoma and radon or pesticide exposure was found. The relatively small number of cases may have prevented a subtle association between lymphoma and the environmental factors from being detected, in addition, it was not recorded whether cats were mainly indoor or outdoor which would affect their exposure to environmental factors. Furthermore, bias due to missing data for the environmental variables is highly probable due to the absence of sufficient public records for some districts. Although FeLV and FIV status were rarely recorded, it was considered less likely that this was subject to bias due to missing not at random as these tests are generally considered chargeable items and hence should have been reliably recorded in the EPR. Nonetheless, due to the sparsity of recording of these variables they were not retained in the models as discussed above. Finally, over interpretation of age and its association with feline lymphoma should be avoided due to the use of age categories in the current study. Age categories were selected in the present study according to biological categories and therefore the reader should appreciate the increase in risk of feline lymphoma as the cat ages from young, middle-aged and older-aged rather than based on the exact category boundaries.

This study reported the prevalence of UK feline lymphoma cases as 48/100,000 cats per year and the incidence as 32/100,000 cats per year. This investigation supports previously reported risk factors for feline lymphoma where age and sex were associated with an increased risk. However, this study also identified an increased risk of feline lymphoma diagnosis in insured cats and a decreased risk of feline lymphoma in vaccinated cats. No association with environmental factors were found. This study suggests older aged, males, insured and unvaccinated cats are at increased risk of being diagnosed with lymphoma in primary practice. Future studies are needed to further explore the role of vaccination and sex in the development of lymphoma in cats in order to further improve our understanding of this disease.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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