Epidemiology of hyperadrenocorticism among 210,824 dogs attending primary-care veterinary practices in the UK from 2009-2014

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Running title: Hyperadrenocorticism in dogs

Structured Summary

Objectives: To estimate prevalence and risk factors for diagnosis with hyperadrenocorticism in dogs attending primary-care veterinary practices in the UK from 2009-2014.

Methods: Cases were identified by searching de-identified electronic patient records from UK primary-care veterinary practices participating in the VetCompass Programme.

Results: The estimated prevalence for hyperadrenocorticism diagnosis in dogs was 0.28% (95% confidence interval 0.25-0.31). Multivariable logistic regression analysis revealed four associated risk factors: breed, breed-relative bodyweight, age and insurance status. The Bichon Frise had 6.5 times the odds (95% CI 3.5-12.1, $P < 0.001$) of hyperadrenocorticism compared with crossbreds. Dogs weighing more than or equal to their breed mean had 1.7 times odds (95% CI 1.3-2.3, $P < 0.001$) of hyperadrenocorticism compared with dogs weighing less than the breed mean. Dogs aged 12.0 years and above showed 5.7 times the odds (95% CI 3.7-8.7, $P < 0.001$) of hyperadrenocorticism compared with dogs aged 6.0-8.9 years. Insured dogs had

4.0 times the odds (95% CI 2.8-5.6, $P < 0.001$) of hyperadrenocorticism compared with non-insured dogs.

Clinical significance: This is the first epidemiological report of a non-referral hospital population of dogs diagnosed with hyperadrenocorticism in the UK and describes important breed, age and bodyweight associations with this disorder which may improve diagnosis and enhance understanding of the underlying pathophysiology.

Keywords hyperadrenocorticism, Cushings, VetCompass, epidemiology, electronic patient record

Introduction

Hyperadrenocorticism results from chronic overproduction of cortisol (Behrend et al., 2015, Melian et al., 2010) and generally results from either a functional pituitary tumour (PTHAC) or adrenal tumour (ATHAC), although other causes are also recognised (Galac et al., 2005, Galac et al., 2008). Traditionally most dogs affected by hyperadrenocorticism show polyuria, polydipsia, polyphagia, muscle atrophy and dermatological changes such as alopecia and cutaneous hyperpigmentation (Ling et al., 1979, Lorenz, 1982, Reusch and Feldman, 1992, White et al., 1989). Studies from the USA estimate the prevalence of PTHAC to be around 0.2% and the incidence of new hyperadrenocorticism cases as 1 to 2 cases/1000 dogs/year (Bruyette et al., 2010, Lourenc et al., 2015, Willeberg and Priester, 1982). To date there have been no studies reporting the prevalence of hyperadrenocorticism for dogs in the UK.

Several risk factors for the development of canine hyperadrenocorticism have been described, including older age and breeds such as the Miniature Poodle, Boxer and Dachshund (Behrend et al., 2015, Burkhardt et al., 2013, Ling et al., 1979, Lourenc et al., 2015, Reusch and Feldman, 1992, Fracassi et al., 2014). Female dogs have been over-represented in some studies describing hyperadrenocorticism but this finding has not been universally identified (Gallelli et al., 2010, Reusch and Feldman, 1992). Due to potential environmental and genetic differences between dog populations across geography and time, the results from non-UK or older studies may be poorly translatable to the current general UK dog population (Wilson and Wade, 2012).

Several studies have described the clinical features of canine hyperadrenocorticism in the UK (Augusto et al., 2012, Helm et al., 2011, Neiger et al., 2002, Kenefick and Neiger, 2008, Barker et al., 2005). These describe a mean age at diagnosis between 8 to 11 years, with toy and terrier breeds overrepresented and suggest female dogs may have an increased risk for ATHAC, but not PTHAC (Helm et al., 2011, Kenefick and Neiger, 2008, Neiger et al., 2002). PTHAC accounts for around 77% of cases of UK canine hyperadrenocorticism, similar to findings in non-UK studies (Augusto et al., 2012, Reusch and Feldman, 1992). However, the majority of these studies describe dogs from referral populations and therefore the results may be poorly generalizable to the wider dog population (Bartlett et al., 2010).

This study aimed to describe the signalment, prevalence and risk factors in dogs diagnosed with hyperadrenocorticism in primary-care practice in the UK that were participating in the

VetCompass Programme from 2009-2014 and to describe diagnostic testing protocols and outcomes for these cases. A main focus of the study was to identify UK breeds at higher risk of diagnosis with hyperadrenocorticism. The study hypothesised that sex was not associated with risk of diagnosis with hyperadrenocorticism.

Materials and methods

The VetCompass companion animal surveillance programme (VetCompass, 2015) collates de-identified electronic patient record (EPR) data from primary-care veterinary practices in the UK for epidemiological research (O'Neill et al., 2014b). Collaborating practices were selected by their willingness to participate and their recording of clinical data within an appropriately configured practice management system (PMS). Practitioners could record summary diagnosis terms from an embedded VeNom Code list (The VeNom Coding Group, 2015) during episodes of care. Information collected related mainly to the owned dog population and included patient demographic (species, breed, date of birth, sex, neuter status, insurance status and bodyweight) and clinical information (free-form text clinical notes, summary diagnosis terms, treatment and deceased status with relevant dates) data fields. EPR data were extracted from PMSs using integrated clinical queries (O'Neill et al., 2014b) and uploaded to a secure VetCompass structured query language (SQL) database.

A cross-sectional study design was used to estimate the prevalence and risk factors for hyperadrenocorticism. The sampling frame for the current study included all dogs with at least one EPR (clinical note, VeNom summary term, bodyweight or treatment) uploaded to the VetCompass database from September 1st, 2009 to August 31st, 2014. Sample size calculations

estimated a cross-sectional study with 41,339 male and 41,339 female animals would have 80% power to detect sex as a risk factor with an odds ratio of 1.2 or greater (two-sided α=0.05) having a 0.2% prevalence in the less-predisposed sex (Epi Info 7 CDC, 2015). Ethics approval was granted by the RVC Ethics and Welfare Committee (reference number 2014/S45).

The hyperadrenocorticism case definition required the dog had a final diagnosis of hyperadrenocorticism (or synonym) recorded in the EPR. The case inclusion criteria relied on the clinical acumen of UK veterinary practitioners to make primary-care diagnoses and did not demand any specific diagnostic protocols for hyperadrenocorticism. This approach also enabled the study to report on the diagnostic protocols that were used across all diagnosed hyperadrenocorticism cases. A two-stage case-finding process was used. In stage one, all study dogs were screened to identify candidate hyperadrenocorticism cases by searching the clinical free-text field (search terms: PDH, ADH, hyperadren, cushing, adrenalec, adrenomeg), the VeNom term field (search term: hyperadrenocorticism) and the treatment field (drug searches: vetoryl, Lysodren, DDD, Mitotane, Trilostane and deprenyl). In stage two, the full EPRs of a random subset of candidate hyperadrenocorticism cases were manually reviewed to decide on case inclusion. Randomisation used the *RAND* function in Microsoft Excel (Microsoft Office Excel 2007, Microsoft Corp.). Additional data were extracted on confirmed hyperadrenocorticism cases that described whether the case was pre-existing (first recorded prior to the study period) or incident (first recorded during the study period), the date of first diagnosis and the laboratory tests used during diagnosis (for incident cases only) and the date and mechanism of any deaths. All dogs that were not identified as candidate hyperadrenocorticism cases during the initial screening were included as non-cases for hyperadrenocorticism in the risk factor analysis.

A *purebred* variable grouped all dogs recorded as a recognisable breed (Irion et al., 2003) as 'purebred' and all other dogs as 'crossbred'. A *breed* variable included 16 categories: the 12 most common breeds overall in the study, two other breeds with ≥ 10 incident hyperadrenocorticism cases, a grouping of all remaining breeds and a grouping of all crossbred dogs. A *KC breed group* variable classified breeds recognised by the Kennel Club (KC) into their relevant KC breed groups (Gundog, Hound, Pastoral, Terrier, Toy, Utility, Working) and all remaining dogs were classified as non-KC recognised. *Neuter* described the status of the dog (entire or neutered) as recorded at the final EPR. *Insurance* described whether a dog was insured at any point during the study period. The age value described the age at the date of first diagnosis for incident hyperadrenocorticism cases and was entered as unknown for pre-existing hyperadrenocorticism cases. For non-case dogs, the age described the age at the mid-point between the dates of the first and final EPRs recorded during the study period. All risk factors except age were included as 'fixed' for both the pre-existing and incident cases i.e. their exposure status was not affected by the date of first diagnosis of hyperadrenocorticism and therefore the risk factor analysis could be interpreted as comparing the odds of 'being a case'. On the other hand, by using the specific age value at the point that the incident cases became first diagnosed with hyperadrenocorticism, the risk factor results for the effect of age could be interpreted comparing the odds at each age group for 'becoming a case' (Robins and Hernán, 2009).

Age (years) was categorised into six groups (< 3.0, 3.0-5.9, 6.0-8.9, 9.0-11.9, ≥ 12.0, not recorded). *Actual bodyweight* described the maximum bodyweight recorded during the study period for dogs older than nine months and was categorised into six groups (0.0-9.9 kg, 10.0-19.9 kg, 20.0-29.9 kg, 30.0-39.9 kg, ≥ 40.0 kg, not recorded). *Breed-relative bodyweight*

characterised dogs with a recorded bodyweight when aged older than nine months as being either below or equal/above the mean adult bodyweight of its breed from the overall dataset. Dogs without a bodyweight value recorded when they were aged older than nine months were included as 'not recorded'. This variable allowed the effect of bodyweight variation within breeds to be assessed. The time contributed to the study for each dog described the period from the dates of the earliest to the latest EPR.

Data checking and cleaning was performed in Excel (Microsoft Office Excel 2007, Microsoft Corp.) and evaluated internal data consistency, missing values and outlier data. All analyses were conducted using Stata Version 13 (Stata Corporation). The period prevalence with 95% confidence intervals (CI) described the probability of dogs having hyperadrenocorticism at any time during the study period and included dogs that were first diagnosed with hyperadrenocorticism prior to the study period (pre-existing cases) as well as those diagnosed for the first time during the study period (incident cases). The CI estimates were derived from standard errors based on approximation to the normal distribution (Kirkwood and Sterne, 2003).

Because the sampling design involved manual verification of a subset of the candidate cases, the count of cases that would have been identified if the entire set of candidate cases had been manually verified was calculated by weighting the verified case numbers by the inverse of the proportion of candidate cases that was manually verified. The estimated total hyperadrenocorticism case count was used to estimate overall and breed prevalence values based on a denominator of all study dogs either overall or by breed respectively. The prevalence CI estimates were derived from standard errors, based on approximation to the normal distribution (Kirkwood and Sterne, 2003). Descriptive statistics characterised the breed, sex,

neuter status, insurance status, age and bodyweight separately for the case and non-case dogs. Diagnostic tests used to support the hyperadrenocorticism diagnosis were reported for incident cases only. The chi-square test was used to compare categorical variables (Kirkwood and Sterne, 2003).

Binary logistic regression modelling was used to evaluate univariable associations between risk factors (*purebred, breed, KC breed group, actual bodyweight, breed-relative bodyweight, age, sex, neuter* and *insurance*) and hyperadrenocorticism. Both pre-existing and incident hyperadrenocorticism cases were included in risk factor analysis. Because breed was a factor of primary interest for the study, *purebred* and *KC breed group* (collinear with breed) and *actual bodyweight* (a defining characteristic of individual breeds) were excluded from multivariable modelling but univariable analysis results were reported. Remaining risk factors with liberal associations in univariable modelling ($P < 0.2$) were taken forward for multivariable evaluation. Model development used manual backwards stepwise elimination. Clinic attended was evaluated as a random effect and pair-wise interaction effects were evaluated in the final model (Dohoo et al., 2009). The area under the ROC curve was used to evaluate the quality of the model fit (non-random effect model) (Dohoo et al., 2009). Statistical significance was set at $P < 0.05$.

Results

The overall dataset comprised 210,824 dogs attending 119 clinics in England. There were 4,846 candidate hyperadrenocorticism cases identified. Manual checking of a random sample of 2,535 (52.3%) candidate cases identified 304 dogs that met the hyperadrenocorticism case definition. Many of the excluded animals included hyperadrenocorticism within differential

diagnosis lists but the disorder was not later confirmed. Of the confirmed hyperadrenocorticism cases, 113 (37.2%) were pre-existing and 191 (62.8%) were incident cases. Following removal of the residual 4,542 candidate cases, there were 304 cases and 205,978 non-cases included in the descriptive and inferential statistics. The median time contributed to the study across all dogs in the study was 0.6 years (interquartile range [IQR]: 0.0-2.1 years, range 0.0-5.0 years).

After accounting for the effects of the subsampling protocol by weighting the verified case numbers by the inverse of the proportion of candidate cases that was manually verified as described in the methods section, the estimated prevalence for hyperadrenocorticism diagnosis in dogs was 0.28% (95% CI 0.25-0.30). The breed prevalence results indicated that the breeds with the highest hyperadrenocorticism prevalence were the Standard Dachshund (2.6%, 95% CI 1.6-4.0) and Bichon Frise (1.6%, 95% CI 1.2-2.2) (Table 2).

Data completion varied between the variables assessed: breed 99.9%, sex 99.5%, neuter status 46.4%, insurance 65.7%, age 99.7% and bodyweight 65.4%. Of the hyperadrenocorticism dogs with complete information available (not including those animals with missing data), 241 (79.3 %) were purebred, 152 (50.0%) were female, 205 (86.1%) were neutered and 161 (53.0%) were insured. The median bodyweight was 12.5 (IQR: 9.1-22.0, range: 2.5-58.7) kg and the median age at diagnosis was 10.9 (IQR: 9.5-12.8, range: 2.4-18.0) years (Figure 1). The most common breeds diagnosed with hyperadrenocorticism were the Jack Russell Terrier (n = 29, 9.5%), Bichon Frise (n = 24, 7.9%), Yorkshire Terrier (n = 22, 7.2%), Staffordshire Bull Terrier (n = 17, 5.6%) and West Highland White Terrier (n = 13, 4.3%) (Table 1).

Of the non-case dogs with information available, 159,422 (77.4%) were purebred, 97,985 (47.8%) were female, 73,497 (78.0%) were neutered and 47,696 (40.3%) were insured. The median bodyweight was 17.8 (IQR: 9.2-28.7, range: 0.7-112.0) kg and the median age was 3.9

(IQR: 1.3-8.0, range: 0.0-30.8) years (Figure 1). The most common breeds among non-hyperadrenocorticism dogs were the Labrador Retriever (n= 17,406, 8.5%), Staffordshire Bull Terrier (n = 16,473, 8.0%), Jack Russell Terrier (n = 13,198, 6.4%) and Cocker Spaniel (n = 7,507, 3.6%) (Table 1). Among the overall study dogs, purebred dogs had a higher probability of being insured than crossbred dogs (41.9% versus 37.4% respectively, $P < 0.001$).

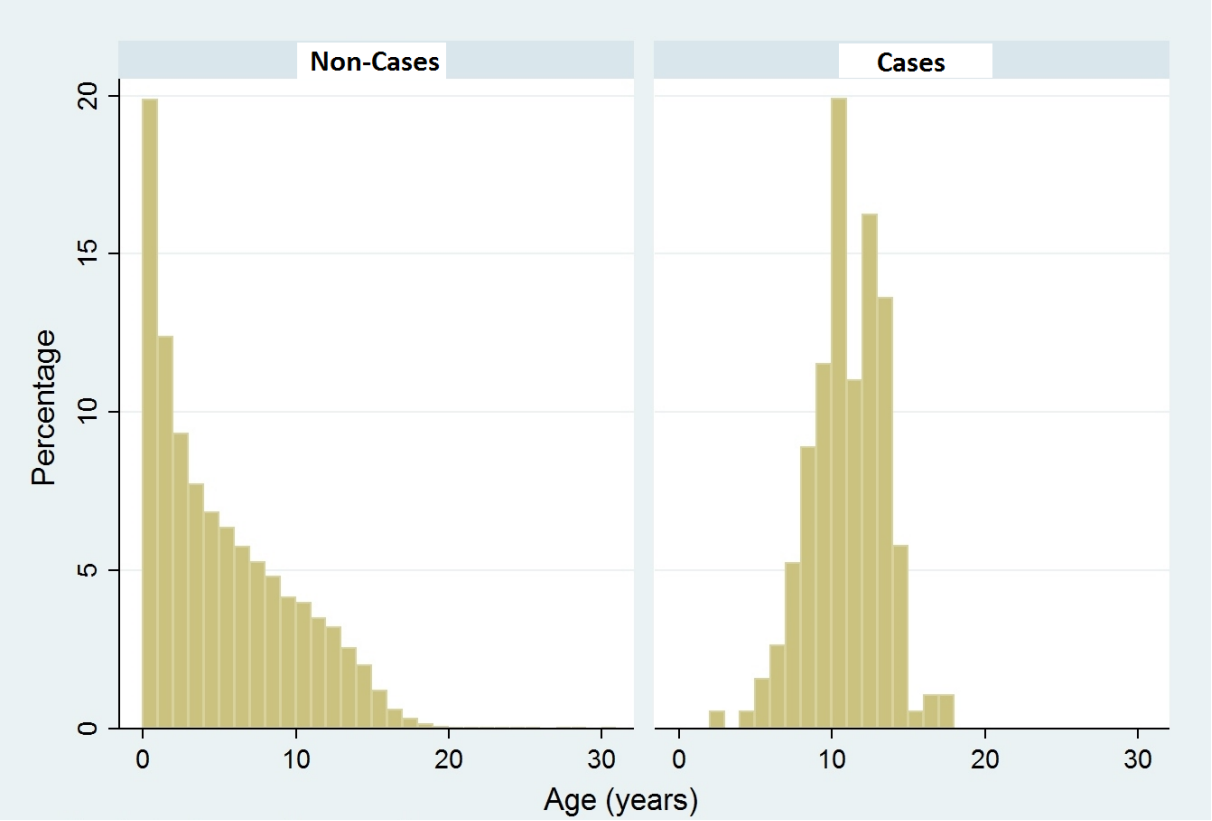

Figure 1. Ages at the mid-point between the dates of the first and final clinical records during the study period for dogs without a diagnosis of hyperadrenocorticism (n = 209,991) and at the date of first diagnosis for dogs with a diagnosis of hyperadrenocorticism (n = 191) attending primary-care veterinary practices in England.

Table 1: Descriptive and univariable logistic regression results for risk factors associated with hyperadrenocorticism in dogs attending primary-care veterinary practices in England. Data on both pre-existing and incident HAC cases were included.

Variable	Category	Case No. (%)	Non-case No. (%)	Odds ratio	95% CI	*P*-value
Purebred status	Crossbred	63 (20.7)	46,425 (22.6)	Base		
	Purebred	241 (79.3)	159,422 (77.4)	1.1	0.8-1.5	0.446
Breed	Crossbred	63 (20.7)	46,425 (22.5)	Base		
	Bichon Frise	24 (7.9)	2,681 (1.3)	6.6	4.1-10.6	< 0.001
	Standard Dachshund	10 (3.3)	702 (0.3)	10.5	5.4-20.5	< 0.001
	Shih-tzu	8 (2.6)	4,051 (2.0)	1.5	0.7-3.0	0.318
	Yorkshire Terrier	22 (7.2)	6,714 (3.3)	2.4	1.5-3.9	< 0.001
	Jack Russell Terrier	29 (9.5)	13,198 (6.4)	1.6	1.0-2.5	0.032
	Chihuahua	2 (0.7)	4,589 (2.2)	0.3	0.1-1.3	0.114
	West Highland White Terrier	13 (4.3)	5,019 (2.4)	1.9	1.0-3.5	0.034
	Other breeds	86 (28.3)	60,440 (29.3)	1.0	0.8-1.5	0.775
	Staffordshire Bull Terrier	17 (5.6)	16,473 (8.0)	0.8	0.4-1.3	0.317
	Cocker Spaniel	7 (2.3)	7,507 (3.6)	0.7	0.3-1.5	0.347
	Cavalier King Charles Spaniel	5 (1.6)	4,355 (2.1)	0.8	0.3-2.1	0.719
	German Shepherd Dog	3 (1.0)	7,025 (3.4)	0.3	0.1-1.0	0.050
	Labrador Retriever	11 (3.6)	17,406 (8.5)	0.5	0.2-0.9	0.019
	English Springer Spaniel	3 (1.0)	4,035 (2.0)	0.5	0.2-1.7	0.309
	Border Collie	1 (0.3)	5,358 (2.6)	0.1	0.0-1.0	0.049
Kennel Club Breed Group	Working	8 (2.6)	11,278 (5.5)	0.5	0.2-1.0	0.047
	Hound	25 (8.2)	9,083 (4.4)	1.9	1.2-2.9	0.006
	Toy	56 (18.4)	24,839 (12.1)	1.5	1.1-2.1	0.013

	Terrier	55 (18.1)	28,621 (13.9)	1.3	0.9-1.8	0.124
	Utility	30 (9.9)	16,894 (8.2)	1.2	0.8-1.8	0.383
	Pastoral	12 (4.0)	15,291 (7.4)	0.5	0.3-1.0	0.039
	Gundog	26 (8.6)	37,590 (18.3)	0.5	0.3-0.7	0.001
	Not KC-recognised	92 (30.3)	62,251 (30.2)	Base		
Actual bodyweight (kg)	< 10.0	105 (34.5)	38,139 (18.5)	3.0	1.5-5.9	0.002
	10.0-19.9	101 (33.2)	35,270 (17.1)	3.1	1.6-6.2	0.001
	20.0-20.9	53 (17.4)	29,711 (14.4)	1.9	1.0-3.9	0.067
	30.0-30.9	27 (8.9)	20,485 (10.0)	1.4	0.7-3.0	0.351
	≥ 40.0	9 (3.0)	9,779 (4.8)	Base		
	Not recorded	9 (3.0)	72,594 (35.2)	0.1	0.1-0.3	< 0.001
Breed-relative bodyweight	< Breed Average	116 (38.2)	73,888 (35.9)	Base		
	≥ Breed Average	179 (58.9)	59,496 (28.9)	1.9	1.5-2.4	< 0.001
	Not recorded	9 (3.0)	72,594 (35.2)	0.1	0.0-0.2	< 0.001
Age* (years)	< 3.0	1 (0.3)	86,930 (42.2)	0.0	0.0-0.1	< 0.001
	3.0 - 5.9	4 (1.3)	43,278 (21.0)	0.1	0.0-0.3	< 0.001
	6.0 - 8.9	32 (10.5)	32,152 (15.6)	Base		
	9.0 - 11.9	81 (26.6)	23,169 (11.2)	3.5	2.3-5.3	< 0.001
	≥ 12.0	73 (24.0)	19,922 (9.7)	3.7	2.4-5.6	< 0.001
	Not recorded	113 (37.2)	527 (0.3)	215.4	144.1-322.0	< 0.001
Sex	Female	152 (50.0)	97,985 (47.8)	Base		
	Male	152 (50.0)	106,999 (52.2)	0.9	0.7-1.1	0.443
Neuter	Entire	33 (10.9)	20,736 (10.1)	Base		
	Neutered	205 (67.4)	73,497 (35.7)	1.8	1.2-2.5	0.003
	Not recorded	66 (21.7)	111,745 (54.32	0.4	0.2-0.6	< 0.001
Insurance	Non-insured	87 (28.6)	70,696 (34.3)	Base		
	Insured	161 (53.0)	47,696 (23.2)	2.7	2.1-3.6	< 0.001
	Not recorded	56 (18.4)	87,586 (42.5)	0.5	0.4-0.7	< 0.001

*incident cases: age at diagnosis, pre-existing cases: no age included, non-cases: age at midpoint of clinical records

Table 2: Estimated prevalence of hyperadrenocorticism in common dog breeds attending primary-care veterinary practices in England. The case counts from a 37.2% sample of dogs were used to estimate the total case count for each breed.

Breed	No. verified cases	Total no. cases (estimated)	Total in study	Prevalence %	95% CI
Crossbred	63	120	47300	0.25	0.2-0.3
Bichon Frise	24	46	2839	1.62	1.2-2.2
Standard Dachshund	10	19	737	2.58	1.6-4.0
Shih-tzu	8	15	4151	0.36	0.2-0.6
Yorkshire Terrier	22	42	6980	0.6	0.4-0.8
Jack Russell Terrier	29	55	13520	0.41	0.3-0.5
Chihuahua	2	4	4619	0.09	0.0-0.2
West Highland White Terrier	13	25	5275	0.47	0.3-0.7
Staffordshire Bull Terrier	17	32	16746	0.19	0.1-0.3
Cocker Spaniel	7	13	7699	0.17	0.1-0.3
Cavalier King Charles Spaniel	5	10	4461	0.22	0.1-0.4
German Shepherd Dog	3	6	7112	0.08	0.0-0.2
Labrador Retriever	11	21	17770	0.12	0.1-0.2
English Springer Spaniel	3	6	4131	0.15	0.1-0.3
Border Collie	1	2	5453	0.04	0.0-0.1

During the protocols used to diagnose the 191 incident hyperadrenocorticism cases, the following diagnostic tests were utilised: ACTH (adrenocorticotrophic hormone) stimulation test 182 (95.3%), urine cortisol-creatinine ratio 53 (27.8%), low dose dexamethasone suppression test (LDDST) 63 (33.0%), high dose dexamethasone suppression test (HDDST) 4 (2.1%), endogenous ACTH assay 8 (4.2%), adrenal ultrasonography 50 (26.2%) and magnetic resonance imaging 2 (1.1%). While differentiation between PTHAC and ATHAC is an important aspect of hyperadrenocorticism diagnosis, this distinction was infrequently recorded in the EPRs of the current study (Behrend et al., 2013).

During the study period, 127 (41.8%) of the 304 dogs with hyperadrenocorticism died from any cause. These deaths were at a median age of 12.7 years (interquartile range 10.9-14.2, range 5.8-17.6). From 119 deceased dogs with information available, 105 (88.2%) of the deaths involved euthanasia. Of the incident cases, 77/191 (40.3%) died during the study period. No significant differences were identified between purebred and crossbred dogs (P = 0.637) or between the individual breed assessed (P = 0.293) for the probability of incident cases dying during the study. However, male incident cases (48/100, 48.0% died) were more likely to die during the study period (P = 0.023) than females (29/91, 31.9%).

Univariable logistic regression modelling identified seven variables with liberally significant ($P < 0.20$) association with hyperadrenocorticism diagnosis: *breed, KC breed group, actual bodyweight, breed-relative bodyweight, age*, *neuter* and *insurance* (Table 1). As explained above, *purebred, KC breed group* and *actual bodyweight* were not considered for multivariable modelling. Based on the results of univariable analysis, no significant difference was detected between purebred compared with crossbred dogs in their odds of hyperadrenocorticism (odds ratio [OR] 1.1, 95% CI 0.8-1.5, P = 0.446). Among the KC breed groups, Hounds (OR 1.9, 95% CI 1.2-2.9, P = 0.006) and Toys (OR 1.5, 95% CI 1.1-2.1, P = 0.013) showed higher odds of hyperadrenocorticism than KC non-recognised dogs, while Gundogs (OR 0.5, 95% CI 0.3-0.7, P = 0.001), Pastorals (OR 0.5, 95% CI 0.3-1.0, P = 0.039) and Working dogs (OR 0.5, 95% CI 0.2-1.0, P = 0.047) had reduced odds of hyperadrenocorticism. Dogs of actual bodyweight < 10.0 kg had 3.0 times the odds (95% CI 1.5-5.9, P = 0.002) and those of bodyweight from 10.0-19.9 kg had 3.1 times the odds of hyperadrenocorticism (95% CI 1.6-6.2, P = 0.001) compared with dogs weighing ≥ 40.0 kg.

Following evaluation using multivariable logistic regression, the final model comprised four risk factors: *breed, breed-relative bodyweight, age* and *insurance*. No biologically significant interactions were identified. The final model was improved by inclusion of the clinic attended as a random effect and these results are reported ($P < 0.001$, rho = 0.094 indicating that the clinic attended accounted for 9.4% of variation). The final non-clustered model showed good discrimination (area under the ROC curve: 0.949). After accounting for the effects of the other variables evaluated, the Bichon Frise showed strong evidence of higher odds of hyperadrenocorticism compared with crossbred dogs (OR: 6.5, 95% CI 3.5-12.1, $P < 0.001$) while the Standard Dachshund (OR: 3.4, 95% CI 0.9-13.3, $P = 0.083$) and Yorkshire Terrier (OR: 1.8, 95% CI 1.0-3.4, $P = 0.055$) showed some evidence of increased odds. The Border Collie (OR: 0.1, 95% CI 0.0-0.9, $P = 0.038$) and Labrador Retriever (OR: 0.3, 95% CI 0.1-0.7, $P = 0.003$)) showed decreased odds of hyperadrenocorticism compared with crossbred dogs. Dogs with bodyweight equal to or higher than their breed mean had a 1.7 times odds of diagnosis compared with dogs weighing below their breed mean (95% CI 1.3-2.3, $P < 0.001$). Increasing age was associated with increasing odds of hyperadrenocorticism; dogs aged 9.0-11.9 years showed 3.9 (95% CI 2.6-6.0, $P < 0.001$) times the odds and dogs aged ≥ 12.0 years showed 5.7 (95% CI 3.7-8.7, $P < 0.001$) times the odds of diagnosis with hyperadrenocorticism compared with dogs aged 6.0-8.9 years. Insured dogs had 4.0 times the odds of diagnosis with hyperadrenocorticism compared with non-insured dogs (95% CI 2.8-5.6, $P < 0.001$) (Table 3).

Table 3: Final mixed-effects multivariable logistic regression model for risk factors associated with diagnosis of hyperadrenocorticism in dogs attending primary-care veterinary practices in England (n = 206,282).

Variable	Category	Odds Ratio	95% CI	P - Value
Breed	Crossbred	Base		
	Bichon Frise	6.5	3.5-12.1	< 0.001
	Standard Dachshund	3.4	0.9-13.3	0.083
	Shih-tzu	1.8	0.7-4.6	0.197
	Yorkshire Terrier	1.8	1.0-3.4	0.055
	Jack Russell Terrier	1.5	0.9-2.6	0.128
	Chihuahua	1.4	0.3-5.8	0.649
	West Highland White Terrier	1.1	0.5-2.3	0.789
	Other breeds	1.0	0.7-1.5	0.900
	Staffordshire Bull Terrier	0.9	0.5-1.8	0.790
	Cocker Spaniel	0.7	0.3-1.7	0.465
	Cavalier King Charles Spaniel	0.6	0.2-2.0	0.445
	German Shepherd Dog	0.4	0.1-1.4	0.172
	Labrador Retriever	0.3	0.1-0.7	0.003
	English Springer Spaniel	0.2	0.0-1.2	0.088
	Border Collie	0.1	0.0-0.9	0.038
Breed- relative bodyweight	< Breed Average	Base		
	≥ Breed Average	1.7	1.3-2.3	< 0.001
	Not recorded	0.0	0.0-0.0	< 0.001
Age* (years)	< 3.0	0.0	0.0-0.2	< 0.001
	3.0 - 5.9	0.1	0.0-0.3	< 0.001
	6.0 - 8.9	Base		
	9.0 - 11.9	3.9	2.6-6.0	< 0.001
	≥ 12.0	5.7	3.7-8.7	< 0.001
	Not recorded	33741.3	10959.1-103883.6	< 0.001
Insurance	Non-insured	Base		
	Insured	4.0	2.8-5.6	< 0.001
	Not recorded	1.1	0.7-1.7	0.754

*incident cases: age at diagnosis, pre-existing cases: no age included, non-cases: age at midpoint of clinical records

Discussion

This study reports the estimated prevalence of diagnosis with canine hyperadrenocorticism in the UK as 0.28%, suggesting hyperadrenocorticism is the third most prevalent endocrinopathy of dogs behind hypothyroidism (0.87%) (Dixon et al., 1999) and diabetes mellitus (0.34%) (Mattin et al., 2014), although methodological differences between the various studies complicate direct prevalence comparisons. Despite several molecular studies that investigated its aetiopathogenesis, the exact cause of hyperadrenocorticism in most dogs remains unknown (Hanson et al., 2010, Kool et al., 2013, Teshima et al., 2009, van Rijn et al., 2010, van Wijk et al., 2014). Access to clinical health data on the large population of dogs in VetCompass offers the intriguing prospect of aetiological and epidemiological research across a range of disorders such as hyperadrenocorticism and of linking these with primary-care diagnosis and health management (Mattin et al., 2014, Kearsley-Fleet et al., 2012, Taylor-Brown et al., 2015, Mattin et al., 2015, O'Neill et al., 2013).

The ACTH stimulation test was by far the most commonly test used during hyperadrenocorticism diagnosis in the current study, with over 95% of diagnoses including this test. The ACTH stimulation test has previously been reported as a commonly-used test for hyperadrenocorticism diagnosis in the UK (Barker et al., 2005, Kenefick and Neiger, 2008, Neiger et al., 2002). The ACTH stimulation test is diagnostically more specific, with reported test specificities ranging from 59% to 93%, but less sensitive than the LDDST (Behrend et al., 2013). The predominance of the ACTH stimulation test in the current study should have resulted in fewer false positive diagnoses of hyperadrenocorticism than if the LDDST was the predominant test. The LDDST is currently considered the screening test of choice for the

diagnosis of hyperadrenocorticism and can assist with differentiating between PTHAC and ATHAC (Behrend et al., 2013, Feldman et al., 1996) but was performed in only one third of cases in the current study. Although diagnostic specificity is lower for LDDST than the ACTH stimulation test, appropriate case selection on those patients presenting with highly suggestive history, clinical signs, and routine haematology and biochemistry should reduce false positives. The ACTH stimulation test may be preferred in primary-care practice to the LDDST because it is faster to perform and more convenient for owners.

The guidelines published in the Diagnosis of Canine Spontaneous Hyperadrenocorticism ACVIM Consensus Statement strongly recommend differentiation between the ATHAC and PTHAC variants of hyperadrenocorticism (Behrend et al., 2013). However, this distinction between PTHAC and ATHAC was infrequently recorded in the EPRs of the current study. The LDDST can differentiate PTHAC from ATHAC in approximately two-thirds of cases and, with specialist equipment and operator skills, adrenal ultrasonography has a high diagnostic sensitivity and specificity (82–100% and 82–99%, respectively) for the diagnosis of ATHAC (Behrend et al., 2015, Benchekroun et al., 2010). Increased awareness of the importance of this differentiation step should improve the management of hyperadrenocorticism-affected dogs.

This study identified evidence that the Bichon Frise (OR 6.5), Standard Dachshund (OR 3.4) and Yorkshire Terrier (OR 1.8) are predisposed to hyperadrenocorticism. Indeed, one in forty Standard Dachshunds and one in sixty Bichon Frise dogs were affected by hyperadrenocorticism in the current study (Table 2). Although familial hyperadrenocorticism has been described in Wire-Haired Dachshunds, spontaneous hyperadrenocorticism still accounts for the vast majority of cases in this breed (Stritzel et al., 2008). Dachshunds have been identified as predisposed in several hyperadrenocorticism studies but the predisposition

identified for the Bichon Frise in the current study is novel (Hanson et al., 2007, Helm et al., 2011, Jensen et al., 1997, Ling et al., 1979, Peterson, 1984). The Border Collie and Labrador Retriever were identified as protected breeds in the current study, with 0.1 and 0.3 times the odds for hyperadrenocorticism respectively compared with crossbreds. The reduced odds of hyperadrenocorticism in the Labrador Retriever shown in the current study is of interest because gundogs in general have previously been reported to have a reduced risk of hyperadrenocorticism in the UK (Barker et al., 2005). However, it is important that practitioners still remain alert for hyperadrenocorticism in non-predisposed breeds that are populous in the general population (e.g. Jack Russell Terrier and Staffordshire Bull Terriers) because these breeds can still contribute substantially to overall hyperadrenocorticism caseloads because of their popularity in the wider dog population (Table 1).

Of the KC breed groups, the Toy group had 1.5 times the odds of hyperadrenocorticism compared with breeds unrecognised by the KC, in line with previous reports (Barker et al., 2005). Two of the three individual breeds (Bichon Frise and Yorkshire Terrier) with evidence of over-representation for hyperadrenocorticism are among the Toy group and, together, these two breeds accounted for 15% of all hyperadrenocorticism diagnoses in this study population. The Hound group also had an increased risk of hyperadrenocorticism, with almost twice the odds of hyperadrenocorticism compared with breeds unrecognised by the KC. This can be explained by the presence of the Standard Dachshund in this group, which accounted for 40% of all Hounds diagnosed with hyperadrenocorticism. The Gundog, Working and Pastoral each had half the odds of hyperadrenocorticism compared with non-KC breed dogs. Members of these groups are generally medium to large bodysize breeds, and the univariable results suggested dogs of larger bodyweight had lower hyperadrenocorticism risk compared with smaller bodysize and toy breed dogs.

Individuals with a bodyweight equal to or higher than their breed mean had almost twice the odds of hyperadrenocorticism compared with dogs weighing below their breed mean. It is possible that dogs with higher average bodyweight are more likely to be overweight and therefore these results may suggest that overweight/obesity may be associated with hyperadrenocorticism in dogs. Previous reports have also suggested that dogs with a higher body condition score were at higher risk of hyperadrenocorticism than dogs with normal or low body condition scores, and an association between obesity and increased hyperadrenocorticism risk is recognised in human medicine (Lund et al., 2006, Tiryakioglu et al., 2010). However, these earlier studies reported associations rather than causal relationships and there are clearly other possible non-causal explanations for this association. For example, increased bodyweight in hyperadrenocorticism cases may be a consequence rather than a contributor to hyperadrenocorticism risk since dogs with hyperadrenocorticism are likely to demonstrate varying degrees of polyphagia and hence have increased risk of weight gain (Ramsey and Ristic, 2007). Future work to collect data on body condition score from dogs within the VetCompass database could help to clarify these associations.

The current study identified increasing risk of hyperadrenocorticism as animals age that has also been reported in other studies (Gallelli et al., 2010, Willeberg and Priester, 1982). The median age at diagnosis of hyperadrenocorticism in the current study was 10.9 years. Multi-centre studies performed in the UK described 37 dogs with a diagnosis of ATHAC having a mean age of 11.5 years (S.D. 1.87 years) and 148 dogs with a diagnosis of PTHAC having a mean age of 9.6 years (S.D. 2.3 years) (Barker et al., 2005, Helm et al., 2011). These ages are similar to the peak cancer incidence in a population of insured UK dogs (Dobson et al., 2002).

Many tumours are caused by spontaneous somatic mutations and are more likely to become clinically significant as individuals age, possibly explaining why the risk of hyperadrenocorticism diagnosis increases with age (Kennedy et al., 2012). It should be noted that the large number of cases without an apparent age shown in Tables 1 & 2 was because pre-existing cases were included in the study as having no recorded 'age-at-diagnosis'.

Although some previous studies reported a female predisposition for hyperadrenocorticism, this finding has often not been confirmed in other studies (Gallelli et al., 2010, Reusch and Feldman, 1992, Kenefick and Neiger, 2008, Neiger et al., 2002). The results of the current study supported the study hypothesis that sex is not a risk factor for a diagnosis of hyperadrenocorticism in dogs. In human medicine, hyperadrenocorticism has a substantial female over-representation, with female-to-male ratios commonly reported as 3:1 but the cause for this female predisposition in humans remains unclear (Boscaro et al., 2001, Huan et al., 2014, Newell-Price et al., 2014). In contrast to male humans who have an increased risk of large corticotroph tumours, large pituitary tumours have been reported as more common in female dogs (Gallelli et al., 2010, Huan et al., 2014, Zilio et al., 2014). This suggests differing aetiopathogenesis of PTHAC between humans and dogs. Larger pituitary tumours are associated with poorer prognosis, so it is possible that sex could affect outcome (Gallelli et al., 2010, Hanson et al., 2007, Kent et al., 2007). Female dogs did not have an increased odds of death compared with males in a recent European study (Fracassi et al., 2014). However, among hyperadrenocorticism cases, the current study identified a higher risk for males than females to die during the study period. Further work is needed to examine associations between sex, aetiopathogenesis and survival in more detail.

Testosterone has been shown to inhibit ACTH and cortisol secretion in rat models (Ajdžanović et al., 2015, Handa et al., 1994) and, if similar physiological pathways exist in dogs, a release of suppression might contribute to hyperadrenocorticism in neutered dogs. However, the current study did not detect an increased odds of a diagnosis of hyperadrenocorticism in neutered dogs after accounting for age and other risk factors. Similarly, a recent abstract describing an American population of dogs diagnosed with hyperadrenocorticism also failed to identify a differing risk of hyperadrenocorticism between neutered and intact dogs (Lourenc et al., 2015). In relation to the use of a standardised ACTH stimulation test using a 'genderless' reference range for the diagnosis of hyperadrenocorticism, it is interesting to note healthy neutered male dogs had higher serum post-ACTH cortisol concentrations than entire males (Frank et al., 2003). It has been suggested entire dogs may require a different reference interval for ACTH stimulation tests and that this group may be currently under-diagnosed if the same reference interval as neutered males is used (Frank et al., 2003).

The finding that insured dogs had increased odds of a diagnosis of hyperadrenocorticism mirrors other studies that show higher diagnostic proportions in insured compared with non-insured dogs, especially in disorders where diagnostic protocols are expensive or complicated (Taylor-Brown et al., 2015, Shoop et al., 2015, O'Neill et al., 2013). Increased disorder diagnosis may result from insured dogs being more likely to undergo diagnostic investigations than non-insured dogs (Egenvall et al., 2009). Hyperadrenocorticism is a chronic disease and treated patients generally require protracted therapeutic monitoring with consequent significant expense. It is estimated the actual lifetime cost of owning a dog in the UK is £16,000-31,000 and it is likely that diagnostic and treatment costs that can be triggered by unexpected illness may be poorly affordable for some owners of uninsured dogs (PDSA, 2015).

The study had some limitations. Due to time constraints, just over half of the possible cases identified in the original search strategy underwent manual verification. This complicated the calculation of prevalence estimates but should not have affected the overall findings of the study because of the sizeable number of cases that were still verified and because a randomised process was used to select the subset of candidate animals that were manually verified. As previously described for other published VetCompass studies, these data were not recorded primarily for research purposes and thus were limited by both missing or incomplete data and potential misdiagnoses (O'Neill et al., 2014a, O'Neill et al., 2014b)(O'Neill et al., 2015, 2013; O'Neill et al., 2014). Many cases did not undergo testing to differentiate between PTHAC and ATHAC, and consequently we have analysed the data by combining PTHAC and ATHAC cases as a general hyperadrenocorticism diagnosis. Although this paper accurately describes the prevalence of diagnosed hyperadrenocorticism recorded in the current study, this value may be an underestimate of the true prevalence of hyperadrenocorticism in this population because some positive cases may not have been diagnosed because of clinical or financial constraints.

In conclusion, this study estimated that hyperadrenocorticism is diagnosed in about one in four hundred UK dogs and that the Bichon Frise, Standard Dachshund and the Yorkshire Terrier show evidence of increased odds compared with other breeds. Dogs diagnosed with hyperadrenocorticism are more likely to be older, weigh under 20kg but be above their average breed bodyweights. These results may assist clinicians to balance their index of suspicion for this disease as well highlighting interesting features of hyperadrenocorticism in the dog which could enhance research into the aetiopathogenesis of the disease in both dogs and people.

Abbreviations

ACTH; adrenocorticotropic hormone

ACVIM; American College of Veterinary Internal Medicine

ATHAC; adrenal tumour hyperadrenocorticism

CI; confidence interval

EPR; electronic patient record

HDDST; high dose dexamethasone suppression test

KC; Kennel Club

LDDST; low dose dexamethasone suppression test

OR; odds ratio

PMS; practice management system

PTHAC; pituitary tumour hyperadrenocorticism

SQL; structured query language

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