Published in final edited form as:

J Small Anim Pract. 2018 November ; 59(11): 681–690. doi:10.1111/jsap.12904.

Canine hyperadrenocorticism associations with signalment, selected comorbidities and mortality within North American veterinary teaching hospitals

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Abstract

OBJECTIVE—To describe a large population of dogs with a diagnosis of hyperadrenocorticism at the time of death in North American veterinary teaching hospitals, and to identify comorbid conditions associated with hyperadrenocorticism.

MATERIALS AND METHODS—Retrospective cohort study of 1519 dogs with hyperadrenocorticism from a population of 70,574 dogs reported to the Veterinary Medical Database. Signalment, presence or absence of hyperadrenocorticism, aetiology of hyperadrenocorticism (if described), frequency of select comorbidities and causes of death were evaluated in dogs with and without hyperadrenocorticism.

RESULTS—Hyperadrenocorticism was more frequent in females. Neutering was associated with a minor, but significant, increase in the odds of hyperadrenocorticism. Hyperadrenocorticism was the presumed cause of death of 393 (25.9%) of affected dogs. When aetiology was specified (527 dogs, corresponding to 34.7% of the cases), pituitary-dependent hyperadrenocorticism [387 (73.4%) out of 527 dogs] was more common than functional adrenocortical tumour [136 (25.8%) out of 527 dogs]. Hyperadrenocorticism was over-represented in certain expected (miniature poodle, dachshund) and unexpected (Irish setter, basset hound) breeds compared with the population at large. Of the select comorbidities investigated, dogs with hyperadrenocorticism were at increased risk for concurrent diabetes mellitus, urinary tract infection, urolithiasis, hypertension, gall bladder mucocoele and thromboembolic disease compared with dogs without hyperadrenocorticism.

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Conflict of interest

The authors of this manuscript have no financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the manuscript.

CLINICAL SIGNIFICANCE—Hyperadrenocorticism is significantly associated with certain comorbid conditions but is not a major cause of mortality in affected dogs. Documented patterns now provide targets for prospective clinical research.

INTRODUCTION

Naturally occurring canine hyperadrenocorticism (HAC), aka canine Cushing's syndrome, is an endocrinopathy characterised by chronic glucocorticoid excess. Naturally occurring HAC can be caused by an adrenocorticotrophic hormone (ACTH)-secreting pituitary tumour leading to excessive cortisol production by the responsive adrenal glands (pituitary-dependent hyperadrenocorticism [PDH]) or by a cortisol-secreting tumour arising in the adrenal cortex (functional adrenocortical tumour [FAT]) (Pérez-Alenza & Melián 2017). Ectopic ACTH secretion and food-stimulated cortisol secretion have also been reported, but appear to be rare (Galac *et al.* 2005, 2008). In dogs, limited evidence suggests that HAC is over-represented in females, that PDH is the most common form overall, that FAT is seen more frequently in larger dogs than in smaller dogs, and that specific breed predilections exist (Ling *et al.* 1979, Willeberg & Priester 1982, Reusch & Feldman 1991, Gallelli *et al.* 2010, Behrend 2015, Pérez-Alenza & Melián 2017). Anecdotally, many North American veterinarians also believe that HAC is diagnosed more frequently among neutered dogs; however, a large epidemiologic study performed in the United Kingdom failed to confirm this when data was controlled for other risk factors (O'Neill *et al.* 2016). To our knowledge, these suppositions have not been documented in a large patient population, including both common and rare breeds, in North America.

HAC most commonly affects middle-aged to older dogs, causing a substantial impact on quality of life, as affected individuals frequently exhibit polyuria/polydipsia, polyphagia, abdominal distension and endocrine alopecia (Behrend 2015). While available medical therapies are effective in controlling clinical signs and may improve survival (Nagata *et al.* 2017), they are expensive and require long-term commitment to regular administration (Barker *et al.* 2005). Additionally, unless successfully surgically treated, HAC is a chronic condition that will persist for the duration of the patient's lifetime (Pérez-Alenza & Melián 2017). The systemic effects of chronic hypercortisolaemia may predispose patients to comorbidities. Previous case series and studies on smaller populations of patients diagnosed with HAC have suggested that HAC in dogs is associated with an increased occurrence of a variety of diseases, including diabetes mellitus, recurrent urinary tract or skin infections, urolithiasis, hypertension, gall bladder mucocoele, poor wound healing, orthopaedic injury, or hypercoagulability and thromboembolic events (Ortega *et al.* 1996, Nichols 1997, Hess *et al.* 1998, 2000, Johnson *et al.* 1999, Bryden *et al.* 2004, Mesich *et al.* 2009, Pace *et al.* 2013, Park *et al.* 2013, Rose *et al.* 2013, Behrend 2015, Fracassi *et al.* 2015). Rates of occurrence and risk factors for these comorbidities have not been systematically described in a large patient population of dogs with HAC regardless of the aetiology. Although, in one of the largest studies to date, the presence of comorbid conditions was not associated with survival times in dogs with PDH (Fracassi *et al.* 2015), comorbidities such as recurrent infections, orthopaedic injury or urolithiasis would negatively impact the quality of life of affected dogs. Even for highly committed and adherent owners, successful treatment of comorbid diabetes mellitus in HAC patients can be challenging, leading to death or euthanasia of the

patient (Hess 2010, Blois *et al.* 2011). It would be valuable for veterinarians to have a more accurate understanding of the risk of these comorbidities to facilitate effective client education.

The purpose of the study reported here was to use the Veterinary Medical Database (VMDB) to describe a large canine patient population with a diagnosis of HAC at the time of death, comorbidities associated with HAC, and mortality associated with HAC in North American veterinary teaching hospitals (VTHs). We hypothesised that female and neutered dogs would be overrepresented and that the occurrence of HAC would vary by breed. We also hypothesised that comorbid diseases previously described would be over-represented among HAC dogs compared with non-HAC dogs at the time of death.

MATERIALS AND METHODS

Data acquisition, HAC diagnosis, comorbidities and cause of death

This was a retrospective cohort study of canine entries from the VMDB (The VMDB does not make any implicit or implied opinion on the subject of the paper or study.). Data were obtained from searches of the VMDBs (<https://vmdb.org>). The VMDB was created in 1964, and contains abstracted records from the VTHs in North America. Summary data that have been entered by the attending clinician for each patient visit are subsequently extracted by medical records personnel and coded for entry into the VMDB. A data set of all entries between 1984 and 2004 for dogs that died or were euthanased during their VTH visits was reviewed to identify those with HAC (Fig. 1). Due to the nature of the data, natural death was not distinguished from euthanasia. The terminal visit was chosen both to ensure that no individual dog was included more than once, and to allow maximal time for the possible comorbidities associated with chronic HAC to occur. We have previously reported causes of death for dogs in this VMDB data set (Fleming *et al.* 2011). For purposes of this study, all diagnoses associated with each dog's terminal VTH visit as well as the cause of death previously identified in the previous study were evaluated. As HAC is a disease of middle-aged to older dogs, all dogs under the age of 1 year were removed from analysis as previously reported (Hoffman *et al.* 2013).

VMDB patient age and body weight are recorded within predefined categorical groups. The database's age categorisation includes six groups for dogs over 1 year of age. Patients with no recorded age were excluded from age-based analyses. VMDB patient weight in pounds (1 pound=0.45 kg) is categorised within seven groups, including an "unknown" group for patients whose weights were not available when the record was abstracted. For size-based analyses, dogs were grouped as small [up to 15 lbs; (up to 6.8 kg)], medium [15.1 to 50 lbs (6.9 to 22.7 kg)], large (50.1 to 100 lbs (22.8 to 45.4 kg)) and giant [over 100 lbs (over 45.4 kg)]. Patients with no recorded weight were excluded from size-based analyses. Breed information are entered into the VMDB by selection from a standardised list of breeds, including mixed breed, and represents owner-reported information. For breed-based analysis, to ensure adequate statistical power, we analysed the 24 most common breeds in our VMDB data set, which includes records of all dogs that died in VTHs between 1984 and 2004. Note that this list is slightly different from the most common breeds in the *overall* VMDB during that time period. Sex and neuter status were extracted as reported into the

VMDB in one of five categories: female, entire; female, spayed; female, unknown; male, entire; male, castrated. Females with no recorded neuter status were excluded from neutering-based analyses.

The Standard Nomenclature of Veterinary Diseases and Operations coding language used by the VMDB includes both broad and refined diagnoses and allows for some degree of overlap and redundancy, thus seven separate codes were used to identify HAC. A diagnosis of HAC was recorded for any dog whose VMDB entry contained one of these seven codes. Dogs without any of these diagnoses were recorded as not having HAC. The VMDB does contain a code indicating iatrogenic hypercortisolism, and dogs with this diagnosis were not included as having HAC. When a code indicating aetiology was present, dogs were stratified into PDH and FAT groups. Diagnoses of eight select comorbidities were recorded for all HAC dogs, including diabetes mellitus (seven codes), urinary tract infection (32 codes), urolithiasis (nine codes), bacterial or fungal skin infection (34 codes), hypertension (one code), gall bladder mucocoele (two codes), ligament or tendon rupture (16 codes) and thromboembolic disease (54 codes). The complete list of 162 precise codes searched is available as Table S1, Supporting Information.

To assess the impact of HAC on mortality, the previously reported (Fleming *et al.* 2011) causes of death among HAC dogs were analysed and aetiology of HAC (when described) was assessed for those dogs for which HAC was the cause of death. Categorical causes of death according to organ system (OS) and pathophysiologic process (PP) (Fleming *et al.* 2011) for dogs with and without a diagnosis of HAC were also tabulated.

Statistical analysis

All statistical analyses described below were completed using the statistical program R (R Core Team 2017). Frequency of HAC for dogs in each size, age, sex and breed category was calculated. Categorical analyses were performed using the chi-square (χ^2) test. These included assessments of frequency of HAC in individual breeds *versus* the population at large, frequency of the eight specified comorbidities in HAC *versus* non-HAC dogs, and causes of death in HAC dogs with FAT *versus* PDH. For multi-variate analyses, a Cox proportional hazard (CPH) model was employed in the R package survival (Therneau & Grambsch 2000). This model allows determination of significant factors that may be influencing an event of interest (HAC diagnosis) as a function of age. In the CPH model, the effects of neuter status and sex on HAC diagnoses were assessed. Odds ratios (ORs) with 95% confidence intervals (CIs) were determined for odds of HAC within each sex category at each age point. Giant and large dogs were collapsed into a single category for sizebased analysis of aetiology. The non-parametric Cochran Mantel Haenszel (CMH) test was also used to control for the effect of age when assessing whether neutering affects the proportion of HAC within breeds (Cochran 1954). A logistic regression using a generalised linear model (GLM) (McCullagh & Nelder 1989) with a binomial distribution was used to determine the effects of HAC on comorbidities using the equation below

$$y = \alpha + \beta_1 \text{sex} + \beta_2 \text{age} + \beta_3 \text{breed} + \beta_4 \text{HAC} + \varepsilon$$

where y is the presence (1) or absence (0) of the comorbidity, and HAC is the presence (1) or absence (0) of HAC. Graphs were plotted using either base R plotting function or the gplots package (Warnes *et al.* 2009).

RESULTS

Overall HAC morbidity

Medical records from a total of 80,958 dogs that died in VTHs between 1984 and 2004 were evaluated (Fig. 1). A total of 866,978 medical records existed in the data set for dogs who had been examined in VTHs for this same period time. Of the 80,958 dogs with a record of death, 10,384 dogs under 1 year of age were excluded, resulting in a total of 70,574 dogs meeting the inclusion criteria; a breakdown of the number of dogs analysed per year can be seen in Table S2. A total of 1519 dogs (2.1% of the studied population of 70,574 deceased dogs over 1 year of age) had a diagnosis of HAC at the time of death. For breed-based analyses, the 24 breeds with the greatest number of entries in the data set were used to ensure adequate numbers of individuals for within-breed analysis. Members of these 24 breeds accounted for 35,942 patients (50.9% of all dogs in the data set) and each breed was represented by a minimum of 680 entries.

Age, sex and neuter status

The 70,574 dogs were comprised of 35,327 females (50.1%), 34,842 males (49.4%) and 405 dogs with no recorded sex (0.6%). Eight hundred seventy-three female dogs had a diagnosis of HAC while 642 males had an HAC diagnosis. Four dogs with HAC had an unidentified sex. At the time of death, 24,938 females (70.6%) and 14,651 males (42.0%) were neutered. Twenty-two females had no recorded neuter status and were removed from these analyses. Of those dogs with a diagnosis of HAC 697 females (79.8%) and 323 males (50.3%) were neutered. For the overall population, frequency of diagnosis of HAC at the time of death increased with increasing age ($P<0.001$, Fig. 2).

Among all individuals, females (2.5%) were significantly more likely than males (1.8%) to have a diagnosis of HAC at the time of death ($P<0.001$). The frequency of HAC at the time of death was greater among neutered than intact dogs for both males [OR=1.40; 95% CI=1.20 to 1.65]; and females (OR=1.67; 95% CI=1.41 to 1.97). However, after controlling for age of an individual in a multi-variate model, the CPH model suggests only a weak effect of sex (HR=-0.115±0.055 SE, $P=0.035$) and neuter status (HR=0.116±0.0577 SE, $P=0.04$) on HAC diagnosis.

Aetiology of HAC (PDH and FAT)

The majority of dogs with HAC at the time of death ($N=992$; 65.3%) had a diagnosis code which did not specify pituitary or adrenal origin. Of those for which the aetiology could be determined ($N=527$), there were 387 dogs (73.4%) with a diagnosis of PDH, and 136 dogs (25.8%) with a diagnosis of FAT. Four dogs had diagnoses of both PDH and FAT (0.76%). There was no effect of size on diagnoses of FAT as assessed by a GLM, but large and medium dogs were significantly more likely to be diagnosed with PDH than small dogs

($P=0.0012$). Small dogs were significantly more likely to have diagnoses of “not specified” HAC than large or medium dogs ($P<0.0001$) (Fig. 3).

Breed variation

Relative frequency of HAC diagnosis at the time of death varied among breeds (Fig. 4, median: 2.22%, range: 0.34% to 4.35%). When the 24 most common breeds in our data set were evaluated, six breeds were found to have HAC frequencies over 3% at the time of death (in descending order of relative frequency, miniature poodle, dachshund, Irish setter, basett hound, miniature schnauzer and toy poodle, $P<0.05$ for all breeds). Conversely, four breeds among these 24 largest sample size breeds were found to have HAC frequencies less than 1% at the time of death (Dobermann pinscher, rottweiler, collie and great Dane, $P<0.004$ for all breeds). Results of the GLM including both breed and body size revealed that breed carries a more significant impact on the frequency of HAC than does size (Table S3).

The effect of neuter status on frequency of HAC diagnosis at the time of death was evaluated within each of the 24 breeds with the largest sample sizes by CMH tests; no significant effect was found for any breed (Fig. 4).

Concurrent diagnoses

The frequency of eight comorbidities purported to be associated with HAC was recorded among all dogs with HAC at the time of death ($N=1519$). One hundred and sixty dogs (10.5% of HAC dogs) had concurrent diabetes mellitus, 101 dogs (6.6%) had concurrent urinary tract infection, 43 dogs (2.8%) had concurrent urolithiasis, nine dogs (0.59%) had concurrent bacterial or fungal skin infection, nine dogs (0.59%) had concurrent hypertension, 24 dogs (1.6%) had concurrent gall bladder mucocoele, five dogs (0.33%) had concurrent ligament or tendon rupture and 93 dogs (6.1%) had concurrent thromboembolic disease. Compared with all dogs in the data set, dogs with HAC were significantly more likely to have comorbid diabetes mellitus, urinary tract infection, urolithiasis, hypertension, gall bladder mucocoele and thromboembolic disease than dogs without HAC at the time of death (all $P<0.0005$). These results remained statistically significant even after controlling for age, sex and breed. By simple categorical χ^2 testing, there was no difference in frequency of the diagnosis of skin infections ($P=0.077$), or ligament or tendon ruptures ($P=1$) between dogs with or without HAC at the time of death (Fig. 5). However, using the GLM to control for sex, age and breed, skin infections were shown to be significantly increased in HAC dogs ($P=0.0027$), while there remained no association between diagnosis of HAC and diagnosis of ligament or tendon ruptures.

Cause of death

HAC itself had been previously identified (Fleming *et al.* 2011) as the *cause* of death for 393 (25.9%) out of the 1519 HAC dogs, of which the majority ($N=283$; 72.0%) had a diagnosis code which did not specify pituitary or adrenal origin. Therefore, HAC was a cause of death for only 0.6% of the overall population. Of HAC dogs for which the aetiology could be determined ($N=110$), there were 21 dogs (19.1%) for which PDH had been identified as the cause of death, and 89 dogs (80.9%) for which FAT had been identified as the cause of death. FAT was significantly more likely to have been identified as the cause of death than

PDH ($P<0.0001$). Of 1126 HAC dogs that did not have HAC as the cause of death, the leading identified OS categorical cause of death was endocrine followed by gastrointestinal and urogenital, while the leading identified PP categorical cause of death was neoplastic followed by metabolic. HAC itself is in OS category “endocrine”, and PP category “metabolic.” Among non-HAC dogs, the leading OS categorical cause of death was neurologic followed by musculoskeletal, while the leading PP categorical cause of death was neoplastic followed by trauma. Among dogs without a diagnosis of HAC, OS endocrine causes of death were the ninth most common out of 11 categories, while PP metabolic causes of death were fourth most common out of nine categories.

Seventy-five HAC dogs, of the 1126 dogs for which a disease other than HAC was identified as the cause of death, had their deaths ascribed to one of the eight select comorbidities. Of these 75 dogs, the cause of death was identified as diabetes mellitus in 39 (52%) dogs, urinary tract infection in 19 dogs (25.3%), thromboembolic disease in 13 dogs (17.3%), urolithiasis in three dogs (4%) and bacterial or fungal skin infection in one dog (1.3%). No dogs with HAC died of hypertension, gall bladder mucocoele or ligament or tendon rupture.

DISCUSSION

Age, sex and neuter status

Not surprisingly, we found that the diagnosis of HAC was more frequent among dogs that were older at the time of death. This is compatible with the description of HAC as a disease of middle-aged and older dogs (Behrend 2015). With the exception of cases of FAT that undergo adrenalectomy or PDH that undergo hypophysectomy (a procedure which had extremely limited availability in North America during the timespan of this study), HAC is also not curable, and so a diagnosis made at any age will persist until the time of death.

HAC was more frequent in females than in males as hypothesised. Females were 1.4 times more likely to have a diagnosis of HAC at the time of death than their male counterparts. However, as female dogs may live longer than male dogs (Michell 1999, Hoffman *et al.* 2013, 2018), once the confounding effect of age at the time of death was controlled, the association of sex and diagnosis of HAC, while remaining significant, became much weaker. Surgical neutering of pet dogs is commonly performed in North America (AVMA n.d., Trevejo *et al.* 2011). Based on anecdotal experience, we expected that HAC would be more frequent in neutered dogs. At first glance, the frequency of HAC at the time of death among neutered males and females is greater than that among their intact counterparts, but analysis shows that neuter status is only marginally associated with HAC after controlling for age. Furthermore, a significant association of neutering on HAC frequency cannot be demonstrated within any individual breed after controlling for age. Because age at neutering is not recorded in the VMDB, it is possible that some dogs in the data would have been neutered later in life, which could impact our analysis; however, our results are similar to those of a recent large epidemiologic study in dogs in the UK, which concluded no significant impact of neutering after controlling for age and other risk factors (O'Neill *et al.* 2016). Previous research has shown that there is a significant lifespan cost of maintenance of reproductive capacity in the domestic dog, and that neutered males and females live longer than their intact counterparts (Kraft 1998, Moore *et al.* 2001, Hoffman *et al.* 2013). The

frequency of HAC increases with age, which leads to an apparent increase of HAC among neutered dogs at the time of the death. Our study suggests that this apparent increase is primarily due to the indirect effect of the association of neutering with longer lifespan (Hoffman *et al.* 2013), rather than a direct effect of neutering on HAC itself.

Human cases of Cushing's syndrome are very rare, with an annual incidence of only two or three cases per million adults, compared with one to two new cases per thousand dogs per year (Gallelli *et al.* 2010). In humans, PDH cases greatly predominate over FAT cases, as in dogs (Steffensen *et al.* 2010). Cases in human females greatly outnumber cases in human males, with ratios from 3:1 to 15:1 cited by various authors (Fauci & Harrison 2009, Valassi *et al.* 2011). No explanation has been found for this disparity. Compared with a human female to male predominance of 3:1 at minimum, the canine female predominance of 1.4:1 in this study seems mild.

Overall HAC morbidity and aetiology of HAC (PDH and FAT)

In our study population, 2.1% of deceased dogs over 1 year of age had a diagnosis of HAC, a proportion 10 times higher than the prevalence reported in dogs presented to primary care veterinary practices in the UK (O'Neill *et al.* 2016). The high prevalence reported here likely reflects differences in prevalence between a general dog population and that presented to referral hospitals, although regional differences between North America and the UK could also be a contributing factor.

Among the dogs of this report for whom the aetiology was specified, PDH (73.4%) was more common at the time of death than FAT (25.8%). Retrospective database studies such as the work presented here have certain weaknesses, deriving from the fact that each data entry represents a single point in time, and the detail of the recorded information is limited. Thus, we know only that dogs had the recorded diagnosis of HAC but are unable to determine whether or not these dogs underwent the diagnostic tests necessary for differentiation between PDH and FAT. The absence of aetiological information may reflect lack of recording at the participating institution, or lack of differentiation of the aetiology in any given dog. However, importantly, dogs with a diagnosis of iatrogenic hypercortisolism were not included in our HAC population. While iatrogenic hypercortisolism existed as a diagnostic option in each case, it remains possible that this diagnosis was sometimes missed. Nevertheless, even with these limitations, the proportions of PDH and FAT we identified compare favourably with previous reports of relative prevalence in dogs (Gould *et al.* 2001, Behrend 2015). FAT has previously been described to be more common in larger dogs, when compared to smaller dogs, although PDH remains more frequent overall (Behrend 2015). We found PDH to be over-represented in larger dogs, with no apparent size effect on FAT. However, the majority of HAC dogs in our study had a diagnosis code for HAC that did not specify adrenal or pituitary origin; specifically, the great majority of smaller dogs had no specified aetiological diagnosis. Clinician bias towards heightened concern for FAT in larger dogs may have led to the use of more differentiating tests in larger dogs than in smaller dogs. Thus, the apparent increase in cases specified as PDH among larger dogs may actually reflect the fact that PDH is the more common aetiology overall, and larger dogs were more likely to have their aetiologies identified.

Breed variation

Our results support our hypothesis that HAC frequency does vary by breed. Compared with limited previous information and anecdotal opinion describing frequency of HAC among various breeds, some high-frequency HAC breeds identified in our analysis (miniature and toy poodles, dachshund and miniature schnauzer) were expected, while other breeds we identified (Irish setter, bassett hound) have not been previously described as high-frequency for HAC (Reusch & Feldman 1991, Barker *et al.* 2005, Behrend 2015). While the over-representation of smallbreed dogs with HAC may partly be an artefact of the longer lifespans of small breeds (Patronek *et al.* 1997, Greer *et al.* 2007), the Irish setter and the bassett hound are both large-breed dogs which exhibited a frequency of HAC >3% at the time of death, despite the shorter average lifespans that might be expected in those breeds. Our GLM analysis confirms that the frequency of HAC remains associated with breed when controlling both for age and for dog size.

Comorbidities

Because of the multi-systemic effects of cortisol, comorbidities are a significant concern in HAC dogs and awareness of comorbidities can influence an owner's decision to treat HAC (Behrend *et al.* 2002, Ramsey & Neiger 2007, Klose *et al.* 2011, Behrend 2015). This is the largest study to date to report comorbidities among a population of HAC dogs. In this population, we were able to confirm prior suspicion that HAC dogs were at increased risk for comorbid diabetes mellitus, urinary tract infection, urolithiasis, hypertension, gall bladder mucocoele, thromboembolic disease and skin infection than dogs without HAC at the time of death, after controlling for age, sex and breed (Ortega *et al.* 1996, Hess *et al.* 1998, 2000, Mesich *et al.* 2009, Hess 2010, Behrend 2015). Surprisingly, we were not able to confirm the purported concurrence between HAC and ligament or tendon ruptures (Lemarie *et al.* 1996, Nichols 1997, Bryden *et al.* 2004). It is important to note that our data are limited to dogs presented to VTHs. We anticipate that rare diseases, or complex presentations of disease, will be more prevalent in this data set than in the population at large as an effect of referral bias (Bartlett *et al.* 2010). As such, HAC dogs with concurrent ligament or tendon ruptures should be subject to this bias, and their absence is particularly noteworthy. Similarly, the rates of HAC comorbidities that we describe in this population are likely to be higher than those seen in the population at large. We also do not know how many of the HAC dogs in our data set were undergoing therapy for their HAC but it is unlikely that a difference in proportion of treated dogs affects the influence of HAC on ligament or tendon ruptures uniquely. Chronic comorbidities (*e.g.* diabetes mellitus) may be more likely to persist to the time of death than acute comorbidities that have the potential to heal (*e.g.* ligament rupture) and, therefore, a study design that requires both conditions at the same time point (*i.e.* death) rather than within the same time period may introduce bias towards chronic *versus* acute comorbidities. Additional comorbidities, such as pancreatitis or renal proteinuria (Barker *et al.* 2005, Mazzi *et al.* 2008), are purported to be associated with HAC. These were not investigated in the present retrospective study because of the challenge of establishing a definitive diagnosis (pancreatitis), or the emerging nature of the diagnosis over the span of time represented by this data set (renal proteinuria). Further investigation into the prevalence of these comorbidities among HAC dogs in general practice, and the mechanisms of the relationships with HAC, is warranted.

Cause of death

A presumptive cause of death had been identified for each of the dogs in this data set as part of a previous report (Fleming *et al.* 2011). In this data set, HAC was the identified cause of death for approximately one-quarter of the cases (25.9%) affected by the disease. This is compatible with reports of long-term survival times for treated HAC patients (Barker *et al.* 2005, Schwartz *et al.* 2008) and confirms that many dogs diagnosed with HAC will eventually die of an unrelated cause. Given that patients presented to VTHs are likely to be more severely ill than patients in the general population, it is possible that deaths due to HAC are even less frequent in the general population than in the data presented here. Additionally, it was not possible to determine for each dog whether death occurred naturally, or whether euthanasia was performed. The only study of a large population of companion dogs in which frequency of euthanasia was compared with frequency of natural death revealed that 86.4% of dogs were euthanased, while 13.6% died naturally (O'Neill *et al.* 2013). Owners may elect euthanasia for reasons of perceived quality of life, perceived prognosis or inability (financial, emotional, or physical) to provide needed care for dogs. As long as euthanasia remains an option for North American dog owners, including euthanased dogs among any population of dogs whose deaths are studied is representative of outcomes that will be encountered by the population at large. Among HAC dogs that did not have HAC as the cause of death, other endocrine conditions, as well as gastrointestinal and urogenital conditions were predominant OS causes of death, in contrast to dogs without HAC, for whom neurologic and musculoskeletal diseases were predominant OS causes of death. This may in part be related to the frequency of neurology referrals to VTHs overall, and the fact that dogs at risk for HAC may be at risk for other endocrinopathies. Among both HAC dogs without HAC as the cause of death, and non-HAC dogs, the predominant PP categorical cause of death was neoplastic. This likely reflects the high prevalence of cancer among dogs overall, as well as the nature of cases referred to VTHs.

Among dogs with HAC as the cause of death for whom an aetiology was specified, the majority (80.9%) had a diagnosis of FAT. While this is compatible with the previous reports describing the risk of caval invasion by FAT (Kyles *et al.* 2003, Massari *et al.* 2011), as well as resistance to medical management of FAT (Feldman *et al.* 1992, Helm *et al.* 2011), this finding must be interpreted with care. It is possible that the aetiology of HAC may be more readily identified and recorded for dogs who die of FAT than for dogs who die of PDH, either because of terminal surgical exploration, or acute and dramatic *ante mortem* clinical signs such as haemoabdomen, which lead to the discovery of FAT. Additionally, long-term survival has been reported for dogs who underwent medical management of FAT (Arenas *et al.* 2014).

In addition to HAC dogs with HAC itself identified as the cause of death, there were 75 HAC dogs that had one of the eight select concurrent diagnoses identified as the cause of death. The interplay between these comorbidities, and the fact that both diseases may have been concurrently treated before death, makes it challenging to determine with certainty which disease contributed more to cause of death. This study was designed to investigate correlation and it is not clear that HAC is the underlying cause of any of the comorbidities with which it is associated. However, if HAC were ultimately determined to be causative of

the development of these select comorbidities, then it could be argued that HAC was the primary cause for death for these additional 75 dogs. In that case, HAC would become the cause of death for 468 (30.8%) of HAC dogs, or 0.7% of the total population.

Limitations

The information available for each dog was limited to that recorded in the abstracted medical record in the VMDB. All cases were seen at VTHs, which are specialty centres primarily staffed by board-certified personnel. It is important to note that the only diagnoses entered into the VMDB by information technology personnel were those that had been originally entered into the medical record by these attending clinicians; that is, there is no part of the VMDB data entry process in which records are scanned for keywords in an automated way. For example, if a dog were presented to the VTH for a complaint of “suspicion of Cushing’s” and the attending clinician ultimately excluded that diagnosis, that historic suspicion would not be part of the information extracted for the VMDB. Thus, diagnoses that appear in this data set are those that the attending clinicians of the VTH specialty services actively entered at the time. Additionally, VTHs are primarily referral centres, where clients anticipate high costs. Thus, it is likely that many clients who undertook such referrals were financially able to pursue recommended diagnostics.

Because of the nature of this data set, we cannot independently confirm the accuracy of any diagnosis, determine the diagnostic criteria that were used to achieve it, or discover whether or how it was treated. The impact of this disadvantage must be evaluated in context. The availability of computerised medical records and powerful computational software enables us to analyse increasingly large and more complex data sets. This statistical power creates new opportunities to challenge dogma, suppositions and conclusions that have previously been based on small studies. The trade-off is that the granularity to which we have grown accustomed in veterinary medicine – the ability to manually review each individual diagnostic or therapeutic choice or result for each individual patient – becomes less feasible. When appropriate questions are asked of these large data sets, that level of individual scrutiny is also less necessary. While we risk incorporating some error into our data when we trust in the accuracy of medical records, the benefit is that we are able to draw broad conclusions regarding large populations, a positive outcome not possible in studies of a few dozen or even a few hundred patients at a time. Individual record review remains an important aspect of retrospective studies in which novel diagnoses, novel treatments, rare side effects or unexpected outcomes are investigated; however, when breed, sex and the diagnosis of a familiar, recognisable endocrinopathy of dogs are being investigated, the medical record is a reliable source of accurate information, primarily subject to random error. Acceptance of this approach is illustrated by numerous examples in the human medical literature (Solberg *et al.* 2008, Allen *et al.* 2013, Yu *et al.* 2014).

In this report, our goals were to describe a large canine patient population with a diagnosis of HAC at the time of death, and the comorbidities and mortality associated with HAC in VTHs. For this we need only demographic information and final diagnoses. Demographic features of this population included those reported by their owners, and diagnoses in these patients were those recorded by attending clinicians and reflected their conclusions or

summaries of the cases. Clinicians in VTHs are typically board-certified specialists, or trainees in each discipline supervised by specialists, practicing state-of-the-art medicine. While it is reasonable to have high confidence in the diagnostic abilities of such referral centres, the data structure does not allow us to know which tests were run on which dogs in which order to ultimately make the diagnosis of HAC that was reported to the VMDB. It is likely that errors exist in any dataset of this size.

A final limitation of this study is the age of the data set described here. Causes of death had been manually identified in this data set as part of a previous report (Fleming *et al.* 2011) and the value of the addition of this cause of death information was perceived to justify the use of an aging dataset for the analysis described here. More contemporary data which included cause of death information were not available to us and no significant changes to the approach to the diagnosis of canine HAC have occurred in the intervening years since these data were captured. While we acknowledge that the diagnosis of certain comorbidities (*e.g.* gall bladder mucocoele) has been refined in recent years, our ability to diagnose comorbid diseases would have affected the patients in this data set with and without HAC similarly. However, it is possible that a selection bias may have been introduced here as, being aware that HAC is associated with certain comorbid conditions, clinicians may be motivated to perform more extensive diagnostic work-ups in dogs with HAC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was completed at the University of Georgia College of Veterinary Medicine, and the Department of Pathology at the University of Washington. This work was partially supported by NIH grants AG044284 (DELP) and T32GM007103 (JMH). The authors would like to acknowledge Steve Austad for his assistance with procurement of the primary data from the VMDB.

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FIG 1.
Flow diagram of cohort selection. VMDB Veterinary Medical Database

FIG 2.

Relative frequency of canine hyperadrenocorticism (HAC) according to sex, neuter status and age at the time of death. Barplot of each age bin for neutered females (red), intact females (yellow), neutered males (blue) and intact males (purple) at time of death. For each age-class, the y-axis shows the percentage of dogs with a diagnosis of HAC at the time of death, among all dogs that died at that particular age. Error bars indicate ± 1 standard error of the mean. Neutered dogs were more likely to have diagnoses of HAC than intact dogs ($P=0.04$). There was significant evidence that frequency of HAC increases with age in both males and females

FIG 3.

Aetiology of hyperadrenocorticism (HAC) in dogs according to size. Bars indicate relative frequency of each HAC aetiology among dogs with HAC at the time of death. Red bars indicate functional adrenal tumour (FAT), blue bars indicate pituitary-dependent hyperadrenocorticism (PDH), and yellow bars indicate HAC of unknown origin. Dogs were grouped as small [up to 15 lbs; (up to 6.8 kg)], medium [15.1 to 50 lbs (6.9 to 22.7 kg)], large [50.1 to 100 lbs (22.8 to 45.4 kg)] and giant [over 100 lbs (over 45.4 kg)]. Error bars indicate ± 1 standard error. Large and medium dogs were significantly more likely to be diagnosed with PDH than small dogs ($P=0.0012$). Small dogs were significantly more likely to have diagnoses of “not specified” HAC than large or medium dogs ($P=0.0003$).

FIG 4.

Breed log-odds and proportions of hyperadrenocorticism (HAC). Breeds are listed in ascending size order according to breed standard adult weights. Left panel indicates proportions of HAC for the 24 breeds with the largest sample sizes. Error bars indicate ± 1 standard error. Right panel indicates results of CMH tests on the association of neuter status at time of death after controlling for age for the same breeds. The log-odds value is the $\log_e$ transform of the odds ratio. Positive values indicate increased strength of association in neutered dogs. Negative values indicate increased strength of association in intact dogs. Error bars indicate 95% confidence intervals. Colours of bars indicate size of dogs (red small, blue medium, green large, yellow giant)

FIG 5.

Proportion of concurrent disease diagnoses at time of death among hyperadrenocorticism (HAC) dogs and non-HAC dogs. Proportion of each diagnosis among all dogs in the group is shown for HAC dogs (blue) and non-HAC dogs (red). Concurrent disease abbreviations are diabetes mellitus (DM), urinary tract infection (UTI), urolithiasis (U), skin infection (SI), hypertension (H), gall bladder mucocoele (GBM), ligament or tendon rupture (LTR) and thromboembolic disease (TE). *** indicates statistically significant difference with a Chi-squared P -value <0.0001 . Error bars indicate ± 1 standard error