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ORIGINAL ARTICLE

Asymptomatic heart valve dysfunction in healthy middle-aged companion dogs and its implications for cardiac aging

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Abstract Heart disease is the leading cause of death in the USA, accounting for about one in every four deaths. Age is the greatest risk factor for heart disease in both people and dogs; however, heart disease is generally not considered as a major cause of morbidity or mortality in dogs. As part of the preliminary selection process for a veterinary clinical trial, 40 companion dogs with no history of cardiac pathology that were at least 6 years old and weighed at least 18 kg underwent a cardiac screening using Doppler echocardiography. Eleven dogs from this cohort were diagnosed with valvular regurgitation by echocardiography, and seven of these cases were of sufficient severity to warrant exclusion from the clinical trial. In only one case was a heart murmur detected by auscultation. Serum alkaline phosphatase levels were significantly higher in the dogs with moderate to severe valvular regurgitation compared to the rest of the cohort. These observations suggest that asymptomatic degenerative valvular disease detectable by echocardiography, but not by a standard veterinary exam including auscultation, may be present in a significant fraction of middle-aged companion dogs, indicating a previously underappreciated similarity between human and canine aging. Further, these data suggest that companion dogs may be a particularly useful animal model for understanding mechanisms of age-related degenerative valve disease and for developing and testing interventions to ameliorate cardiac disease. Future studies should address whether dogs with asymptomatic valve disease are at higher risk for subsequent morbidity or early death.

Keywords Dogs · Cardiac aging · Degenerative valve disease · Regurgitation · Echocardiogram

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Introduction

Age is the greatest risk factor for nearly every major cause of mortality in developed countries (Kaeberlein et al. 2015). In particular, this is true for heart disease, which is the leading cause of death in the USA. Between the ages of 40 and 60, the risk of dying from heart disease for a typical American adult increases about eightfold, which is greater than the increase in risk associated with having high blood pressure, high

cholesterol, and diabetes combined. In addition to cardiovascular disease and cardiomyopathy, degenerative valve disease (DVD) plays an important role in age-related cardiac morbidity in people (Zeng et al. 2016). It is estimated that more than one out of every eight people over age 75 have moderate to severe DVD (Nkomo et al. 2006), and DVD accounts for 10 to 20% of all cardiac surgeries in the USA (Maganti et al. 2010). Furthermore, its worldwide prevalence is expected to increase dramatically as the aged population increases, raising significant public health concerns (d'Arcy et al. 2011).

Animal models play an essential role in the study of heart disease, and species used in this line of research include mice, rats, rabbits, dogs, sheep, and swine, among others. In contrast, DVD induction has not been achieved in rodents to date (Houser et al. 2012), and their size limits the potential for surgical intervention. Given these limitations, most of the available models of DVD create cardiac pathology in dogs and swine through surgically severing the *chordae tendineae* (Houser et al. 2012), rather than studying DVD in the context of normative aging. While the hemodynamic changes thus created mimic the situation in DVD, the fact that this intervention is performed in young animals in an acute fashion limits its usefulness as a model for changes associated with normative aging, which include gradual age-related alterations to the myocardium in addition to gradual valvular changes (Akasheva et al. 2015; Jones and Zook 1965; Fairweather 1992). Additionally, all of these models are studied in captive animals maintained in laboratory conditions, which fail to recapitulate important features of the natural environment.

In this context, companion dogs provide a potentially powerful model for understanding how and why DVD is both a cause and consequence of aging. In this species, DVD accounts for roughly 75% of all heart disease (Anonymous 2010; Detweiler and Patterson 1965; Das and Tashjian 1965; Buchanan 1977), with age also being a significant risk factor (Whitney 1974). While there have been several studies focusing on individual high risk breeds such as Cavalier King Charles Spaniels (Pedersen et al. 1999a) and Dachshunds (Olsen et al. 1999), and one more recent study that focused on the prevalence of mitral valve disease in dogs diagnosed in a primary care setting (Mattin et al. 2015), the most recent screening studies of DVD prevalence in the general dog population seem to date from the 1960s (Jones and Zook 1965; Detweiler and Patterson 1965; Detweiler et al. 1961). As trivial valvular regurgitation is not uncommon in older dogs and not all dogs with diagnosed valvular regurgitation demonstrate clinical signs of cardiac disease during their lifetimes (Yuill and O'Grady 1991; Borgarelli et al. 2008), it is often assumed that this is a normal part of the aging process, and the extent to which asymptomatic regurgitation and DVD contribute to morbidity and mortality in dogs remains unclear.

It is generally accepted that older, small dogs are more commonly affected by DVD than young, large dogs, and inheritance studies reveal an increased risk of clinical signs among male dogs (Anonymous 2010; Olsen et al. 1999; Swenson et al. 1996). A retrospective study of more than 111,967 dogs treated at primary care veterinary practices in the UK found a prevalence of diagnosed mitral valve disease of 0.4% and a prevalence of heart murmur consistent with DVD but not sufficient to warrant diagnosis of 3.5% (Mattin et al. 2015). However, the above values are derived from the VetCompass database (VetCompass 2013), which only captures veterinary record data from clinical practice, where screening in the absence of symptoms usually consists of a clinical exam including auscultation. We note that it has been demonstrated that the presence of mild valvular regurgitation in dogs cannot be reliably detected by auscultation and that considerable variation in the ability to detect heart murmurs associated with valvular regurgitation exists among individual veterinarians (Pedersen et al. 1999b).

In addition, when comparing these clinical data to data obtained by post-mortem examination, the measured prevalence of DVD can increase quite dramatically. For instance, Detweiler and Patterson (1965) found a prevalence of 391 out of 4831 dogs (8.1%) seen in private veterinary practice based on clinical diagnosis and/or post-mortem examination. Based on post-mortem examination only, Jones and Zook (Jones and Zook 1965) found that 139 out of 404 dogs (34.4%) showed valvular disease, and that the prevalence was strongly correlated with age. These observations would thus seem to indicate that the prevalence of degenerative valve disease in dogs is generally underestimated in clinical practice.

As mentioned above, the available epizootiological screening studies of DVD prevalence in the general dog population now date from over 50 years ago, which raises concerns as to their comparability to data from the contemporary dog population (Mattin et al. 2015). As part of the Dog Aging Project, we are performing

veterinary clinical studies to identify and assess genetic, environmental, and pharmacological interventions with the potential to delay aging and promote healthy longevity in companion dogs (Kaeberlein 2015; Kaeberlein et al. 2016). The cohort described in this report represents animals that were nominated by their owners to participate in a clinical trial aimed at assessing the safety and efficacy of low-dose rapamycin treatment in companion dogs; the outcomes of that trial will be described elsewhere. All of the dogs considered for that trial are companion dogs at least 6 years old and at least 40 lb (18 kg) in weight.

Aside from age and weight, a criterion for enrollment in this study was the absence of significant pre-existing health conditions, which we ensured through extensive health screening of candidate dogs. In addition to a standard veterinary exam and blood chemistry analysis, each of these dogs received a detailed cardiac exam including Doppler echocardiography. Here, we report the observation that, although none of the dogs had a prior clinical history of diagnosed cardiac abnormalities, 11 out of 40 dogs (27.5%) examined in this study presented with cardiac valve regurgitation, only one case of which was also detected as a heart murmur. The cases of DVD represent the most common cause of exclusion from the subsequent rapamycin intervention. Our findings suggest that asymptomatic DVD is a relatively common feature of normative aging in larger companion dogs, raising the question as to whether these age-associated changes contribute to morbidity and mortality to a greater extent than has been previously appreciated, and leading us to conclude that the privately owned domestic dog may be a suitable model for studying the mechanisms of spontaneously occurring age-related DVD, its impact on subsequent morbidity and mortality, and potential therapeutic strategies.

Methods

Forty companion dogs from the greater Seattle area were recruited into this study and completed the initial study exam. Dogs were recruited through an internet-based nomination form on the Dog Aging Project website (www.dogagingproject.com). Dogs were selected for further consideration if they met the following enrollment criteria: (1) the owners agreed to bring the dog to the study veterinary clinic three times during an 11-week period, (2) the dog weighed at least 18 kg, (3) the dog was at least 6 years old, and (4) the dog did not have any prior or current significant health problems or current medication that might be contraindicated with the study drug (rapamycin/sirolimus). Based on the information provided by the owners, 83 dogs were identified whose owners lived within approximately 50 miles of the study clinic and who met the study criteria for weight and age. Seventeen of these dogs were excluded at this stage based on owner-reported pre-existing health conditions or current medications that might be contraindicated with the study medication, and six dogs were excluded at the recommendation of the clinical trial monitor (SM), based on owner-reported information regarding animal personality and/or aggression. None of the dogs excluded at this stage were excluded because of heart disease including DVD. The remaining dogs were invited to participate in the study on a rolling basis, based primarily on distance from the study clinic, with those owners living closest to the clinic invited first. Out of 60 owners invited to participate in the study, 48 were scheduled for an initial exam, ten were non-responsive to email follow-up, and two were unable to schedule an appointment at a mutually agreeable time. Seven owners canceled their appointments prior to enrollment, and one failed to show up and was non-responsive to follow-up communication.

All of the owners completed a written informed consent form prior to their first study-related veterinary visit. Upon meeting the above criteria and completion of informed consent, all dogs attended a first study-related veterinary exam at the VCA Specialty Clinic in Lynnwood, WA between August 2015 and December 2015. All procedures were approved by the University of Washington Institutional Animal Care and Use Committee (IACUC) under protocol #4359-02.

At the first examination, a medical history was taken and physical examination performed for each dog. A standard minimum database including a complete blood count, chemistry profile, serum total T4 and free $T4_{ED}$ concentration, and urinalysis were performed on each dog using a commercial reference laboratory (Antech Diagnostics Inc., Kent, WA 98032). Standard echocardiography with continuous ECG monitoring was performed by a board-certified veterinary cardiologist using a GE Vivid i, GEMS Ultrasound machine with a 3.0-MHz phased-array transducer (GE Healthcare, Seattle, WA 98199). Standard right parasternal short axis, right parasternal long axis, and left apical views were obtained while the dogs were gently restrained in lateral

recumbency without sedation or anesthesia. Color flow Doppler was used to determine the presence/absence of valvular regurgitation. If valvular regurgitation was detected, continuous wave (CW) spectral Doppler imaging was used (in the left apical view) to measure the peak regurgitant velocity and velocity-derived pressure gradients. The average of three consecutive regurgitant profiles was then calculated. The severity of the valvular regurgitation was subjectively classified as trace, mild, moderate, or severe for each dog. CW Doppler recordings were made at a sweep speed of 100 mm/s.

All age and weight values are given as mean ± standard deviation. To test for significance in differences between normal and abnormal groups, we used a two-tailed *t* test. To test for enrichment of specific breeds among dogs with regurgitation, we used a one-tailed Fisher's exact test. Testing for normal distribution was done using the Shapiro-Wilk test. Non-normal data were first analyzed using non-parametric tests. In the case of alkaline phosphatase (ALP), we were able to achieve normal distribution with a reciprocal transformation ($1/x$) and present both non-parametric and parametric *P* values. All statistical analyses were carried out using *R* (Team RC 2015).

Results

Forty companion dogs were recruited from the greater Seattle area to participate in a 10-week randomized, double-blind, and placebo-controlled veterinary clinical trial to assess the safety and efficacy of low-dose rapamycin treatment on healthy aging. Breed, sex, and reproductive status were not considered when enrolling dogs into this study. Twenty-two (55%) dogs were female and 18 (45%) were male. Only four (10%) dogs were reproductively intact. Owner reports indicated that 23 (57.5%) dogs were purebreds and 17 (42.5%) were mixed breeds. The owner-reported breed distribution is shown in Table 1. The average age and weight for the 40 dogs in the study cohort was 9.0 ± 1.8 years and 30.5 ± 7.0 kg, respectively.

Out of the 40 dogs to receive an initial examination, 11 (28%) showed abnormal valvular function by echocardiogram (Table 2). All of these cases involved valvular regurgitation, with four dogs (10%) showing trace or mild regurgitation and seven dogs (18%) showing moderate to severe regurgitation. The seven cases with moderate or severe regurgitation were excluded from the remainder of the study, while the four dogs with trace or mild valvular regurgitation were allowed to continue in the study, the results of which will be presented elsewhere.

Table 1 Breed distribution in this study

	Entire cohort	Normal echo	Regurgitation
Australian Cattle Dog	2	0	2
Border Collie	3	0	3
Chow	1	1	0
Doberman	1	0	1
English Bulldog	1	1	0
Golden Retriever	3	3	0
Great Dane	1	1	0
Greyhound	1	1	0
Labrador Retriever	6	6	0
Newfoundland	1	0	1
Siberian Husky	1	1	0
Standard Poodle	1	1	0
Wheaten Terrier	1	1	0
Mixed breed	17	13	4
Total	40	29	11

Breeds are owner reported. Entire cohort refers to the number of dogs of the indicated breed in the study cohort. Normal echo indicates no regurgitation noted during echocardiography

The age and weight values for the dogs showing DVD were 8.3 ± 0.3 years (mean ± 1 SE) and 27.0 ± 2.8 kg, while the age and weight values for the dogs with normal echocardiograms were 9.1 ± 0.3 years and 31.0 ± 1.2 kg. The normal and abnormal groups did differ in age ($t_{32} = 2.14$, $P = 0.04$), but not in weight ($t_{13.5} = 0.63$, $P = 0.54$).

Among the 23 owner-reported purebred dogs in the cohort, 16 (70%) had normal echo and 7 (30%) showed valvular regurgitation as determined by echocardiography. At least one breed, the Border Collie, appeared to be more likely to have regurgitation than expected by chance ($n = 3$ dogs with regurgitation; 0 healthy dogs, Fisher's exact test $P < 0.02$) (Table 1). The other breeds in this group included the Australian Cattle Dog ($n = 2$), Doberman Pinscher ($n = 1$), and Newfoundland ($n = 1$). While there was no overlap between the breeds diagnosed with regurgitation and the breeds without diagnosis, sample sizes are too small to identify the enrichment for other specific breeds. The remainder of the dogs showing valvular regurgitation were owner-reported

Table 2 Animals presenting with valvular disease in this study

Breed	Age (years)	Weight (kg)	Sex	Description
Border Collie	6	26	FS	[a]Significant mitral valve regurgitation. Heart murmur detected at exam
Doberman	6	24	FS	Mild aortic and tricuspid valve regurgitation. No detectable heart murmur
Newfoundland	7	47	MN	[a]Significant tricuspid valve regurgitation. No detectable heart murmur
Mixed breed	8	29	FS	[a]Significant mitral valve regurgitation. No detectable heart murmur
Border Collie	8	20	MN	[a]Moderate tricuspid valve regurgitation. No detectable heart murmur
Australian Cattle Dog	8	20	MN	[a]Significant mitral valve regurgitation. No detectable heart murmur
Australian Cattle Dog	8	27	FI	[a]Moderate tricuspid valve regurgitation. No detectable heart murmur
Mixed breed	8	27	MN	Mild tricuspid valve regurgitation. No detectable heart murmur
Border Collie	9	19	FS	[a]Significant mitral valve regurgitation. No detectable heart murmur
Mixed breed	9	34	MN	Mild mitral valve regurgitation. No detectable heart murmur
Mixed breed	10	44	MI	Mild aortic valve regurgitation. No detectable heart murmur

Among 40 companion dogs older than 6 years of age and weighing at least 18 kg, 11 showed undiagnosed cardiac disease by echocardiography. Of these, only one had a detectable heart murmur in a follow-up examination. *FS* female spayed, *FI* female intact (not spayed), *MN* male neutered, *MI* male intact (not neutered)

[a] Animal was excluded from the study due to pre-existing cardiac disease considered outside of the normal decline in cardiac function with aging

mixed-breed dogs, representing four out of 17 (24%) total mixed-breed dogs in our cohort. Interestingly, all of the mixed-breed dogs with one exception displayed trace or mild regurgitation, while all of the purebred dogs with one exception displayed moderate or severe DVD.

Alkaline phosphatase (ALP) has been described as being increased in humans with degenerative valve disease (Yang et al. 2015), and it has been argued that this is due to ALP inducing calcification of valve interstitial cells (Mathieu et al. 2005). In this light, we tested whether there were differences in ALP between the dogs excluded from the trial for subclinical DVD and those who continued in the trial. While all values were within the reference range, ALP activities were significantly higher in the dogs excluded for subclinical DVD as compared to those accepted (50 ± 68.81 vs. 24 ± 4.97; $P = 0.015$, Kruskal-Wallis test). Reciprocal transformation ($1/x$) resulted in a marginal decrease in the P value ($t_{52} = -2.59$, $P = 0.012$).

Discussion

As part of the Dog Aging Project rapamycin intervention trial, we assessed the baseline health and cardiac function in a cohort of 40 companion dogs living in the Seattle area that were at least 6 years old and at least 18 kg in weight. Of these, more than 25% showed regurgitation due to DVD on an echocardiogram, and seven of these dogs were graded as having moderate or severe regurgitation sufficient to exclude them from the trial. Only one of these dogs had a heart murmur that was also detected by a standard examination including auscultation. Since echocardiograms are not routinely performed on asymptomatic companion dogs, this may suggest that the prevalence of cardiac disease in middle-aged, larger-sized companion dogs is underestimated. This is consistent with findings from earlier studies that found a considerably higher prevalence of DVD in necropsied dogs from the general population (Jones and Zook 1965). Given the similarities between DVD in dogs and humans and the lack of availability of a suitable animal model of human DVD, we argue that the privately owned companion dog is an intriguing model for this disease in humans. Our findings regarding the prevalence of asymptomatic DVD in our cohort would indicate the existence of a large number of potential study subjects in the companion dog population, which, unlike other common animal models, share the human environment. DVD is a significant health concern in the older population, occurring in approximately 10–15% of people over age 75 (Nkomo et al. 2006; Maganti et al. 2010).

Although our cohort was not a random selection of companion dogs, we feel that the observed prevalence of cardiac disease in this study likely serves as a reasonable proxy for the larger population of companion dogs

in this size and age range throughout the USA. Of note, the dogs in our study were required to be relatively healthy and not receiving medications that might interact with the study drug, including heart medication, in order to be invited for the initial veterinary appointment. Thus, the population of sick, older companion dogs was not included in our sample set, which could lead to an underestimation within our cohort of the true prevalence of cardiac disease within the overall companion dog population. This exclusion may also have influenced our finding that dogs excluded for DVD were significantly younger than those who were accepted; if asymptomatic DVD is exerting an impact on the health of dogs under 6 years of age, it is possible that such dogs are less likely to make it to an older age than those who are not affected at that age. It is also possible that, because we were explicitly recruiting dogs without pre-existing health conditions, owners with dogs having clinically diagnosed heart disease did not apply to participate, and that such self-selection may have been more common in older dogs.

It is perhaps noteworthy that among the total of 12 dogs excluded from the rapamycin clinical trial for which this initial examination was performed, seven were excluded due to DVD identified at the time of the screening echocardiogram. The second most common finding was asymptomatic hypothyroidism, which resulted in exclusion in two cases.

While certain dog breeds are at risk for specific types of heart disease, age-related cardiac dysfunction is generally not considered a major cause of morbidity and mortality in companion dogs. Our data suggest that undiagnosed DVD is likely quite common in middle-aged companion dogs. Whether this contributes to subsequent disease or death in these dogs remains unknown and will be important to assess in future longitudinal studies. In one study of human adults with an average age of 54 ± 10 years, subclinical DVD affected 15–20% of individuals (Singh et al. 1999). Valve repair and replacement surgery is commonly performed in clinically affected people and comprises nearly 50% of all cardiac surgeries in octogenarians (Freeman et al. 1991). In contrast, valve repair and replacement surgery is not commonly available for dogs, where medical management to reduce strain on the heart is the commonly used treatment (Anonymous 2005).

As is the case in humans, the valvular changes observed in canine age-related DVD are usually myxomatous in nature, even though they tend to be accompanied by more fibrotic changes and less calcification in dogs than is seen in human DVD (Buchanan 1977). Nevertheless, the pathology of age-related canine DVD is sufficiently similar to that found in humans for it to constitute a useful model for the latter: macroscopic findings in both species include enlarged and/or thickened valve leaflets, elongated *chordae tendineae*, and interchordal hooding of the mitral valve. There may also be changes to the tricuspid valve in both species, which are usually less pronounced. In late stages, ruptured *chordae tendineae* and secondary fibrosis of the leaflets are commonly observed. Microscopically, there are disruptions of collagen, as well as depositions of glycosaminoglycans in the affected leaflets, and the collagen fibrils appear disorganized and spiraling under the electron microscope in both human and canine DVD (Pedersen and Haggstrom 2000; Pomerance and Whitney 1970).

It should be noted that DVD in both dogs and humans is normally a slowly progressive disease, with severe changes only developing in old age. Human studies have found that only about 5 to 10% of patients diagnosed with DVD will require surgery over a period of 6 to 13 years after diagnosis (Pedersen and Haggstrom 2000). In this context, it may be that the sporadic and slowly progressive form of DVD seen in older large dogs is a closer approximation of human DVD than the early-onset and more aggressive form observed in some small breeds, particularly Cavalier King Charles Spaniels (Pedersen et al. 1999a), which appears to be highly heritable and therefore may have a different underlying molecular mechanism than sporadic age-related DVD in other dogs (Swenson et al. 1996). In contrast, DVD risk in Dachshunds has been linked to a narrow chest shape (Olsen et al. 1999), while a low anterior-posterior chest diameter is associated with increased DVD risk in humans (Schutte et al. 1981; Udoshi et al. 1979), which could indicate that this breed in particular may also be an interesting potential model for human DVD.

In addition to uncovering an unexpectedly high rate of asymptomatic DVD in our study cohort, the results presented here illustrate one of the major challenges associated with clinical assessment of interventions designed to promote healthy longevity. Because the study population consists of normally aging individuals, criteria must be established prior to enrollment defining what level of disability or disease is considered abnormal for the cohort and, therefore, grounds for exclusion

from the study. Because age is the greatest risk factor for nearly every major cause of disease and disability in both humans and companion dogs (Pitt and Kaeberlein 2015), progressive declines in functional capacity and progressive increases in disease incidence are certain to be present as the age of the study population increases. Defining "normal" aging from "abnormal" morbidity is non-trivial, and there are currently no established metrics for doing so. For this study, we relied upon the expertise of a veterinary cardiologist to determine whether the disease was atypical for a normally aging dog; however, we recognize that this is a necessarily imperfect approach given the lack of data on echocardiographic parameters among the aging, asymptomatic companion dog population.

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