



## AN ABSTRACT OF THE THESIS OF

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Title: Treatment and Characterization of Valvular Pulmonic Stenosis and Coronary  
Artery Anatomy in English Bulldogs and French Bulldogs

Abstract approved:

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Pulmonic stenosis (PS) is one of the most commonly encountered congenital cardiac defects in canine patients. English bulldogs have an increased risk of PS as compared to other dog breeds and have a higher reported prevalence of type II/type B/dysplastic pulmonic valve morphology. It has been shown that the dysplastic PS morphology may carry a more guarded prognosis and be less amenable to percutaneous balloon valvuloplasty (BV) procedures in canine and human patients. Due to the potentially decreased success of BV procedures in canine patients with a more dysplastic valvular phenotype, the ideal treatment for these cases may not be fully determined based on the currently published literature. There are breed predispositions observed with PS and French bulldogs may be more frequently affected with PS. Evaluation and characterization of the specific PS morphology observed in French bulldogs is lacking in veterinary medicine.

Case reports have provided evidence that English bulldogs and boxer dogs have an increased risk for anomalous coronary artery anatomy that may cause or

complicate PS treatment. There is a possible association with coronary artery anomalies in the French bulldog breed, although information on prevalence in this breed is lacking in the currently available veterinary literature. The primary aim of this research study was to assess the pulmonic valve morphology and prevalence of coronary artery aberrancy in English and French bulldogs with severe PS (trans-pulmonic valve pressure gradient  $>80\text{mmHg}$  based on Doppler echocardiography) using echocardiography, angiography, and computed tomography (CT) imaging modalities. A secondary study aim was to evaluate demographic data for all English and French bulldogs with PS (mild, moderate, or severe) that presented to the Oregon State University Veterinary Teaching Hospital as compared to the population of non-bulldog breeds diagnosed with PS (mild, moderate, or severe) in the same time frame. These populations were further analyzed to specifically evaluate the bulldog and non-bulldog breeds with severe PS that underwent BV procedures. Data collection and evaluation included population characteristics, electrocardiography, echocardiography, and surgical records. A third study aim was to investigate BV procedural success and survival data between bulldog and non-bulldog breeds undergoing BV for severe PS.

Medical records were reviewed for French and English bulldogs presented to Oregon State University Veterinary Teaching Hospital (OSU VTH) for heart murmur evaluation or imaging prior to BV. Dogs were included if they had severe PS (pressure gradient  $>80\text{ mmHg}$  estimated via Doppler echocardiography) and confirmation of their coronary artery anatomy by either left-sided angiography or CT imaging. Echocardiographic, angiographic, and CT images were reviewed. The

pulmonic annulus (PA), aortic annulus (Ao), PA:Ao and Ao:PA ratios, valvular anatomy, and coronary anatomy were assessed on each imaging modality by three observers (CS, KS, DDS) and averaged. The echocardiographic images were reviewed and measurements of right ventricular (RV) systolic function, chamber dimensions, and wall thickness were performed by 1 reviewer (CS). All non-bulldog breeds that were diagnosed with severe PS were also identified and echocardiographic data was assessed in the group that underwent BV procedures. Owners, referring veterinarians, or both were contacted to obtain outcome and survival information on all included patients to determine if they were alive/dead, date and cause of death if available, and if clinical signs of cardiac disease were observed. Data were tested for normality using the D'Agostino & Pearson omnibus normality test. Continuous variables were compared by Mann-Whitney U tests, ANOVA, or unpaired t-tests as indicated and categorical data was compared using a Fisher's exact test. Survival times were calculated from the date of birth reported in the medical records to the date of natural death or euthanasia within the observational study period. Kaplan-Meier survival analysis was performed to compare cardiac and all-cause mortality.

The results of this study show that in the population of French and English bulldogs that presented to the OSU VTH, there was an increased prevalence of markedly dysplastic pulmonic valve morphology with the majority of cases displaying mild annular hypoplasia, which was different than the non-BD population. None of the French bulldogs (0/13) that underwent definitive coronary artery imaging had evidence of coronary artery anomalies while 6/8 English Bulldogs were

definitively diagnosed with an R2A coronary artery anomaly. Within the group of dogs diagnosed with an R2A coronary artery anomaly, BV procedures were not performed in any of the patients and they had a poor mean long-term prognosis of 26 months. Indices of RV systolic function were decreased in the BD breeds in comparison to the non-BD breeds. There were significant differences observed in procedural success at the 1-month post-BV echocardiographic recheck and long-term follow up with the BD group having lower BV success than the non-BD group.

The results of this study indicate that BV performed on French and English bulldogs has a lower success rate than non-bulldog breeds. This lower success rate may be related to difference in valve morphology identified on echocardiographic, CT, and angiographic images. In addition, bulldogs appear to have a shorter survival time compared to non-bulldog breeds and this may be related to the differences in RV systolic function identified by echocardiography. Lastly, French bulldogs did not have the same high prevalence of anomalous coronary artery anatomy as English bulldogs in the population of dogs evaluated at the OSU VTH.

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Treatment and Characterization of Valvular Pulmonic Stenosis and Coronary Artery  
Anatomy in English Bulldogs and French Bulldogs

by  
Courtney C. Smith

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APPROVED:

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Courtney C. Smith, Author



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## **Chapter 1**

### **Introduction**

Pulmonic stenosis is one of the most commonly encountered congenital cardiac defects in canine patients and is reported to be within the top three most common congenital cardiac lesions in dogs.<sup>2-8</sup> Several published studies on canine congenital cardiac defects place the overall prevalence of congenital heart defects in dogs at 0.68-21% depending on the specific study population signalment (age and breed) and location (tertiary veterinary hospital or shelter).<sup>2, 3, 9</sup> In people it is well accepted that the etiology of congenital heart disease is multifactorial with environmental and genetic factors playing important roles.<sup>10</sup> Similar to cardiac defects in people<sup>11</sup> there is strong suspicion that many of the cardiac defects in veterinary medicine have a primary genetic origin. Breed specific genetic abnormalities include conotruncal defects in the keeshond,<sup>12-14</sup> subvalvular aortic stenosis in the Newfoundland,<sup>15</sup> and patent ductus arteriosus in the miniature poodle<sup>16-18</sup> and the Dutch Stabyhoun.<sup>19</sup>

There is evidence of a hereditary cause of pulmonic stenosis (PS) in beagles<sup>20</sup> as well as in people.<sup>21</sup> Besides the beagle breed, potential purebred breed predispositions to PS that are reported include the boxer, Boykin spaniel, English bulldog, French bulldog, miniature schnauzer, Scottish terrier, German shepherd, West Highland white terrier, wirehaired fox terrier, Cavalier King Charles spaniel, and Chihuahua breeds.<sup>2, 3, 22, 23</sup> There has also been a slightly increased male predisposition reported with PS in dogs.<sup>6, 24-26</sup> A specific genetic defect has not been isolated in any breed of dog with PS and the results of breeding within a colony of beagles with PS revealed evidence that the inheritance pattern was not a simple Mendelian trait.<sup>20</sup> The morphological characteristics of PS lesions vary. Specific syndromes that often include PS with

characteristic morphology have been identified in people<sup>27</sup> and specific types of PS morphology have been identified in people and dogs.<sup>20, 24</sup>

### *Pulmonic valve anatomy and development*

The normal pulmonic valve is a tri-leaflet structure with a right cusp, left cusp, and anterior cusp that opens during ventricular systole and closes during diastole. The development of the cardiac valves begins with migration of a population of endothelial cells that originates within the developing heart tube.<sup>28</sup> The migrating endothelial cells combine with the extracellular matrix (cardiac jelly) to form structures called cardiac cushions that ultimately lead to the formation of the atrioventricular valves and the semilunar (aortic and pulmonic) valves.<sup>29</sup> Additional cellular migrations are integral in the semilunar valve formation and include a second cell population of neural crest cells that migrate from the branchial arches to the distal outflow tracts as well as a third population of cells that originate from the secondary heart field. The neural crest cell population merges with the endocardial cushion tissue and forms the aortopulmonary septation complex that plays an important role in initiation of the division between the pulmonary and systemic circulations as well as development of the semilunar valves.<sup>30, 31</sup> The cardiac cushions in the outflow tracts remodel and form thin, tapered leaflets with a single endothelial cell layer that surrounds a central matrix of collagen, elastin, and glycosaminoglycans.<sup>28, 29</sup>

The cellular migration and differentiation processes that ultimately lead to development of the outflow tracts, great vessels, and the semilunar valves are extraordinarily complex with multiple cellular signaling pathways playing different roles in a precisely orchestrated process that is still incompletely understood. Disruption within any one of these signaling pathways can

lead to malformation of the semilunar valves. Defects in a G-protein-coupled, chemokine receptor (CXCR7) found in the endocardial cushion mesenchymal cells result in pulmonic and aortic stenosis in a mouse embryo model caused by increased bone morphogenic protein signaling triggered excessive mesenchymal cell proliferation.<sup>32</sup> Additional research has shown that deletion of exon 2 in *Ptpn11*, a gene that encodes a tyrosine kinase phosphatase (SHP-2), results in dysplastic aortic and pulmonic valves.<sup>33</sup> Other defects within the *PTNP11* gene have been linked to specific human syndromes including Noonan and Leopard,<sup>28, 34</sup> that commonly include PS within their spectrum of developmental abnormalities.

### *Pulmonic stenosis morphology*

In people the morphology of valvular PS is most commonly divided into 2 categories. The most commonly observed morphology involves commissural fusion of the leaflets with variable degrees of leaflet thickening and a characteristic systolic appearance of the leaflets with doming leaflets and a central opening.<sup>35, 36</sup> The other less commonly observed valve abnormality is described as dysplastic, which consists of 3 distinct valve cusps with little commissural fusion, but severely thickened leaflets with limited mobility that create the stenosis.<sup>27, 36</sup> Annular hypoplasia is commonly observed in conjunction with the dysplastic morphology. Expansion of the intermediate layer of loose connective tissue (spongiosa layer) within the pulmonic leaflets as well as an embryonic-appearing connective tissue phenotype have been observed histopathologically on dysplastic human leaflets.<sup>27</sup> Initial veterinary literature classified PS morphology in beagles according to the human morphological classification system,<sup>20</sup> with type I morphology encompassing commissural fusion and normal pulmonic annulus dimensions and type II morphology encompassing dysplastic leaflets with marked thickening, lack of



commissural fusion, and annular hypoplasia. In this same study, the researchers also identified a population of dogs with intermediate lesions that did not fit completely within either of these defined morphology types. More recent veterinary reports classify PS in dogs as type A, which is similar to the previously described type I (Figure 1A) or type B PS (Figure 1B), which is similar to the previously described type II (dysplastic) morphology.<sup>24,37</sup> Within the veterinary literature, there are conflicting reports regarding which PS morphology is most common. Some reports provide evidence that the dysplastic morphology is most common while others describe type A as the most commonly observed.<sup>3, 20, 24</sup>

### *Clinical signs and diagnosis*

The degree of valvular stenosis is variable and the pressure gradient (PG) across the stenosis determines the risk of developing clinical signs, secondary right ventricular (RV) remodeling, and treatment recommendations in veterinary medicine. Dogs are classified as having mild PS with trans-pulmonic PG < 50mmHg, moderate PS with PG between 50-80mmHg, and severe PS with PG >80mmHg.<sup>3, 22, 37-39</sup> Dogs with PS are frequently diagnosed at a young age due to the characteristic presence of a left basilar systolic murmur that is present at birth.<sup>2, 3, 22</sup> Many dogs are asymptomatic on initial evaluation, while others present with clinical signs of exercise intolerance, syncope, or signs of right-sided congestive heart failure (R-CHF).<sup>2, 22</sup> Other findings on physical examination can include visualization of a prominent jugular pulse, detection of arrhythmias, and, in dogs that have developed R-CHF, ascites, pleural effusion, and/or cachexia.<sup>3</sup>

Thoracic radiographs often reveal evidence of right-sided cardiac enlargement, post-stenotic dilation of the main pulmonary artery, variable dilation of the proximal left pulmonary

artery, and under-circulation of the pulmonary vasculature. Electrocardiographic findings commonly include evidence of RV enlargement, rightward deviation of the mean electrical axis, and occasionally right atrial (RA) enlargement. Routine hematologic testing usually does not reveal any significant abnormalities, unless there are concurrent congenital cardiac defects that would lead to right-to-left intracardiac shunting and resultant polycythemia.<sup>3, 23, 40</sup> Cardiac troponin I is a protein component of the cardiac sarcomere and has been described as a sensitive and specific marker for myocardial injury.<sup>41</sup> Evaluation of this biomarker in dogs with severe PS prior to and post treatment with balloon valvuloplasty (BV) revealed elevated cardiac troponin I concentrations in 7 out of 23 dogs at baseline and in 14 out of 16 dogs post-BV.

The test of choice for diagnosing PS is transthoracic echocardiography. Two-dimension (2D) echocardiography allows identification of concentric RV hypertrophy with hypertrophy of the RV papillary muscles, secondary muscular narrowing of the RV outflow tract, thickened and/or doming pulmonic leaflets, pulmonic annular hypoplasia, post-stenotic dilation of the main pulmonary artery, RA dilation, and decreased left-sided chamber dimensions due to under-filling.<sup>3</sup> Color flow Doppler can be used to assess for tricuspid valve regurgitation, which may be secondary to the RV remodeling or reflect concurrent tricuspid valvular dysplasia, and can evaluate for the presence of and severity of pulmonic valvular insufficiency. Continuous wave spectral Doppler interrogation of the RV outflow tract reveals increased turbulence and blood flow velocity across the stenotic lesion, which is used to estimate the RV to pulmonary artery PG by the Bernoulli principle. Angiocardiography can also be used to diagnosis PS,<sup>26, 42</sup> but requires sedation or general anesthesia and is more commonly used during BV.

### *Natural history*

The natural history of PS in dogs depends on the severity of the lesion as well as the presence of concurrent congenital cardiac defects. Concurrent congenital defects are seen in approximately 16-32.5% of dogs that have PS with the most common being ventricular septal defects (in both perimembranous and muscular locations), patent foramen ovale, tricuspid valve dysplasia, left to right shunting patent ductus arteriosus (PDA), subvalvular aortic stenosis, and persistent left cranial vena cavae.<sup>2, 22, 23, 40, 43-45</sup>

Dogs diagnosed with mild PS may live a normal lifespan without development of cardiac failure.<sup>2, 3, 37</sup> Most cases of moderate valvular stenosis live a normal lifespan, although there have been several studies that have revealed a higher incidence of clinical signs and risk of sudden cardiac death and R-CHF in dogs with moderate PS as compared to dogs with mild PS.<sup>2, 25</sup> Dogs with severe PS have an increased risk of death either secondary to cardiac arrhythmias or R-CHF without palliative treatment to reduce the degree of stenosis.<sup>2, 22, 25</sup> Similar findings in people with PS have also been observed with cases of mild PS rarely needing treatment and patients with moderate to severe PS often displaying clinical signs secondary to cardiac dysfunction.<sup>46-48</sup> Current recommendation in people diagnosed with PS is to treat moderate and severe PS.<sup>46, 49, 50</sup> The recommendations in veterinary medicine with treatment of moderate PS remain less well defined although it is generally recognized that dogs with moderate PS and clinical signs benefit from BV procedures.<sup>2</sup>

### *Treatment options and recommendations*

Treatment recommendations for severe PS in veterinary medicine (defined by the transpulmonic PG or RV systolic pressure) include a minimally invasive catheter-based procedure

called BV,<sup>37, 49</sup> more invasive cardiovascular surgical procedures such as valvulotomy, partial valvectomy, patch grafting over the outflow tract, or conduit placement.<sup>3, 26, 51-53</sup> Alternatively, the use of antiarrhythmic medications such as beta-blockers has been described as sole therapy.<sup>2, 45, 54</sup> Recently the development and experimental use of autologous valve conduits with a self-expanding stent in beagles has been described, although its clinical use in patients with PS has not been evaluated.<sup>55</sup> Percutaneous stent implantation has also been described in 2 dogs with dysplastic PS morphology.<sup>56</sup> Most percutaneous BV procedures are performed with a single balloon catheter, although the use of a single higher pressure balloon catheter, double balloon catheter technique in dogs and people,<sup>54, 57, 58</sup> cutting balloon catheter,<sup>59</sup> or hour-glass shaped balloon catheter have been described.

Percutaneous BV procedures are the most commonly recommended and performed palliative procedures for PS in dogs and people.<sup>37, 60</sup> Treatment of PS with a surgical-hybrid balloon catheter procedure was described initially in the human literature in 1952, but the current commonly used percutaneous technique was first reported in a child with PS in 1982.<sup>46, 61</sup> Prior to the first percutaneous BV procedure performed in a child, it was performed in an English bulldog with severe PS in 1980 who survived the procedure and lived for 2 years prior to developing of signs of R-CHF.<sup>62</sup> Additional reports of BV procedures in veterinary medicine soon followed.<sup>63, 64</sup> Both short and long term follow up of canine patients that have undergone BV procedures has revealed generally favorable results with decreased clinical signs and a decreased risk of cardiac death.<sup>2, 65</sup> BV procedure success varies based on individual published reports, but the general consensus is that a successful procedure is one in which the PG across the pulmonic valve is reduced by 50%<sup>37</sup> or the final PG is less than 64 mmHg, although other published reports have labeled success with a final PG less than 80mmHg.<sup>45, 54</sup>

### *Literature review*

In a report of 30 dogs that underwent BV procedures, 18 of the dogs were classified as having type A morphology and had an overall success rate of 100% in regards to procedural survival with resolution of clinical signs immediately after the BV procedure and a 94.4% one year survival rate.<sup>24</sup> There were 12 dogs with type B morphology which had a 66.6% success rate following BV with a 66.6% one year survival rate. In the population of dogs with type A morphology, 16 had a trans-pulmonic valve PG <75mmHg post-operatively. In the population of dogs with type B morphology, 2 died during the procedure and 2 attempts were aborted, leaving 8 dogs that ultimately underwent the BV procedure with only 2 of 8 dogs having a PG <75mmHg at the one year follow up.

There is an additional veterinary case report of BV procedures attempted in a population of 18 dogs with PS.<sup>38</sup> The BV procedure was successfully performed in 16 cases (1 case was aborted and another died during the procedure) with 66% of the treated cases surviving up to 4 years post BV. Overall 54% of the dogs maintained a PG reduction of 30-70% of the original PG. Severe valvular stenosis reoccurred in 3 of the 16 cases.

Another case report evaluating BV procedures in a population of 40 dogs revealed an overall mean PG reduction of 46% following BV with a mean pre-BV PG of 124mmHg and a 6 month post-BV mean PG of 67mmHg.<sup>45</sup> Six of the dogs had clinical signs of R-CHF on presentation and of these dogs one died during the BV procedure, 2 were euthanized due to refractory R-CHF signs at 4 months and 18 months post-BV procedure, 2 died post-operatively after a patch graft surgical procedure, and 1 died under anesthesia for an unrelated condition. Follow up was available on 30 of the cases and revealed that in 24 cases, the dogs either became

or remained asymptomatic for the study period (up to 8 years post BV with a mean follow up time of 2.7 years).

The largest published veterinary study looked at 172 dogs diagnosed with PS in which 107 dogs underwent BV and 65 dogs did not undergo BV.<sup>2</sup> This case series found an overall survival benefit in dogs with severe PS that underwent a BV procedure, with a median survival time (MST) of 92 months in the population of dogs that did not undergo a BV procedure compared to an undefined MST in the BV population because 50% of the BV population dogs did not reach a primary endpoint (most were still alive at the end of the study period). In this same study, comparison between BV population survival based on valve morphology (type A or type B) revealed a MST of 69 months in dogs with type B morphology and an undefined MST in dogs with type A morphology since 50% of the dogs did not reach a primary study endpoint.

Procedural mortality has been reported to be 0-7.5% based on larger published case series in veterinary medicine.<sup>2, 24, 38, 45</sup> Causes of procedural mortality include malignant ventricular arrhythmias leading to unresponsive ventricular fibrillation, paradoxical cerebral emboli due to concurrent right to left shunting defects, and rupture of vascular structures leading to fatal hemorrhage.<sup>37, 45, 54</sup> Other potential complications of BV include ligation of the vessel used for introducer placement and catheter access, mild hemorrhage/bruising, ventricular and supraventricular arrhythmias, transient to permanent right bundle branch block patterns, dynamic right ventricular outflow tract obstruction due to infundibular hypertrophy and obstruction, valve hemorrhage and edema, and mild discomfort from vascular access.<sup>37, 66</sup> Potential reasons that dogs with severe PS do not undergo palliative therapies are related to lack of definitive

diagnosis, lack of follow up, owner's preference, financial burden, or a lack of clinical symptoms of cardiac disease.<sup>22, 25</sup>

In people, reported pediatric success rates without re-intervention at 1 year post procedure was 100% in a group of 70 patients with a more typical doming PS morphology and 91.3% in a group of 73 patients with dysplastic morphology.<sup>35</sup> An additional report in people provided long-term follow up data revealing evidence of continued procedural success in the majority of cases (unsuccessful procedure classified in 2/53 patients).<sup>67</sup> An overall peak-to-peak PG decrease from a mean of 74mmHg to 20mmHg post BV was observed. Procedural mortality in published human reports is 0.24-0.25%.<sup>59, 68</sup>

Reported poor prognostic indicators in veterinary medicine in dogs with severe PS (with or without BV procedure performance) include type B morphology,<sup>2, 24</sup> clinical signs at the time of diagnosis, younger age at diagnosis, and severity of the PG in dogs without BV treatment (identified as >60mmHg in a study).<sup>25</sup> Procedural factors associated with an unsuccessful outcome in both human and veterinary literature include: a balloon catheter diameter to annulus ratio outside of a 1.2-1.5 range (with 1.3 providing the best results in a veterinary study),<sup>50, 60, 69</sup> dysplastic valvular morphology,<sup>2, 24, 35, 36</sup> and clinical signs of R-CHF at the time of BV.<sup>2</sup>

#### *Right ventricular function and evaluation*

Hemodynamic consequences related to the RV outflow tract obstruction caused by PS are numerous and primarily related to RV remodeling.<sup>3</sup> Increased RV systolic pressure triggers concentric RV hypertrophy and leftward deviation or flattening of the interventricular septum which impairs left ventricular filling and function. A component of the RV hypertrophy is excessive fibrous tissue accumulation, which creates increased myocardial stiffness leading to

diastolic filling impairment and elevated filling pressures. Severe PS also limits cardiac output due to the fixed nature of the obstruction, inducing compensatory retention of sodium and water via neurohormonal pathways. Decreased RV coronary blood flow has been documented in dogs with PS<sup>70</sup> and may predispose a patient to hypoxia, ischemia, and subsequent malignant arrhythmias leading to clinical signs of syncope and sudden cardiac death. Additionally, the high velocity turbulent blood flow across the stenotic pulmonic valve creates post-stenotic dilation of the main pulmonary artery. The impaired diastolic function of the RV, impaired outflow capacity and increased systolic pressures, as well as the effects of RV remodeling can lead to tricuspid valve regurgitation, RA dilation, and R-CHF.<sup>3</sup> It is possible that a more severe PG in dogs induces more severe changes to RV function. Quantitative evaluation of systolic and diastolic RV function could help provide additional prognostic information in determining procedural success guidelines and potential parameters for palliative management post initial BV procedure. In addition, this could also help determine which patient populations might obtain more significant benefit from a BV procedure, particularly in dogs with moderate PS or dogs with R-CHF, in which no survival benefit has yet to be found with BV procedures.<sup>2, 38, 45</sup>

Echocardiographic assessment of the RV is problematic in that the triangular shape of the RV is difficult to model. It is often incompletely imaged in routine echocardiographic imaging planes, and neither the inflow nor outflow tracts can be imaged simultaneously. Additionally, quantitative data and reference intervals for the RV have not been as thoroughly studied as have left ventricular measurements.<sup>71-73</sup> Prognostic implications obtained from qualitative RV size and function data in people are becoming well recognized and utilized.<sup>74, 75</sup> Echocardiographic methods of evaluating RV size include direct measurement of wall thickness and chamber dimensions at end-diastole and end-systole in various imaging windows. Normal reference



ranges have been published, although reference ranges utilizing data from larger populations are lacking.<sup>76</sup> Measurements of RV systolic function that have been evaluated in dogs include M-mode derived tricuspid annular plane systolic excursion (TAPSE), percent fractional area change (FAC) which is considered the 2D measurement of RV ejection fraction, tissue Doppler imaging (TDI)-derived systolic myocardial velocity of the lateral annulus of the tricuspid valve (S'), and speckle-tracking echocardiography-derived strain and strain rate.<sup>71, 73</sup> A recent study provided reference ranges in a population of normal dogs although comparison of obtained measurements to a gold-standard such as cardiac magnetic resonance imaging or cardiac CT, as has been performed in people, was not performed.<sup>71</sup> There is published data evaluating RV function parameters in dogs with pulmonary hypertension<sup>73</sup> and RV dimensions in dogs with heartworm disease.<sup>77</sup> Evaluation and measurements of RV dimensions and systolic function in a veterinary population with PS, to the author's knowledge, has not been published.

### *Coronary artery anomalies*

English and French bulldogs have an increased risk of PS as compared to other dog breeds.<sup>1, 2, 24, 78</sup> There are also case reports of English bulldogs and boxer dogs that have been found to have anomalous coronary artery anatomy. Frequently these anomalous coronary arteries are characterized by a single origin right coronary ostium with the right coronary artery giving rise to the left main coronary artery. The anomalous course of the left coronary artery encircles the pulmonic root in the region of the pulmonic valve.<sup>1, 79</sup> This lesion is commonly referred to as an R2A coronary artery anomaly based on human classification systems (Figure 2).<sup>1</sup> The circumpulmonary course of the left coronary artery may cause the primary stenotic lesion or play a secondary role (i.e. underlying valvular PS with an additional stenotic component caused by the encircling coronary artery). A potential hypothesis for the observed R2A coronary artery

anatomy and concurrent PS is that the circumpulmonary left main coronary artery causes compression of the conus arteriosus in utero resulting in a hypoplastic pulmonary annulus and PS.<sup>78, 80</sup> In cases of severe PS with R2A coronary artery anatomy there is concern and risk that balloon catheter inflation to dilate the stenosis can damage the left main coronary artery and cause fatal avulsion and hemorrhage or potential myocardial ischemia and infarction.<sup>22, 45, 79, 81</sup> Current recommendations in English bulldogs and boxers breeds with severe PS is to perform definitive coronary artery imaging prior to performing a BV procedure with a left-sided angiogram, cardiac gated CT imaging, or transesophageal echocardiography.<sup>22, 45, 82</sup> Current recommendations for treatment of dogs diagnosed with an R2A anomaly and severe PS include not performing a BV procedure<sup>81</sup> or performing a more conservative procedure without any significant upsizing of the balloon catheter maintaining a 1:1 ratio of the balloon diameter to pulmonic valve annulus.<sup>83</sup> The R2A coronary artery anomaly has been described in other dog breeds such as a mongrel dog without evidence of clinical signs of pulmonic stenosis observed at necropsy in 1959,<sup>84</sup> bull mastiff,<sup>45</sup> Brittany spaniel,<sup>85</sup> and in a mixed breed dog at the Oregon State University Veterinary Teaching Hospital (OSU VTH) (unpublished data). A report of a different coronary artery anomaly in English bulldogs and a boxer has been published, which describes a single left coronary ostium, absent right coronary ostium, and an anomalous circumpulmonary course of the right coronary artery in dogs with concurrent PS.<sup>86</sup> A similar case with a single origin left coronary artery with an anomalous circumpulmonary course of the right coronary artery has also been diagnosed in an English springer spaniel at the OSU VTH (unpublished data). An additional case report in an English bulldog with PS that was successfully treated with BV revealed the presence of a single right coronary ostium that gave rise to coronary

vessels that lacked circumpulmonary branches (consistent with human morphologically characterized R2B or R2C coronary artery anomalies; Figure 2).<sup>80</sup>

Based on personal observation of patients referred to the OSU VTH there may be a perceived association of an increased risk of anomalous coronary artery anatomy in the French bulldog breed with concurrent PS, although the initial veterinary reports did not report this finding.<sup>42, 81</sup> There are also a few case reports of coronary artery anomalies in French bulldogs, although definitive diagnosis with coronary artery imaging was not always performed in these cases.<sup>82</sup> At the OSU VTH, the author has had French bulldogs with severe PS referred specifically for coronary artery imaging prior to BV procedures, which is in contrast to the general population of dogs referred to the OSU VTH for management of severe PS. It has been suggested that there may be a genetic predisposition in the English bulldog breed for coronary artery anomalies,<sup>1</sup> and it is unknown if other brachycephalic breeds such as the French bulldog also share this same predisposition. Anomalous coronary artery anatomy may or may not be involved in the pathogenesis of PS and it is currently unknown if there is increased prevalence in English bulldogs and boxers that do not have PS. The performance of definitive imaging of clinically normal English bulldogs to determine the incidence of coronary artery anomalies would help further clarify the role of the R2A anomaly in PS in English bulldogs. Since there can be variable branching seen with anomalous coronary artery anatomy, definitive coronary artery imaging could be useful in determining the feasibility of BV procedures in cases previously determined to be uncorrectable with a percutaneous procedure. With the increasing popularity of the French bulldog breed and increased cases reports of PS in this breed (evidenced by the lack of significant numbers of French bulldogs observed in early reports of PS<sup>3, 38</sup> and increasing

numbers identified in a more recent report)<sup>2</sup> there is a potential that definitive coronary artery assessment could reveal an increased risk for diagnosis of coronary artery anomalies.

### *English and French bulldogs with pulmonic stenosis*

The French bulldog and English bulldog breeds are more commonly diagnosed with type II/B PS morphology with evidence of severe thickening of pulmonic valve leaflets as well as annular hypoplasia.<sup>2, 24</sup> As previously indicated, it has been shown that this morphologic type may carry a more guarded prognosis and may be less amenable to BV procedures.<sup>2, 24, 36, 54</sup> Long-term follow up in a large case series of dogs with dysplastic morphology is lacking in veterinary medicine, and the ideal treatment for these cases has not been fully determined based on the currently published information. These type II/B dogs might benefit from surgical management of their PS rather than percutaneous BV procedures, although long term follow up with BV procedures is lacking. It has been observed that early interventions in infants or young children with critical PS (defined as PG >80mmHg) prior to adverse RV remodeling and clinical signs leads to improved outcomes.<sup>87</sup> With this in mind, more aggressive surgical management at an earlier age in these affected dogs might similarly lead to improved outcomes. Due to the differences in morphology and response to BV procedures, success may need to be redefined in cases of valvular dysplasia as compared to cases that have more evidence of commissural fusion.

### *Study aims*

The primary aim of this retrospective research study was to further classify valvular morphology of severe PS in French bulldogs as compared to English bulldogs with the hypothesis that French bulldogs commonly present with more severe pulmonic valve leaflet thickening and are not as predisposed to anomalous coronary artery anatomy as English

bulldogs. Comparisons of valvular morphology, degree of pulmonic annular hypoplasia, and prevalence of coronary artery anomalies were used to better describe morphological characteristics in the two breeds. A secondary study aim was to compare the French and English bulldog PS population to the entire population of non-bulldog breeds presenting to the Oregon State University Veterinary Teaching Hospital (OSU VTH). Evaluation of demographic data included age at presentation, clinical signs, PS severity, and presence of anomalous coronary artery anatomy and concurrent congenital heart defects. In addition, a weight-matched population of non-bulldog breeds with severe PS that underwent BV was compared to the bulldog population that underwent BV. Demographic data, electrocardiographic data, and echocardiographic data of RV dimensions, PS morphology, and systolic function were evaluated. The third aim of this study was to evaluate the bulldog and non-bulldog populations that underwent BV procedures to determine BV procedural success and long-term survival in the populations.

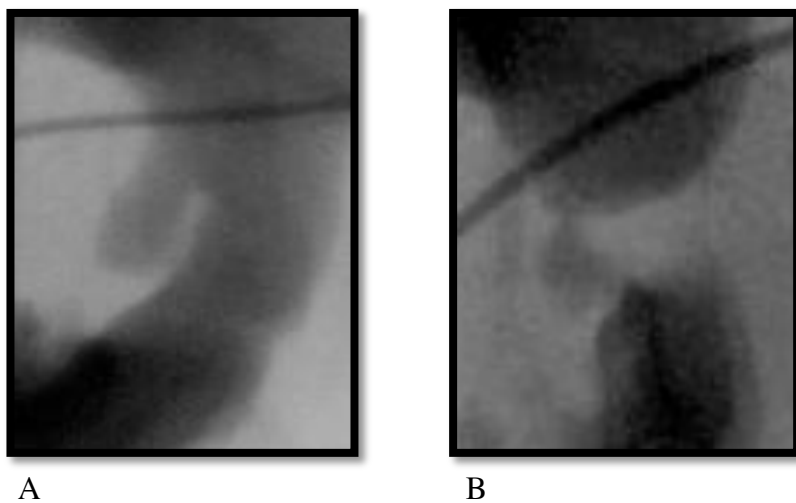


Figure 1: Angiographic appearance of valve morphology

Figure 1 caption: Right-sided angiograms displaying features of type 1 or type A valvular pulmonic stenosis (A) and features of type II or type B valvular pulmonic stenosis (B).

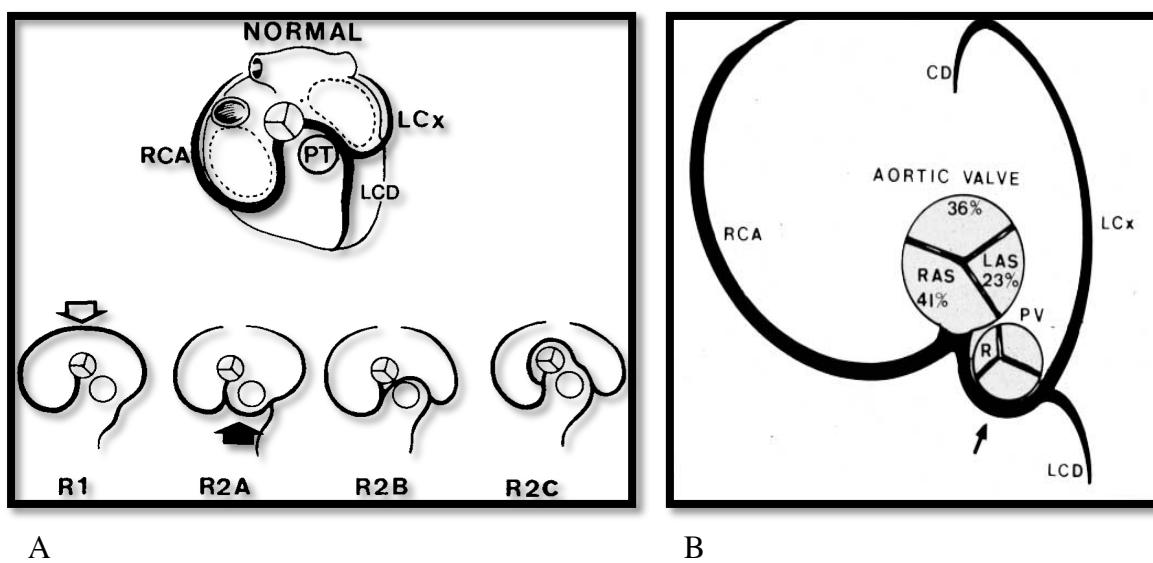


Figure 2: Coronary Anatomy

Figure 2 caption: Schematic diagram of (A) normal coronary artery ostia and branching along with the described single right coronary ostium defects as described in people and (B) a magnified image of the single right ostium coronary artery anomaly that gives rise to a circumpulmonary left main coronary artery, commonly referred to as a R2A anomaly.<sup>1</sup>

## **Chapter 2**

### **Materials and Methods**

#### *Patient populations*

Medical records of dogs referred to the Oregon State University Veterinary Teaching Hospital (OSU VTH) for cardiovascular diagnostic evaluation and treatment between August 2005 and June 2016 were retrospectively reviewed to identify bulldog breed (BD) dogs with a diagnosis of severe valvular PS that underwent advanced imaging of coronary vasculature. Inclusion criteria were being an English bulldog (EB) or a French bulldog (FB) breed, a diagnosis of severe PS (trans-pulmonic PG  $> 80$ mmHg measured via continuous wave Doppler echocardiography), and either a thoracic/cardiac-gated computed tomography (CT) study or a left-sided angiogram performed at the OSU VTH to definitively determine coronary artery vascular anatomy.

To further evaluate the secondary study aim, all cases of dogs diagnosed with PS in the same time frame were identified and case records were retrospectively reviewed. Inclusion criteria for this population included diagnosis of PS of any severity, a complete case record with dog and owner information, clinical history, physical exam findings, and a complete echocardiographic study with 2D and Doppler echocardiographic images providing the trans-pulmonic valve PG, localization of the obstruction, and valve morphology available for review. Dogs with severe complex congenital defects such as tetralogy of Fallot were excluded. Dogs with concurrent congenital defects that did not appear to be causing significant cardiovascular compromise were included in the study. Dogs were separated into two groups: 1) a combined group of English and French bulldogs (BD-PS) and 2) all non-bulldog dogs (NonBD-PS) for comparisons of group demographics, presence of concurrent congenital defects, and prevalence



of anomalous coronary artery anatomy. Dogs were also stratified by severity of PS as mild (PG <50mmHg), moderate (PG 51-79 mmHg), or severe (PG >80 mmHg).

A subpopulation of all of the dogs diagnosed with PS was created that identified only dogs with severe PS (PG >80 mmHg) that underwent BV procedures. In addition to the above criteria, additional inclusion criteria for this subpopulation included a BV procedure performed at the OSU-VTH, body weight less than 21kg, and 1-month follow up echocardiography performed at the OSU-VTH. This was to allow for documentation of echocardiographically measured trans-pulmonic PG prior to and post-BV treatment (24 hours and approximately one month post-BV). Dogs were divided into bulldog (BD-BV) and non-bulldog (NonBD-BV) groups for comparison of RV function, pulmonic valve morphology, trans-pulmonic PG, BV outcome, and survival.

All data from the records and cardiovascular examinations were reviewed by a single investigator (CS). Data extracted from the records included date of birth, breed, weight, date of initial presentation, presence of clinical signs on presentation, presence and type of heart murmur, diagnostic imaging modalities used to obtain diagnosis, date of BV procedure if applicable, number of BV procedures if more than one performed, use of any cardiac medications, date of most recent follow-up, and date of death if noted in the record.

### *Electrocardiography*

Electrocardiograms (ECG) were performed in the majority of patients on initial presentation using a standard 10 lead electrocardiograph,<sup>a</sup> with the dog positioned in right lateral recumbency. ECG recordings were reviewed by a single investigator (CS) in the dogs that underwent BV procedures or in BD that underwent advanced diagnostic imaging. The ECG

recordings were analyzed and the following measurements and assessment were recorded: rhythm diagnosis, presence of RV or RA enlargement patterns, mean electrical axis, presence of supraventricular arrhythmias (atrial premature complexes and/or supraventricular tachycardia), and presence of ventricular arrhythmias (ventricular premature complexes and/or ventricular tachycardia). Criteria for RV and RA enlargement patterns were determined based on previously published guidelines.<sup>88, 89</sup>

### *Echocardiography*

The diagnosis of PS was made via echocardiography by either a board-certified cardiologist or a cardiology resident supervised by a board-certified cardiologist in all dogs. PS severity and suspected valve morphology were determined based on previously published guidelines.<sup>2, 24</sup> All dogs underwent a complete echocardiographic examination using one of two ultrasound units,<sup>b, c</sup> which included transthoracic 2D B-mode, M-mode, and spectral and color flow Doppler. Images were acquired with phased-array transducers specific to the ultrasound machine systems. For all measurements, 3 consecutive measurements were performed and averaged when feasible. Conventional echocardiographic views were used to collect standard diagnostic information regarding the left-sided cardiac chambers and function using previously described methods.<sup>90</sup> Right-sided measurements and RV functional indices were also evaluated on the retrospective data using previously described methods.<sup>71, 72</sup> Specific measurements obtained from a right parasternal short axis apical image included RV free wall thicknesses, RV chamber dimensions, and interventricular septal thickness, each performed at end-diastole and end-systole. The right parasternal short axis basilar imaging plane was used for the pulmonic valve annulus maximal diameter measurement, subjective evaluation of the level of RV outflow

tract obstruction (subvalvular, valvular, or supra-valvular), degree of pulmonic leaflet thickening (mild, moderate, severe), the location of thickening within the leaflets (leaflet base, leaflet tips, or generalized), presence of doming/tethered leaflets, and presence of decreased systolic excursion of the leaflets. Maximum trans-pulmonic velocity was measured via continuous wave Doppler and an average of 3 consecutive measurements was recorded when feasible or an average of at least 3 representative measurements was recorded. Aortic diameter was also measured using the right parasternal short axis basilar imaging window according to previously described methods.<sup>91</sup> RV free wall thickness and RV chamber dimensions were measured at end-diastole and end-systole and the maximum RA diameter was measured at end ventricular systole from the right parasternal 4 chamber long axis window. From the left apical imaging plane, the endocardial borders of the RV were traced at end-diastole and end-systole to acquire the end-diastolic and end systolic RV area. The RV fractional area change (FAC) percent was calculated using the equation:  $FAC\% = (RV \text{ diastolic area} - RV \text{ systolic area}) / RV \text{ diastolic area}$ . When measurement data was available or anatomical M-mode image post-processing capability was available, tricuspid annular plane systolic excursion (TAPSE) and peak systolic annular velocity (S') measured at the lateral aspect of the TV annulus were recorded.

### *Balloon valvuloplasty*

The surgical and anesthetic records for all dogs undergoing BV procedures were evaluated. Data recorded from the record included the size of the balloon dilation catheter used for the procedure, trans-esophageal echocardiography (TEE) measurements if this imaging modality was performed, and invasive RV pressure measurements prior to BV and post-BV. The maximum diameter of the pulmonic annulus in any imaging plane was measured on TEE images.

The trans-pulmonic PG as assessed using standard echocardiography prior to discharge from the hospital (within 24 hours of the BV procedure) and at the one-month follow up visit were also recorded. Balloon valvuloplasty procedures were performed according to previously outlined protocols<sup>37, 54</sup> with a modified Seldinger approach for introducer placement when feasible.

#### *Thoracic/cardiac-gated computed tomography*

Computed tomography (CT) imaging studies that were performed in the BD breed dogs were evaluated by 3 reviewers (CS, KS, DS). In all but 1 of the EB, an ECG-gated, contrast-enhanced CT study of the thorax including the heart were performed. In the first EB to undergo CT imaging, cardiac ECG-gating software was not available so non-gated contrast-enhanced CT was performed. All of the BD breed dogs received sedation prior to induction of general anesthesia with varying protocols based on clinician and anesthesiologist preference. Anesthesia was maintained with isoflurane delivered in oxygen and heart rate, respiratory rate, end-tidal CO<sub>2</sub>, oxygen saturation, and non-invasive blood pressure were monitored and recorded. Mechanical ventilation was performed in all cases. The studies were performed with a breath hold facilitated by applying 10-12 cm H<sub>2</sub>O of positive end-expiratory pressure to the airway and pausing mechanical ventilation during CT image acquisition to decrease respiratory motion. All CT examinations were performed using a 64-slice multidetector computed tomography (MDCT) scanner<sup>d</sup> with animals positioned in sternal recumbency. An unenhanced CT scan was performed from the thoracic inlet to the caudal border of the lungs followed by a contrast-enhanced or contrast-enhanced ECG-gated scan of the heart and proximal great vessels. Nonionic iodinated contrast agent<sup>e</sup> was administered using a 3 injection protocol and scan settings were performed according to previously described methods.<sup>92</sup> Multiplanar reconstructions of the heart were

performed to allow for visualization of the cardiac structures in standard CT tomographic views (dorsal, sagittal, and transverse planes) as well as views that approximated standard trans-thoracic echocardiographic derived views (short axis and long axis 1, 2, 3, 4, and 5 chamber views). Oblique reconstructions of the great vessels and the respective outflow tracts were also performed. Digital Imaging and Communications in Medicine viewer software<sup>f</sup> was used to evaluate all images. Specific 3D reconstruction software<sup>g</sup> was also used to evaluate coronary artery anatomy.

Image analysis and CT measurements were performed on 4 chamber long axis images that maximized the left ventricular outflow tract and aortic valve anatomy. The maximum diameter of the aortic valve was measured at the level of the annulus (Figure 3). The maximum diameter of the pulmonic valve was measured at the annulus and leaflet morphology was subjectively assessed by the reviewers as normal, mild, moderate, or severely thickened from a modified 4-chamber long axis image that optimized the right ventricular outflow tract (Figure 4). Normal valve leaflet thickness was subjectively defined as leaflets with a very thin and slender shape with minimal appearance observed in any imaging plane. Annular ratios were then calculated and included the aorta: pulmonary artery (Ao:PA) ratio and its inverse (PA:Ao). An Ao:PA ratio  $< 1.2$  and a PA:Ao ratio of  $< 0.8$  were considered normal.<sup>24, 39, 76</sup> Coronary artery anatomy was visualized and tracked in multiple imaging windows, with the 4 chamber long axis image optimizing the left ventricular outflow tract (Figure 5) and a basilar transverse imaging window (Figure 6) being the most commonly used to determine the specific origin and branching pattern of the coronary arteries.

### *Angiography*

Left-sided angiography was performed on 5 of the BD patients according to previously described protocols<sup>42, 54</sup> and right-sided angiography with subsequent levo-phase imaging<sup>1</sup> was reviewed on all of the BD animals that underwent either a left-sided angiogram or a BV procedure with a right-sided angiogram. Angiograms were reviewed by 3 reviewers (CS, KS, DS) and measurements of the maximum diameter and the aortic valve and pulmonic valve were made at their respective annulus (Figures 7 and 8). Pulmonic leaflet morphology was also subjectively evaluated as normal, mild, moderate, or severely thickened as outlined above for CT imaging.

### *Procedural outcome and survival*

Owners, referring veterinarians, or both were contacted to obtain follow up information on all of the identified patients to determine survival status (alive or dead), date and cause of death if available, and if clinical signs of cardiac disease were observed at any time. Cardiac deaths were classified as natural death or death from euthanasia due to refractory right-sided congestive heart failure (R-CHF) or sudden death without evidence of preceding clinical signs (suspected to be caused by a malignant arrhythmia). Patients were censored if death was secondary to left-sided congestive heart failure, no documentation of cause was obtained, or if death was related to other non-cardiac diseases/injuries. Presence of clinical signs, death, and pre-procedural trans-pulmonic PG were compared to post-BV results to determine procedural success. Procedural success was defined as an echocardiographically measured trans-pulmonic PG reduction of at least 50% of the pre-BV PG and/or a final PG of less than 64 mmHg at the one month recheck.

*Statistical analysis*

Statistical analysis was performed by standard computer statistical software<sup>h</sup> with statistical significance set at  $P < 0.05$ . Data were tested for normality using the D'Agostino & Pearson omnibus normality test. Continuous data, such as age, weight, echocardiographic measurements, average ECG heart rate, and PG at specific time points, were compared by Mann-Whitney U tests, unpaired t-tests, or ANOVA as indicated based on normality. Categorical data such as breed, PS severity, sex, and ultimate procedural success, were compared using a Fisher's exact test. Survival times were calculated from the date of birth reported in the medical records to the date of natural death or euthanasia within the observational study period. Dogs were right-censored in the survival analysis if they were alive at the end of the observational period. The cause of death was verified in the medical records if reported, direct communication with owners, or via communication with referring veterinarians. All-causes of mortality ("all-cause mortality") and cardiac-attributed mortality ("cardiac mortality") indicated by death due to R-CHF or a suspected arrhythmia were used separately for final data analysis. No distinction was made regarding natural death from the underlying cardiac disease or euthanasia related to the underlying cardiac disease. Kaplan-Meier survival curves were constructed to estimate survival functions.<sup>93, 94</sup>

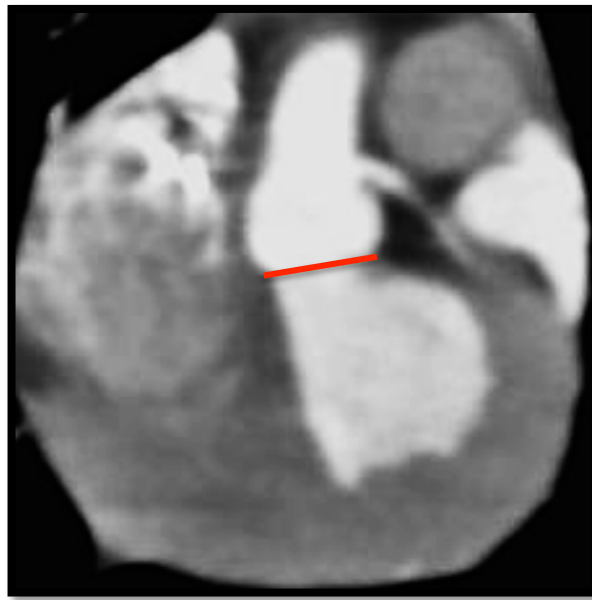


Figure 3: CT aortic valve annulus measurement

Figure 3 caption: Sagittal CT image with contrast highlighting the left ventricle, aorta, and left coronary artery in an English bulldog. The red line indicates the location of the aortic annulus measurement.



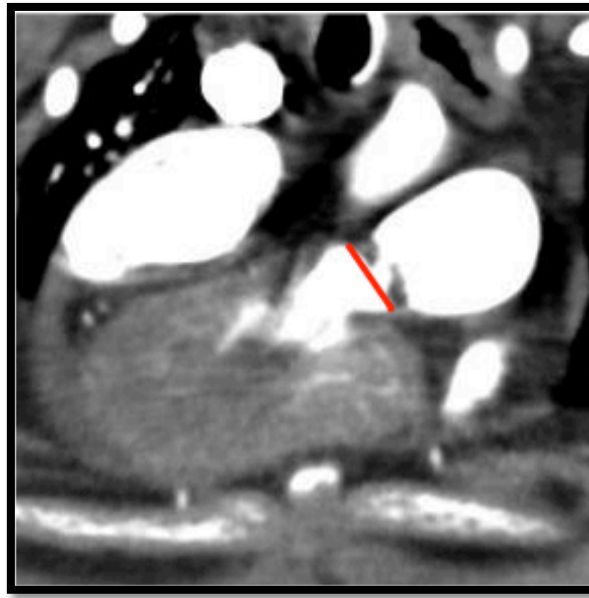


Figure 4: CT pulmonic valve annulus measurement

Figure 4 caption: Sagittal CT image with contrast highlighting the right ventricular outflow tract, pulmonic valve annulus, and main pulmonary artery in a French bulldog. The red line indicates the location of the pulmonic annulus measurement.

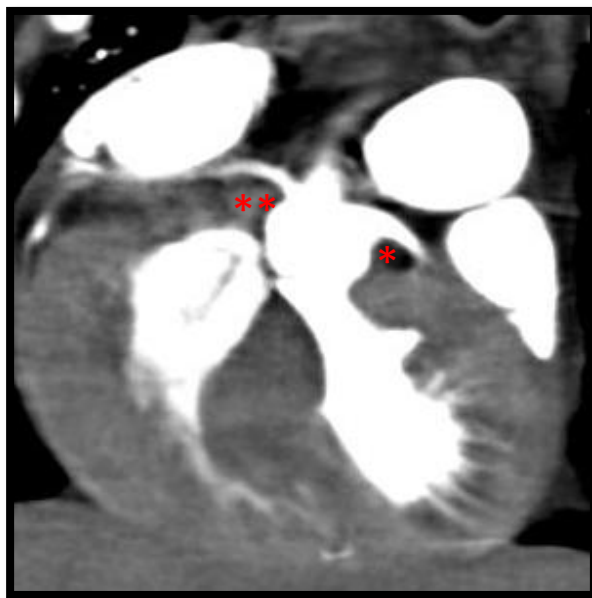


Figure 5: CT imaging of coronary artery ostia (sagittal)

Figure 5 caption: Sagittal CT image with contrast highlighting the coronary artery ostia (\*left ostium and \*\*right coronary ostium) in an English bulldog with normal coronary arteries.

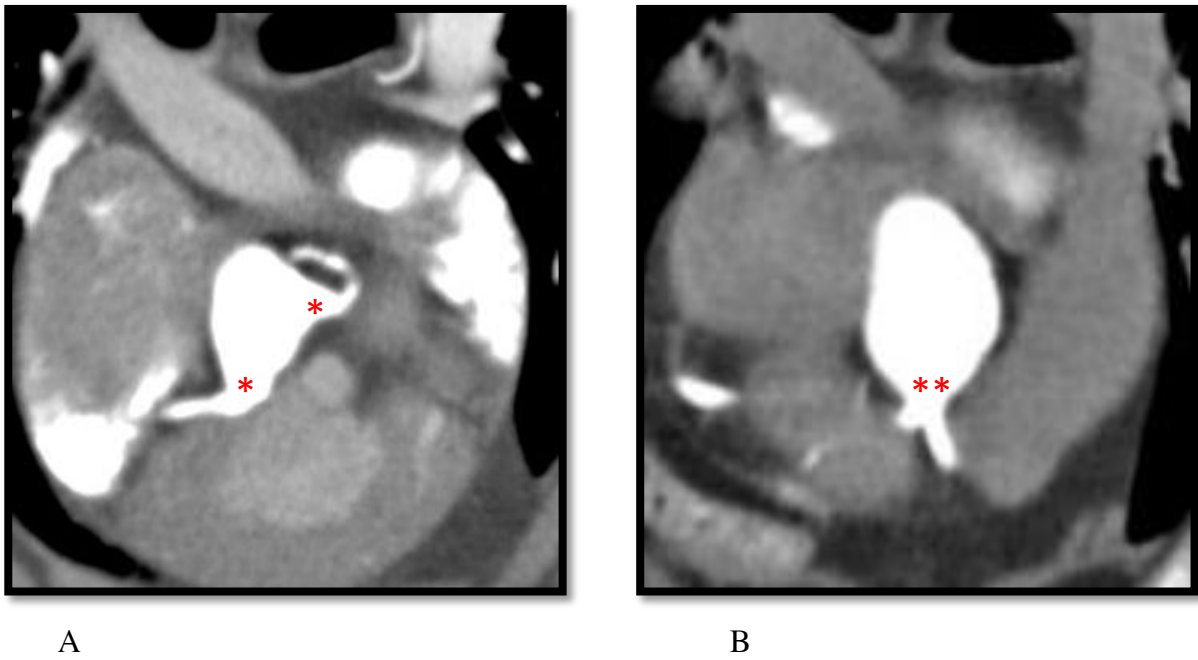


Figure 6: CT imaging of coronary ostia (oblique)

Figure 6 caption: Oblique contrast CT images of the aortic root with coronary artery origins visualized in a normal French bulldog (\* both coronary ostia; A) and an English bulldog diagnosed with a R2A coronary artery anomaly (\*\* single coronary ostium; B).

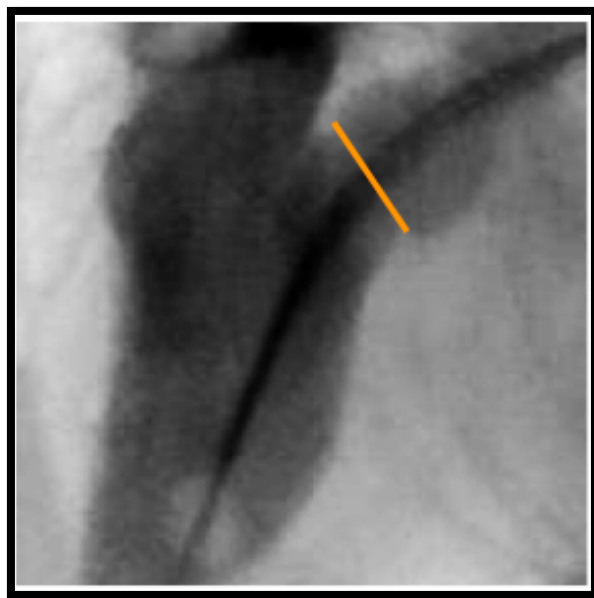


Figure 7: Angiographic aortic valve annulus measurement

Figure 7 caption: Left-sided angiogram highlighting the left ventricle and aorta. The orange line indicates the location of the aortic annulus measurement.

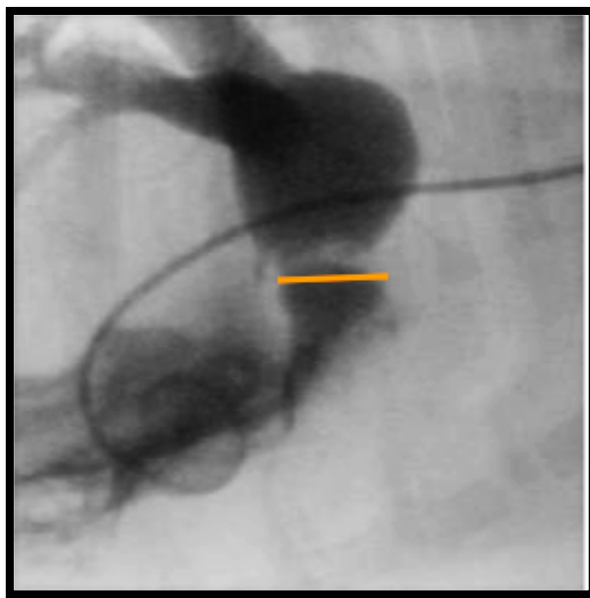


Figure 8: Angiographic pulmonic valve annulus measurement

Figure 8 caption: Right-sided angiogram highlighting the right ventricular outflow tract, pulmonic valve annulus, and main pulmonary artery in a French bulldog. The orange line indicates the location of the pulmonic annulus measurement.

## **Chapter 3**

### **Results**

#### *Aim 1: Comparison of French and English bulldog populations*

The medical records search initially identified 29 dogs that were either French or English bulldog breeds with severe PS. Lack of definitive coronary artery imaging excluded 6 EB and 2 FB, leaving 21 dogs in the BD population consisting of 8 EB and 13 FB (Table 1). There were no significant differences between the groups in regards to sex, age at presentation, average heart rate on a rhythm strip electrocardiogram (ECG), or if cardiac related death at the end of the study period was observed. When evaluating the EB population at the conclusion of the study period, 4 were deceased, 3 were alive, and 1 was lost to follow up. Of the 4 EB that were deceased at the end of the study period, all were diagnosed with an R2A coronary artery anomaly and had been euthanized secondary to R-CHF without BV performance. Of the 3 EB that were still alive, 2 had undergone BV with normal coronary artery anatomy and 1 had been diagnosed with an R2A coronary anomaly with no BV procedure performed. All 3 were reported to be well by owners.

When evaluating the FB population group at the conclusion of the study period 5 of the 12 FB, which had undergone BV procedures, were deceased- 1 during immediate recovery from BV, 1 at home within 1 week of a BV procedure, and 2 secondary to R-CHF and/or cardiac arrhythmias. One FB died following surgical management (exact procedure was not available from the owners) of a heart base tumor, not directly related to pulmonic stenosis and was censored from the study population at 35 months of age (29 months post BV). There were 7 FB alive and reported to be well at the conclusion of the study (6 that underwent BV and 1 that had not undergone BV) and 1 FB was lost to follow-up. All of the BD that were alive at the end of the study period were relatively young with only short-term follow-up available. The FB median

age at conclusion of the study was 28.25 months (range 9-68 months) and EB median age was 22 months (range 11-27 months). There was a significant difference between the groups in regards to mean weight ( $P= 0.0151$ ) with the EB having a greater mean weight on initial presentation (13.6 kg) compared to the mean weight of the FB on initial presentation (8.71 kg). Clinical signs related to pulmonic stenosis/right-sided cardiac disease were noted in 6 of the FB, which was significantly greater ( $P = 0.0456$ ) than EB, none of which had clinical signs. Documented clinical signs in the FB group included syncope ( $n=2$ ), exercise intolerance ( $n=3$ ), and R-CHF ( $n=1$ ).

The imaging modalities used to assess coronary artery anatomy for dogs in each group are listed in Table 2. All dogs had echocardiography performed. Seven EB and 10 FB had CT imaging performed with 6 EB and 1 FB only having a CT study performed to determine coronary artery anatomy. One EB had both a CT and a left-sided angiogram performed. One EB and 3 FB only had left and right-sided angiograms performed. All dogs that underwent a BV procedure (2 EB and 12 FB) had right-sided angiograms performed. None of the EB diagnosed with anomalous coronary artery anatomy underwent BV.

There were significant differences between groups for both the CT derived aortic valve annulus width and pulmonic valve annulus width (Table 3). When these variables were indexed to body weight, they remained statistically significant. The mean Ao:PA and PA:Ao ratios identified 3/7 EB and 0/10 FB with pulmonic annular hypoplasia using this imaging modality. Due to the small number of dogs that underwent left-sided angiography (2 EB and 3 FB), measurements made from left and right-sided angiograms were combined for analysis (Table 4). There was no significant difference between the width of the aortic valve annulus, width of the

pulmonic valve annulus, and Ao:PA or PA:Ao ratios between groups. Based on angiographic measurements 1/3 EB and 3/12 FB met classification criteria for pulmonic annular hypoplasia using this imaging modality.

To compare measurements obtained from all the imaging modalities, the EB and FB groups were combined (Table 5). Transesophageal echocardiography was used intra-operatively for pulmonic annulus diameter measurement in many of the patients that underwent BV. For aortic annulus width, transthoracic echocardiography yielded a statistically larger measurement than CT and angiography, the latter of which were not different from each other. There were no significant differences between the imaging modalities in regards to pulmonic annulus measurements. There was significant difference in the annular ratios with transthoracic echocardiography yielding a larger Ao:PA and smaller PA:Ao ratio. This resulted in a difference in the number of dogs identified with pulmonic annular hypoplasia with CT and angiography identifying 3/17 dogs and 4/15 dogs, respectively, compared with 18/21 dogs via transthoracic echocardiography.

All imaging modalities were assessed collectively to provide a subjective analysis of the pulmonic leaflet morphology for each dog and agreed upon by the three evaluators (CS, KS, DS). The FB population had more dogs with severe leaflet thickening and decreased leaflet motion during systole compared to the EB population (Table 6). The EB population tended to have normal to mildly thickened leaflets with leaflet doming during systole, indicating a component of leaflet commissural fusion.



*Aim 1: Coronary artery anatomy in French and English bulldogs*

Coronary anatomy was clearly observed and diagnosis of an anomalous single origin coronary artery could be performed on all dogs that underwent a thoracic CT or left-sided angiographic study. In the population of included BD, 6/8 EB and 0/13 FB were diagnosed with a R2A coronary artery anomaly, which was significantly different ( $P= 0.0002$ ). In dogs diagnosed with a R2A anomaly, a conservative BV procedure was discussed and offered to owners but was not elected in any of the cases.

*Aim 2: Pulmonic stenosis population demographics*

The initial search for dogs with a diagnosis of PS of any severity within the medical database of the OSU-VTH identified 181 individual cases (34 bulldogs and 147 non-bulldog breeds). There were 2 dogs excluded due to the diagnosis of tetralogy of Fallot. The BD-PS group included 18 English bulldogs and 16 French bulldogs. The NonBD-PS group included 19 pit bulls, 10 Chihuahuas, 9 Cavalier King Charles spaniels, 6 miniature schnauzers, 5 each of German shepherd dog and Pomeranian breeds; 4 boxers, 3 each of beagle, bichon frise, English springer spaniel, golden retriever, and Labrador retriever breeds; 2 each of Australian shepherd, Bernese mountain dog, German wirehaired pointer, Newfoundland, rat terrier, shih tzu breeds; and 1 each of the following breeds: Australian cattle dog, Basset hound, Belgian malinois, border terrier, Boston terrier, Boykin spaniel, Brittany spaniel, chow chow, coonhound, dachshund, doberman, English cocker spaniel, French mastiff, German pinscher, German shorthair pointer, giant schnauzer, Irish setter, Irish water spaniel, Jack Russell terrier, miniature pinscher, papillion, Rhodesian ridgeback, vizsla, West Highland white terrier, Wheaton terrier, whippet,

and Yorkshire terrier. There were 23 mixed breed dogs weighing  $\leq 15\text{kg}$  and 10 mixed breed dogs weighing  $>15\text{kg}$ .

There was no significant difference between the BD-PS and NonBD-PS groups in regards to the presence of concurrent congenital defects. In the BD-PS group, there were a total of 12 separate congenital cardiac defects diagnosed concurrently with pulmonic stenosis in 10 dogs which included 4 dogs with small right to left shunting atrial defects most consistent with a patent foramen ovale (PFO), 1 dog with a right to left shunting atrial septal defect (ASD) suspected to be a cranial vena cava sinus venosus defect with partial anomalous pulmonary venous return, 2 dogs with small left to right shunting perimembranous ventricular septal defects (VSD), 2 dogs with suspected tricuspid valve dysplasia (TVD), 1 dog with bicuspid valvular aortic stenosis, 1 dog with a persistent left cranial vena cava (PLCVC), and 1 dog with a forme fruste ductus arteriosus diagnosed via contrast CT.

In the NonBD-PS group, there were 54 separate congenital cardiac defects diagnosed concurrently with pulmonic stenosis in 43 dogs including 14 dogs with suspected TVD, 7 dogs with suspected PFO, 7 dogs with perimembranous VSD, 5 dogs with 1 or more muscular VSD, 4 dogs with left to right shunting patent ductus arteriosus (PDA), 4 dogs with subvalvular aortic stenosis, 3 dogs with suspected mitral valve dysplasia (MVD), 2 dogs with valvular aortic stenosis, 2 dogs with PLCVC, 2 dogs with right to left shunting ASD (secundum type), and 1 dog each with cor triatriatum dexter, pulmonary vein stenosis, aortopathy/aortic aneurysm, and aortopulmonary collateral vessels.

Comparison of the BD-PS and NonBD-PS groups revealed no statistical differences in sex distribution, weight, severity of pulmonic stenosis, whether or not a BV procedure was

performed in dogs with severe PS, or as previously identified, the presence of concurrent congenital cardiac defects (Table 7). There was a significant difference ( $P = 0.0126$ ) observed between the groups in the age of presentation to the OSU VTH with a mean younger age of presentation in the BD-PS group (13.2 months) compared to the NonBD-PS group (25.5 months). There was also a significant difference in the definitive diagnosis of aberrant coronary artery anatomy ( $P = < 0.0001$ ) with 6 BD-PS diagnosed with a R2A anomaly and 2 NonBD-PS diagnosed with an aberrant coronary artery origin. Of the NonBD-PS with abnormal coronary anatomy, 1 shepherd mixed breed dog had a R2A anomaly and 1 English springer spaniel had a single origin left coronary artery anomaly.

*Aim 2: Comparison of balloon valvuloplasty procedure populations*

The records query for bulldog and non-bulldog patients that underwent a BV at OSU VTH and met the further defined inclusion criteria based on weight, BV performance, and follow up identified 16 BD-BV dogs (2 EB and 14 FB) and 44 NonBD-BV dogs out of a total of 85 NonBD-BV dogs. There was no significant difference between the BD-BV and NonBD-BV groups in regards to sex distribution, mean body weight, age at the time of BV, presence of additional congenital cardiac defects, or clinical signs on presentation. The reported clinical signs at presentation prior to BV in the BD-BV group included exercise intolerance ( $n=3$ ), syncope ( $n=2$ ), and R-CHF ( $n=2$ ). Clinical signs observed in the NonBD-BV group included exercise intolerance ( $n=9$ ), syncope ( $n=4$ ), and R-CHF ( $n=2$ ).

Concurrent congenital cardiac defects in the BD-BV group included 6 defects observed in 5 dogs: small perimembranous VSD, suspected TVD, PLCVC, forme fruste ductus arteriosus, suspected PFO, and an ASD (cranial sinus venosus type) with partial anomalous pulmonary

venous return. Concurrent congenital cardiac defects were observed in 16 of the NonBD-BV dogs and included 4 dogs with TVD, 4 dogs with suspected PFO, 2 dogs with muscular VSD, and individual cases of perimembranous VSD, suspected MVD, a left to right PDA, pulmonary vein stenosis with aortopulmonary collateral vessels, PLCVC, and an ASD (secundum type). Systolic anterior motion of the mitral valve and concentric left ventricular hypertrophy were observed in 6 of the NonBD-BV dogs on initial echocardiography.

There were 8 dogs in the NonBD-BV group and 5 dogs in the BD-BV group that were not receiving any cardiac medications at the time of presentation and were not prescribed any cardiac medications upon hospital discharge. Atenolol was the most commonly prescribed cardiac medication observed in both BV groups. In the NonBD-BV group, 11 cases were receiving atenolol on presentation, 17 were discharged from the hospital following BV with atenolol, and 2 were started on atenolol at the 1 month recheck. Other medications being received or prescribed in the NonBD-BV cases included enalapril (n=2), furosemide (n=2), propranolol (n=1), sotalol (n=3), mexiletine (n=3), and digoxin (n=1). Four dogs were receiving more than one cardiac medication. In the BD-BV group, 3 cases were receiving atenolol on presentation, 4 were discharged following BV with atenolol, and 1 was started on atenolol at the 1 month recheck. Other cardiac medications in the BD-BV group included enalapril (n=1), furosemide (n=2), and spironolactone (n=1). One BD was receiving more than one medication.

Comparison of pre-operative ECG recordings between the BD-BV and NonBD-BV groups revealed no significant difference in mean heart rate (Table 8). There were 42 ECG recordings available for review in the NonBD-BV group and 15 in the BD-BV group.

Arrhythmias were observed in 4 NonBD-BV dogs (supraventricular tachycardia, n=1; ventricular

premature contractions (VPCs), n=3; atrial premature contractions (APCs), n=2) and in 4 BD-BV dogs (APCs and VPCs, n=1; Mobitz type 1 second degree atrioventricular block, n=2; atrial standstill, n=1). An epicardial pacemaker was placed in the dog with atrial standstill prior to BV. The most commonly observed rhythm in the NonBD-BV group was sinus rhythm (n=22) followed by sinus arrhythmia (n=21), and sinus tachycardia (n=1). The most common rhythm diagnosis in the BD-BV group was sinus rhythm (n=7) followed by sinus arrhythmia (n=5), sinus tachycardia (n=1), and atrial standstill (n=1). Criteria for right ventricular enlargement (RVE) were observed in 33 of the NonBD-BV cases, 4 cases had a single criteria for RVE enlargement, and 5 cases had no criteria for RVE. There were 4 NonBD-BV cases that had criteria for RA enlargement. In comparison, there were 13 BD-BV cases with criteria for RVE, 1 BD-BV case with a single criterion for RVE, and 1 dog with no criteria for chamber enlargement. None of the BD-BV cases had criteria for RA enlargement.

Transthoracic echocardiography revealed a significant difference ( $P = 0.0231$ ) in the pulmonic valve annulus size (Table 9) with the BD-BV group having a smaller mean annulus (1.02 cm) width than the NonBD-BV group (mean annulus width 1.19 cm). There was no significant difference in the mean aortic valve annulus measurements between the 2 groups (BD-BV mean 1.44 cm and NonBD-BV mean 1.41 cm). There was a significant difference ( $P = <0.0001$ ) for both the mean Ao:PA (BD-BV ratio 1.41 and NonBD-BV ratio 1.19) and PA:Ao (BD-BV ratio 0.72 and NonBD-BV ratio 0.87) with the BD-BV group showing a more significant degree of annular hypoplasia as a component of their PS morphology. Subjective grading and analysis of the echocardiographic pulmonic valve morphology in the BD-BV and NonBD-BV groups revealed evidence of more severe thickening of the leaflets, specifically at the base of the leaflets. In addition, the BD-BV group showed more subjective leaflet immobility

during systole compared to the NonBD-BV group. In contrast, the NonBD-BV group had more cases of mild leaflet thickening and leaflet tip doming during systole.

Echocardiographic variables of RV chamber dimensions, wall thickness, and systolic function were performed and compared between the BD-BV and NonBD-BV groups (Table 9). When evaluating RV systolic function indices, the BD-BV group had a significantly ( $P = 0.0091$ ) lower mean FAC of 38.4% compared to the NonBD-BV group which had a mean FAC of 50.6%, which could reflect overall decreased RV systolic function in this population. The other two RV function indices evaluated (TV S' and TAPSE) did not reveal statistically significant differences, although there was a slight trend toward decreased mean values in the BD-BV group (TV S' of 7.82 cm/sec and TAPSE of 0.86 cm) as compared to the NonBD-BV group (TV S' of 11.0 cm/sec and TAPSE of 1.0 cm). The BD-BV group had significantly larger RV internal systolic (BD-BV mean 0.94 cm, NonBD-BV mean 0.68 cm;  $P = 0.0305$ ) and diastolic dimensions (BD-BV mean 1.4 cm, NonBD-BV mean 1.03 cm;  $P = 0.0248$ ) as measured from the right parasternal 4-chamber long axis view. The BD-BV group also had significantly thicker interventricular septal wall dimensions at end-diastole (BD-BV mean 0.97 cm, NonBD-BV mean 0.83 cm;  $P = 0.0270$ ) and larger maximum RA internal dimensions (BD-BV mean 2.88 cm, NonBD-BV mean 2.28 cm;  $P = 0.0129$ ).

The range of balloon catheter diameters used in the two groups included larger diameter catheters in the NonBD-BV group (Table 8). The average balloon catheter size was not different between the 2 groups (data not shown). The balloon catheter diameter to trans-thoracic or trans-esophageal pulmonic annulus diameter ratio was derived for each case. There was a significant

difference in the pulmonic annulus to balloon catheter diameter measured with both trans-thoracic and trans-esophageal imaging modalities between the 2 groups.

### *Aim 3: Procedural success*

Procedural success was evaluated between the 2 groups (Table 10). There was no significant difference between the groups in regards to both the initial mean trans-pulmonic valve PG prior to the BV procedure or the 24-hour post BV echocardiographically measured trans-pulmonic PG. There was a significant difference ( $P = 0.0096$ ) between the groups in procedure success evaluated at the one-month echocardiographic recheck with the BD-BV group having lower success (4/12 dogs) than the NonBD-BV group (31/42). The BD-BV group had a significantly ( $P = 0.005$ ) higher mean PG at the one-month recheck (86 mmHg) than the NonBD-BV group (54.4 mmHg). Two deaths occurred within each group during the immediate recovery period following BV.

There was 1 BD-BV and 5 NonBD-BV cases that underwent more than one BV procedure, with 1 of the NonBD-BV cases undergoing 3 BV procedures (data not shown). The reasoning for additional BV procedures was a lack of successful decrease in the trans-pulmonic valve PG in all cases. Success according to previously outlined goals in the population of dogs with >1 BV procedure was only observed in 1 NonBD-BV dog following the second BV procedure (PG decreased by 50% of the initial trans-pulmonic PG), although the overall trans-pulmonic PG in this patient remained just over 100mmHg. In the other patients, a decrease from the pre-procedural trans-pulmonic PG was observed, but did not meet classification goals for a successful procedure.

### *Aim 3: Survival analysis*

Survival statistics were evaluated for 4 specific groups of dogs presenting with or diagnosed with severe PS by the OSU VTH (Figure 9). A total of 44 NonBD breed dogs that underwent BV procedures, 23 NonBD breed dogs that did not undergo BV procedures (NonBD-nonBV), 16 BD breed dogs that underwent BV procedures, and 7 BD breed dogs that did not undergo BV procedures (BD-nonBV) met study inclusion criteria. There was statistically significant difference between the curves when all groups were compared in regards to all-cause mortality ( $P = 0.0009$ ) and cardiac mortality ( $P = 0.0003$ ). The survival curves for dogs that underwent BV procedures (NonBD-BV versus BD-BV groups) were compared (Figure 10) and there was a significant difference between all-cause mortality ( $P = 0.0005$ ) and cardiac mortality ( $P = 0.0084$ ). In regards to all-cause mortality and cardiac mortality, the BD-BV group showed a shorter median survival time (MST) of 67 months for both analyses when compared to the NonBD-BV group, which had an undefined MST because 50% of the NonBD-BV population dogs did not reach a primary study endpoint (due to missing follow-up data or were still alive at the end of the study period). There were a total of 7 deaths in the NonBD-BV group with 3 of these deaths attributed to cardiac causes. Two of the deaths were within the immediate post-procedural period and 1 death was euthanasia secondary to severe R-CHF at 58 months of age, approximately 49 months following BV procedure. There were 6 deaths observed in the BD-BV group with 5 of these events directly attributed to cardiac causes. Two of the deaths were observed within the immediate post-procedural period and 3 deaths were observed at 3 months, 4 months, and 57 months post BV procedure. All of the BD-BV cases that died of cardiac disease were showing clinical signs at the time of presentation- 1 with R-CHF and atrial standstill, 1 with R-CHF, and the other dog with evidence of mild exercise intolerance. The 1 dog in the NonBD-



BV group that died of cardiac disease was not showing any evidence of cardiac disease on initial presentation. All 4 of the dogs that died within the post-procedural period had evidence of advanced/concurrent congenital cardiac disease with either signs of R-CHF (n=3), evidence of SVT and ventricular ectopy (n=1), or an ASD that led to a suspected paradoxical embolic episode (n=1).

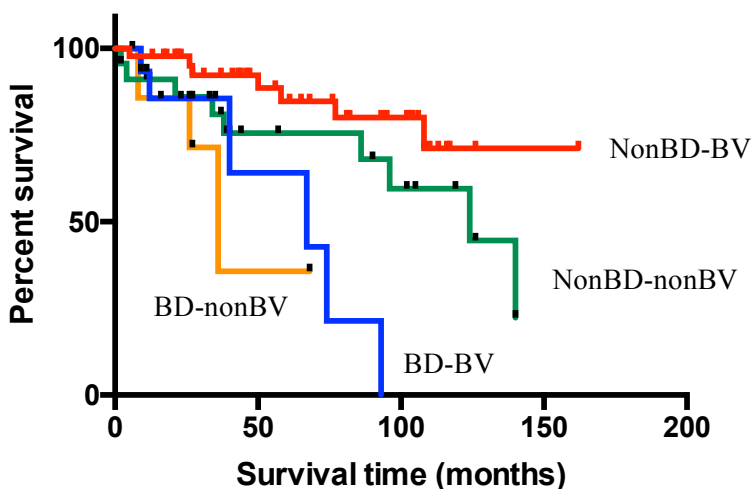
Kaplan Meier survival curves were also constructed to compare survival differences between the BD and NonBD dogs with severe PS that did not undergo BV (Figure 11). There was no significant difference ( $P = 0.0960$ ) in all-cause or cardiac mortality between the groups. All-cause mortality MST in the BD group that did not undergo any BV procedures was 36 months and the MST in the NonBD population was 124 months. In regards to cardiac mortality, the MST in the BD group not undergoing BV was 36 months and the MST in the NonBD population was 140 months. There were 23 dogs meeting inclusion criteria within the NonBD-nonBV group (9 total deaths with 8 cardiac related deaths) and 7 dogs in the BD-nonBV group with 4 total deaths that were cardiac related. There were 9 NonBD-nonBV dogs alive at the end of the study period (range of 11- 126 months of age) and 6 dogs were lost to follow up. There were 2 BD-nonBV dogs still alive at the conclusion of the study: 1 EB with a confirmed R2A anomaly alive at 27 months of age and 1 FB with confirmed normal coronary anatomy alive at 68 months of age. There was 1 BD-nonBV lost to follow-up. All 4 of the BD-nonBV cardiac deaths were observed in EB with R2A coronary artery anomalies, with an average 8-36 month survival.

Kaplan Meier survival curves were constructed to compare dogs within their breed groupings that did or did not undergo a BV for severe PS. NonBD breed dogs that underwent BV

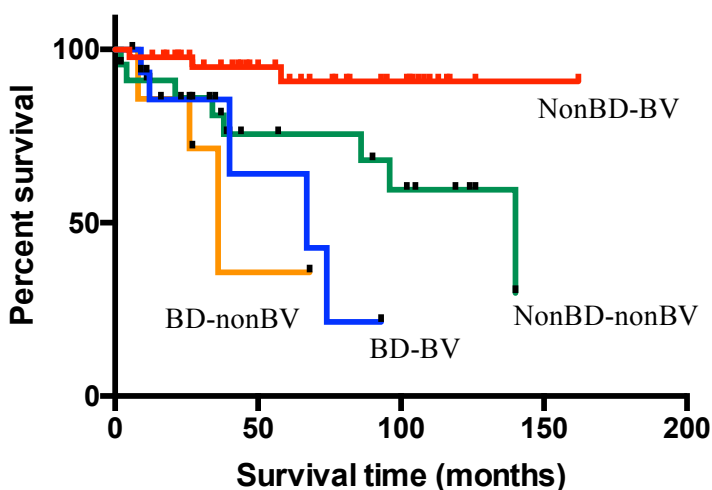
procedures showed no difference in all-cause mortality ( $P = 0.1186$ ) with a MST that was undefined due to less than 50% of the population reaching a primary study endpoint as compared to the NonBD dogs that did not undergo a BV that had a MST of 124 months. There was a significantly longer MST observed in regards to cardiac mortality ( $P = 0.0217$ ; Figure 12) with improved survival seen in the dogs that underwent BV (NonBD-BV group had an undefined MST compared to the MST of 140 months observed in the NonBD-nonBV group). As previously mentioned, there were 3 cardiac deaths in the NonBD-BV group (2 immediately post-procedure and 1 in the follow-up period 49 months post BV) and there were 8 cardiac deaths in the NonBD-nonBV group. Within the NonBD-nonBV group there were 5 deaths attributed to R-CHF and 3 deaths attributed to sudden cardiac death. The age at the time of death in the NonBD-nonBV group was between 2-140 months. Ultimately 6 of the NonBD-nonBV dogs were lost to follow up and 9 dogs were alive at the end of the study (range of 11-126 months of age).

Comparison of survival in BD breed dogs with severe PS that did or did not undergo BV procedures (Figure 13) revealed no significant difference in either all-cause mortality or cardiac mortality ( $P = 0.4994$ ). There were small numbers of dogs in both groups: 16 dogs that underwent BV with 5 cardiac deaths and 7 dogs that did not undergo BV procedures with 4 cardiac deaths. When the 2 dogs of the BD-BV group that died in the immediate post-procedural period were removed from analysis, cardiac mortality survival curves provided evidence of a potential beneficial trend in the performance of a BV procedure ( $P = 0.1748$ ), although the difference in survival between the 2 groups remained statistically insignificant. The MST for the BD-BV group was 74 months and 36 months for the BD-nonBV group, indicating the performance of a BV procedure did not provide a clear survival benefit for these breeds.

Dogs that underwent BV procedures (BD and NonBD breeds) were combined and compared to all dogs (BD and NonBD breeds) with severe PS that did not undergo BV procedures (Figure 14). There were 60 dogs in the BV group and 30 dogs in the NonBV group. There was no significant difference between the groups in regards to all-cause mortality ( $P = 0.1351$ ), but there was significantly longer MST (undefined for the BV group and 140 months in the NonBV group) when cardiac mortality was examined ( $P = 0.0188$ ). This provides evidence that BV procedures provide survival benefit in dogs with severe valvular PS when evaluating the canine population as a whole.



A

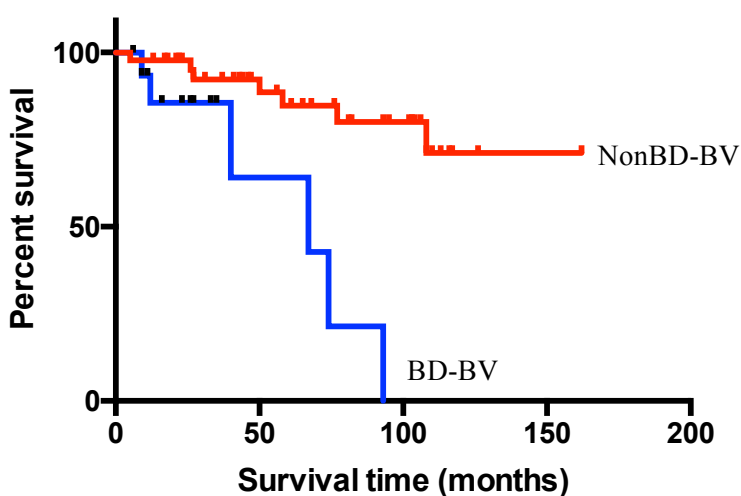


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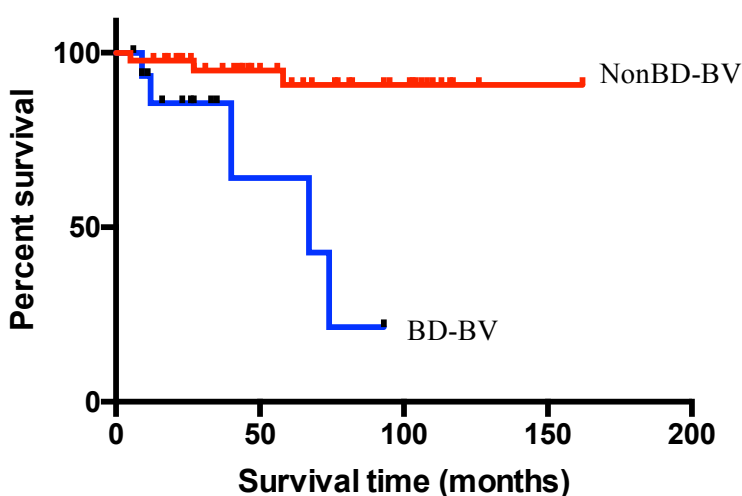
Figure 9: Kaplan Meier survival curves: all populations with severe PS

Figure 9 caption: Kaplan Meier survival curves displaying survival proportions of (A, top) non-bulldog breeds with severe pulmonic stenosis that underwent balloon valvuloplasty (red line;  $n = 44$ ; censored  $n = 37$ ; events  $n = 7$ ), bulldog breeds with severe pulmonic stenosis that underwent balloon valvuloplasty (blue line;  $n = 16$ ; censored  $n = 10$ ; events  $n = 6$ ), non-bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (green line;  $n = 23$ ; censored  $n = 14$ ; events  $n = 9$ ), and bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (yellow line;  $n = 7$ ; censored  $n = 3$ ; events  $n = 4$ ) with regard to all-cause mortality ( $P = 0.0009$ ) and (B, bottom) non-bulldog breeds with severe pulmonic stenosis that underwent balloon valvuloplasty (red line;  $n = 44$ ; censored  $n = 41$ ; events  $n = 3$ ), bulldog breeds with severe pulmonic stenosis that underwent balloon valvuloplasty (blue

line; n = 16; censored n = 11; events n = 5), non-bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (green line; n = 23; censored n = 15; events n = 8), and bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (yellow line; n = 7; censored n = 3; events n = 4) with regard to cardiac mortality ( $P = 0.0003$ ).



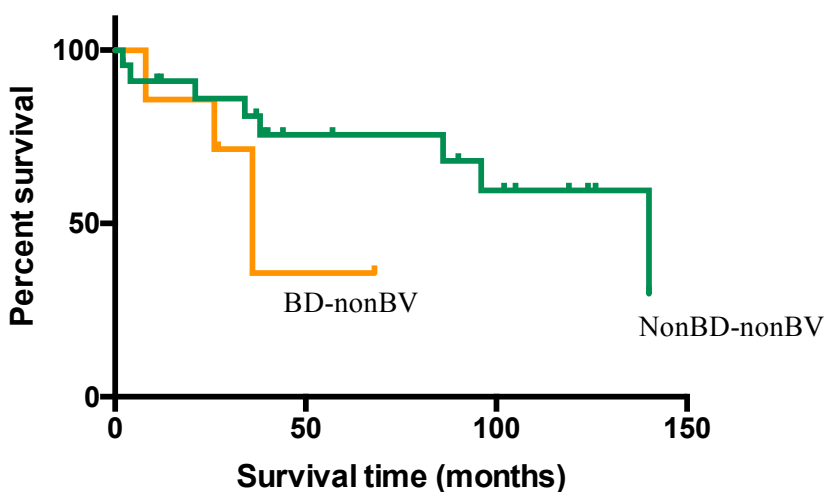
A



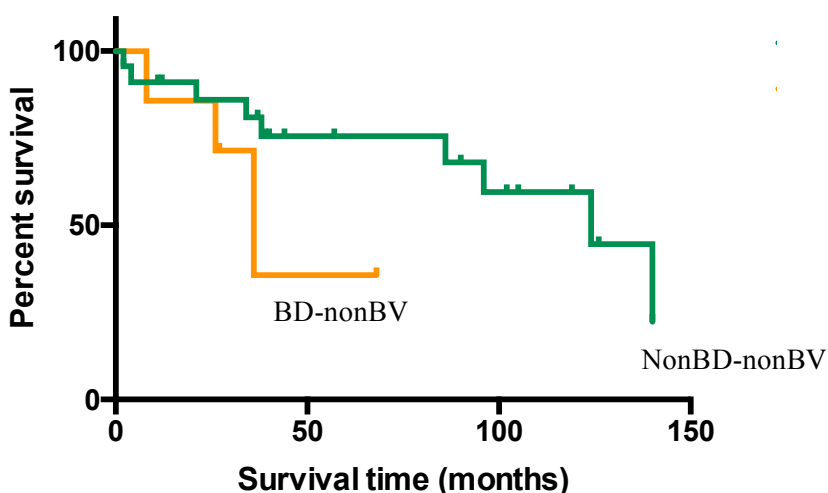
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Figure 10: Kaplan Meier survival curves: BV populations

Figure 10 caption: Kaplan Meier survival curves displaying survival proportions of (A, top) non-bulldog breeds with severe pulmonic stenosis that underwent balloon valvuloplasty (red line;  $n = 44$ ; censored  $n = 37$ ; events  $n = 7$ ) and bulldog breeds with severe pulmonic stenosis that underwent balloon valvuloplasty (blue line;  $n = 16$ ; censored  $n = 10$ ; events  $n = 6$ ) with regard to all-cause mortality ( $P = 0.0005$ ) and (B, bottom) non-bulldog breeds with severe pulmonic stenosis that underwent balloon valvuloplasty (red line;  $n = 44$ ; censored  $n = 41$ ; events  $n = 3$ ) and bulldog breeds with severe pulmonic stenosis that underwent balloon valvuloplasty (blue line;  $n = 16$ ; censored  $n = 11$ ; events  $n = 5$ ) with regard to cardiac mortality ( $P = 0.0084$ ).



A



B

Figure 11: Kaplan Meier survival curves: non-BV populations

Figure 11 caption: Kaplan Meier survival curves displaying survival proportions of (A, top) non-bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (green line;  $n = 23$ ; censored  $n = 14$ ; events  $n = 9$ ), and bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (yellow line;  $n = 7$ ; censored  $n = 3$ ; events  $n = 4$ ) with regard to all-cause mortality ( $P = 0.0960$ ) and (B, bottom) non-bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (green line;  $n = 23$ ; censored  $n = 15$ ; events  $n = 8$ ), and bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (yellow line;  $n = 7$ ; censored  $n = 3$ ; events  $n = 4$ ) with regard to cardiac mortality ( $P = 0.0960$ ).

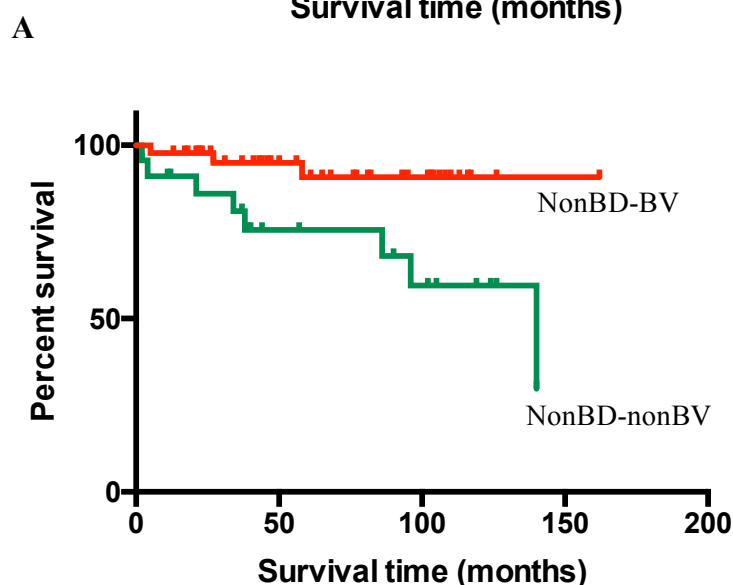
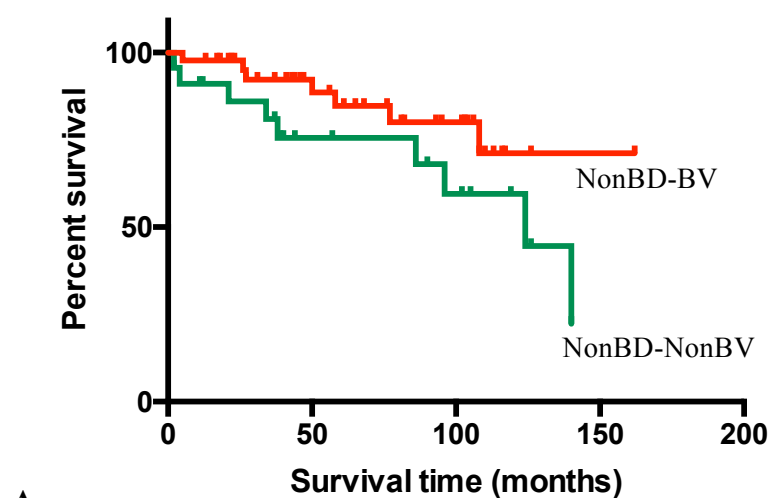
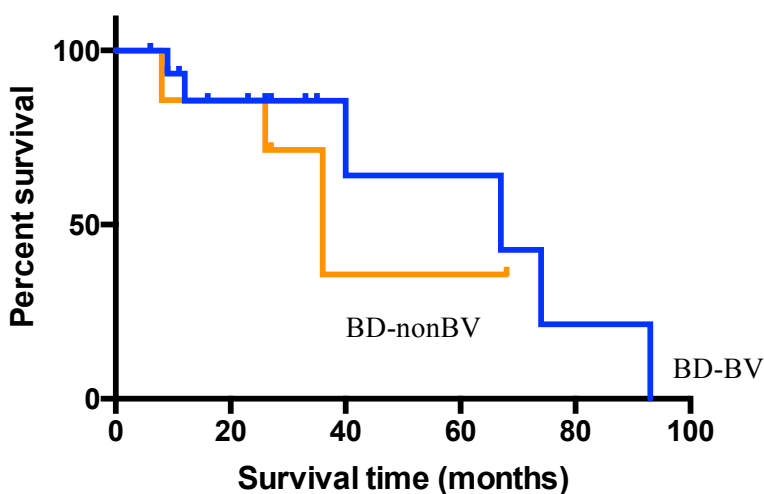


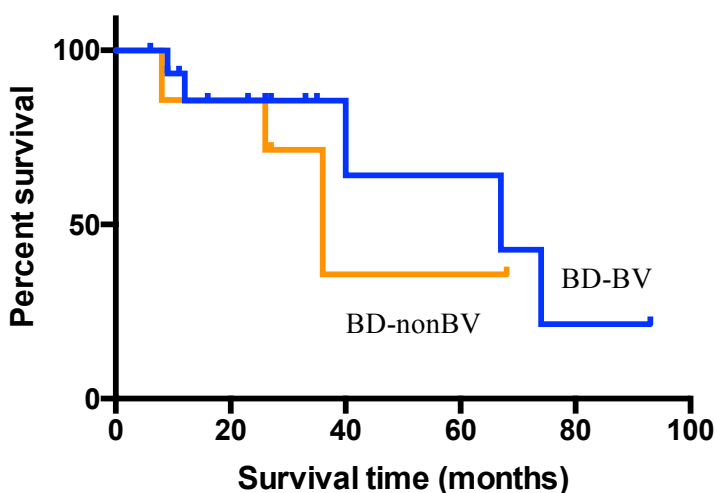
Figure 12: Kaplan Meier survival curves: non-bulldog populations

Figure 12 caption: Kaplan Meier survival curves displaying survival proportions of (A, top) non-bulldog breeds that underwent balloon valvuloplasty (red line;  $n = 44$ ; censored  $n = 37$ ; events  $n = 7$ ) and non-bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (green line;  $n = 23$ ; censored  $n = 14$ ; events  $n = 9$  with regard to all-cause mortality ( $P = 0.1186$ ) and (B, bottom) non-bulldog breeds that underwent balloon valvuloplasty (red line;  $n = 44$ ; censored  $n = 41$ ; events  $n = 3$ ) and non-bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (green line;  $n = 23$ ; censored  $n = 15$ ; events  $n = 8$  with regard to cardiac mortality ( $P = 0.0217$ )).





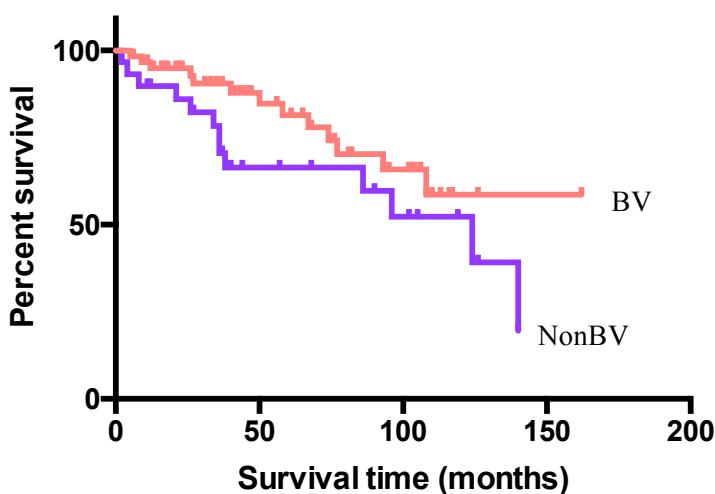
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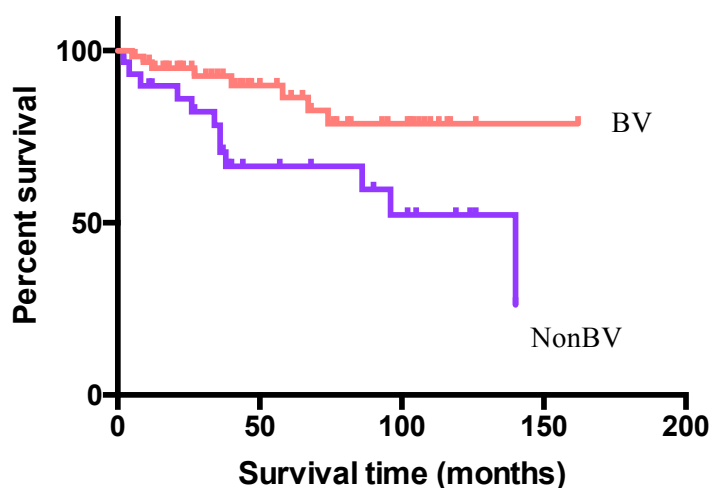
B

Figure 13: Kaplan Meier survival curves: bulldog populations

Figure 13 caption: Kaplan Meier survival curves displaying survival proportions of (A, top) bulldog breeds with severe pulmonic stenosis that underwent balloon valvuloplasty (blue line;  $n = 16$ ; censored  $n = 10$ ; events  $n = 6$ ) and bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (yellow line;  $n = 7$ ; censored  $n = 3$ ; events  $n = 4$ ) with regard to all-cause mortality ( $P = 0.4994$ ) and (B, bottom) bulldog breeds with severe pulmonic stenosis that underwent balloon valvuloplasty (blue line;  $n = 16$ ; censored  $n = 11$ ; events  $n = 5$ ) and bulldog breeds with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (yellow line;  $n = 7$ ; censored  $n = 3$ ; events  $n = 4$ ) with regard to cardiac mortality ( $P = 0.4994$ ).



A



B

Figure 14: Kaplan Meier survival curves: combined populations

Figure 14 caption: Kaplan Meier survival curves displaying survival proportions of (A, top) all dogs with severe pulmonic stenosis that underwent balloon valvuloplasty (pink line;  $n = 60$ ; censored  $n = 47$ ; events  $n = 13$ ) and all dogs with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (purple line;  $n = 30$ ; censored  $n = 17$ ; events  $n = 13$ ) with regard to all-cause mortality ( $P = 0.1351$ ) and (B, bottom) all dogs with severe pulmonic stenosis that underwent balloon valvuloplasty (pink line;  $n = 60$ ; censored  $n = 52$ ; events  $n = 8$ ) and all dogs with severe pulmonic stenosis that did not undergo a balloon valvuloplasty procedure (purple line;  $n = 30$ ; censored  $n = 18$ ; events  $n = 12$ ) with regard to cardiac mortality ( $P = 0.0188$ ).

Table 1: Descriptive statistics (mean  $\pm$  SD) of dogs with definitive coronary imaging

Parameter	English bulldog	French bulldog	P value
Cases meeting inclusion criteria	8	13	
Weight (kg)	13.6 $\pm$ 4.26	8.71 $\pm$ 3.95	0.015*
Sex	7 males (87.5%)	10 males (76.9%)	0.549
Age at presentation (months)	7 $\pm$ 3.70	16.4 $\pm$ 18.3	0.580
Average ECG heart rate (bpm) <sup>1</sup>	134 $\pm$ 15.1	120 $\pm$ 29.7	0.138
Clinical signs of cardiac disease at presentation	0	6 (46.2%)	0.046*
Cardiac death at the end of the analysis period/all-cause death	4/4	4/5 <sup>3</sup>	

\* significant difference

<sup>1</sup>ECG not performed on 1 EB and 2 FB

<sup>3</sup>cardiac death includes 2 FB that either died immediately after the procedure (1 dog) and within 1 week of the procedure (1 dog)

bpm, beats per minute; ECG, electrocardiogram; kg, kilograms

Table 2: Bulldogs undergoing specific imaging modalities

<b>Diagnostic Modality</b>	<b>English bulldogs n = 8</b>	<b>French bulldogs n = 13</b>
Echocardiography	8	13
Computed tomography	7	10
Left-sided angiography	2	3
Right-sided angiography	3	12

Table 3: CT valve morphology (mean  $\pm$  SD) in bulldog populations

Parameter	English bulldog	French bulldog	P value
Total number of dogs with imaging modality	7	10	
Ao annulus (cm)	1.5 $\pm$ 0.11	1.13 $\pm$ 0.22	0.002*
PA annulus (cm)	1.25 $\pm$ 0.21	1.04 $\pm$ 0.19	0.035*
Ao:PA ratio	1.22 $\pm$ 0.19	1.09 $\pm$ 0.09	0.239
PA:Ao ratio	0.83 $\pm$ 0.12	0.93 $\pm$ 0.09	0.219
Dogs classified with annular hypoplasia	3	0	

\* significant difference

Ao, aortic; cm, centimeters; PA, pulmonic artery

Table 4: Angiographic valve morphology (mean  $\pm$  SD) in bulldog populations

Parameter	English bulldog	French bulldog	P value
Total number of dogs with imaging modality	3	12	
Ao annulus (cm)	1.41 $\pm$ 0.21	1.18 $\pm$ 0.21	0.1714
PA annulus (cm)	1.18 $\pm$ 0.09	1.09 $\pm$ 0.2	0.5363
Ao:PA ratio	1.21 $\pm$ 0.23	1.09 $\pm$ 0.16	0.3824
PA:Ao ratio	0.85 $\pm$ 0.14	0.93 $\pm$ 0.13	0.4220
Dogs classified with annular hypoplasia	1	3	

Ao, aortic; cm, centimeters; PA, pulmonic artery

Table 5: Comparison of bulldog pulmonic and aortic annulus size (mean  $\pm$  SD)

Parameter	CT	Angio	TTE	TEE	P value
Total number of dogs n = 21	17	15	21	12	
Ao annulus (cm)	1.28 $\pm$ 0.26	1.23 $\pm$ 0.23	1.53 $\pm$ 0.31		0.0024*
PA annulus (cm)	1.13 $\pm$ 0.22	1.11 $\pm$ 0.18	1.06 $\pm$ 0.23	0.93 $\pm$ 0.23	0.0830
Ao:PA ratio	1.14 $\pm$ 0.15	1.11 $\pm$ 0.17	1.46 $\pm$ 0.26		< 0.0001*
PA:Ao ratio	0.89 $\pm$ 0.11	0.92 $\pm$ 0.13	0.70 $\pm$ 0.12		< 0.0001*
Dogs classified with annular hypoplasia	3	4	18		

\* significant difference

Angio, angiography; Ao, aortic; cm, centimeters; CT, computed tomography; PA, pulmonic artery; TEE, transesophageal echocardiography; TTE, transthoracic echocardiography

Table 6: Combined valve morphology and coronary artery anatomy

Parameter	English bulldogs n = 7	French bulldogs n = 13	P value
Subjective valve thickening (yes/total dogs)	Normal: 2 Mild: 4 Moderate: 0 Severe: 1 Unable to interpret: 1	Normal: 0 Mild: 0 Moderate: 2 Severe: 11	
Anomalous coronary artery origin (R2A)	6	0	0.0002*

\* significant difference



Table 7. Descriptive statistics (mean  $\pm$  SD) of all dogs with pulmonic stenosis

Parameter	Bulldog breed	Non-bulldog breed	P value
Total cases identified	34 (18 EB, 16 FB)	145	
Sex	26 males (76.5%)	90 males (62%)	0.1135
Weight (kg) <sup>1</sup>	11.4 $\pm$ 6.6 range: 2.7-35.0	15.2 $\pm$ 12.5 range: 0.47-54.3	0.6165
Age at presentation (months)	13.2 $\pm$ 19.8 range: 2-85	25.5 $\pm$ 35.5 range: 2-291	0.0126*
Pulmonic stenosis severity	Mild: 3 (8.8%) Moderate: 2 (5.9%) Severe: 29 (85.3%)	Mild: 18 (12.4%) Moderate: 18 (12.4%) Severe: 109 (75.2%)	Mild: 0.5582 Moderate: 0.2766 Severe: 0.2062
BV performance	16 (47.1%)	86 (59.3%) <sup>2</sup>	0.1941
Additional congenital cardiac defects	10 dogs (29.4%)	43 dogs (29.7%)	0.9777
Anomalous coronary artery anatomy (diagnosed with definitive imaging)	6 dogs (17.6%)	2 dogs (1.4%)	< 0.0001*

\* significant difference

<sup>1</sup>weight was not recorded for 10 NonBD patients

<sup>2</sup>all cases of dogs with severe PS that had a BV performed; further inclusion criteria was applied to this group for other analyses; refer to materials and methods for clarification

BV, balloon valvuloplasty; EB, English bulldog; FB, French bulldog; kg, kilograms

Table 8. Demographic and balloon catheter measurement (mean  $\pm$  SD) data

Parameter	BD-BV group	NonBD-BV group	P value
Total cases identified	16	44	
Sex	10 male (62.5%)	28 male (63.6%)	0.9356
Weight (kg)	8.73 $\pm$ 3.6 (range 2.6- 20)	7.73 $\pm$ 4.6 (range 2.7-15.2)	0.2263
Age at time of BV (months)	16.3 $\pm$ 20.8	11.6 $\pm$ 11.2	0.8193
ECG heart rate (bpm) <sup>1</sup>	123 $\pm$ 30.1	117 $\pm$ 36.4	0.6054
Clinical signs at presentation	7 (43.8%)	15 (34.1%)	0.6028
Dogs with additional congenital cardiac defects	5 (31.3%)	16 (36.4%)	0.7134
Balloon catheter diameter range (mm)	10- 20	10- 25	
Balloon catheter to PA annulus (TTE derived) ratio	1.42 $\pm$ 0.17 range: 1.22-1.88	1.3 $\pm$ 0.13 range: 1.0-1.5	0.0370*
Balloon catheter to PA annulus (TEE derived) ratio <sup>2</sup>	1.56 $\pm$ 0.16 range: 1.36-1.88	1.36 $\pm$ 0.11 range: 1.2-1.5	< 0.0001*
Absolute difference between paired TTE and TEE PA measurements (cm) <sup>2</sup>	0.14 $\pm$ 0.09 range: 0.02-0.3	0.1 $\pm$ 0.07 range: 0- 0.3	0.1233
Dogs that underwent >1 BV procedure	1	5	
Subjective valve thickening via echocardiography	Mild to moderate: 3 Severe: 13	Mild to moderate: 38 Severe: 6	

\* significant difference

<sup>1</sup> ECG not recorded on 1 BD and 2 NonBD<sup>2</sup> measurement not available for 2 BD and 13 NonBD

bpm, beats per minute; BD-BV, bulldogs that underwent a balloon valvuloplasty procedure; BV, balloon valvuloplasty; cm, centimeters; ECG, electrocardiogram; kg, kilograms, mm, millimeters; NonBD-BV, non-bulldog breed dogs that underwent a balloon valvuloplasty procedure; PA, pulmonic artery; TEE, transesophageal echocardiography; TTE, transthoracic echocardiography

Table 9: Echocardiographic data (mean  $\pm$  SD) in balloon valvuloplasty populations

Parameter	BD-BV group	NonBD-BV group	P value
PA annulus (cm)	1.02 $\pm$ 0.2	1.19 $\pm$ 0.26	0.0231*
Ao annulus (cm)	1.44 $\pm$ 0.29	1.41 $\pm$ 0.36	0.8077
Ao:PA ratio	1.41 $\pm$ 0.2	1.19 $\pm$ 0.17	< 0.0001*
PA:Ao ratio	0.72 $\pm$ 0.1	0.87 $\pm$ 0.12	< 0.0001*
FAC (%) <sup>1</sup>	38.4 $\pm$ 11.2	50.6 $\pm$ 14.9	0.0091*
TV S'(cm/sec) <sup>2</sup>	7.82 $\pm$ 4.19	11.0 $\pm$ 3.68	0.0820
TAPSE (cm) <sup>3</sup>	0.86 $\pm$ 0.32	1.0 $\pm$ 0.29	0.2249
LV FS (%)	35.9 $\pm$ 12.7	42.2 $\pm$ 13.9	0.2566
RA Max diameter (cm) <sup>4</sup>	2.88 $\pm$ 0.88	2.28 $\pm$ 0.71	0.0129*
Short axis RVPWd (cm)	1.17 $\pm$ 0.27	1.21 $\pm$ 0.4	0.9045
Long axis RVPWd (cm)	1.19 $\pm$ 0.3	1.22 $\pm$ 0.37	0.8653
Short axis RVDd (cm)	1.11 $\pm$ 0.54	0.93 $\pm$ 0.43	0.2602
Long axis RVDd (cm)	1.4 $\pm$ 0.66	1.03 $\pm$ 0.48	0.0248*
IVSd (cm)	0.97 $\pm$ 0.26	0.83 $\pm$ 0.03	0.0270*
IVSs (cm)	1.03 $\pm$ 0.3	0.96 $\pm$ 0.28	0.262
Short axis RVPWs (cm)	1.49 $\pm$ 0.31	1.51 $\pm$ 0.42	0.8783
Long axis RVPWs (cm)	1.46 $\pm$ 0.3	1.46 $\pm$ 0.38	0.9794
Short axis RVDs (cm)	0.71 $\pm$ 0.4	0.66 $\pm$ 0.43	0.3952
Long axis RVDs (cm)	0.94 $\pm$ 0.54	0.68 $\pm$ 0.41	0.0303*

\* significant difference

<sup>1</sup>measurement data not available for 22 NonBD<sup>2</sup>measurement data not available for 8BD and 31 NonBD<sup>3</sup>measurement data not available for 7BD and 14 NonBD<sup>4</sup>measurement data not available for 2BD and 2 NonBD

Ao, aortic; BD-BV, bulldogs that underwent a balloon valvuloplasty procedure; cm, centimeters; FAC, percent fractional area change; IVSd, interventricular septal diastolic dimension; IVSs, interventricular septal systolic dimension; NonBD-BV, non-bulldog breed dogs that underwent a balloon valvuloplasty procedure; LV FS, left ventricular fractional shortening; PA, pulmonic artery; RA Max, right atrial maximum dimension; RVDd, right ventricular diastolic chamber dimension; RVDs, right ventricular systolic chamber dimension; RVPWd, right ventricular posterior wall diastolic dimension; RVPWs, right ventricular posterior wall systolic dimension, TAPSE, M-mode derived tricuspid annular plane systolic excursion; TV S', tissue Doppler derived systolic myocardial velocity

Table 10: Pressure gradients (mean  $\pm$  SD) in the bulldog and non-bulldog groups

Parameter	BD-BV group	NonBD-BV group	P value
Pre BV max PG (mmHg)	143 $\pm$ 40.3	158 $\pm$ 55.6	0.5801
Invasive RV PG (mmHg) <sup>1</sup>	120 $\pm$ 62.4	110 $\pm$ 60.5	0.7663
24-hour post BV PG (mmHg) <sup>2</sup>	81.4 $\pm$ 34.3	66 $\pm$ 31.2	0.0991
1-month post BV PG (mmHg) <sup>3</sup>	86 $\pm$ 29.9	54.4 $\pm$ 22.6	0.0005*
Last follow-up PG (mmHg) <sup>4</sup>	73.8 $\pm$ 23.6 follow-up range: 9-46 months post BV	74.6 $\pm$ 45.8 follow-up range: 7-97 months post BV	0.8201
Mean PG decrease from pre-BV to 1 month post BV <sup>5</sup>	40.5% $\pm$ 26.2%	55.6% $\pm$ 21.8%	0.0647
Dogs meeting criteria for success 1-month post BV (yes/total dogs)	4/12 (33.3%)	31/42 (73.8%)	0.0096*
Dogs meeting criteria for success at last follow up >1 month post BV (yes/total dogs)	2/6 (33.3%)	24/34 (70.6%)	0.0169*
Dogs that died in the hospital following BV or within 1 week of discharge	2 (12.5%)	2 (4.5%)	

\* significant difference

<sup>1</sup> measurement data not available for 1 BD and 19 NonBD

<sup>2</sup> measurement data not available for 2 BD and 2 NonBD

<sup>3</sup> measurement data not available for 5 BD and 7 NonBD

<sup>4</sup> measurement data not available for 10 BD and 14 NonBD

<sup>5</sup> measurement data not available for 4 BD and 3 NonBD

BD-BV, bulldogs that underwent a balloon valvuloplasty procedure; BV, balloon valvuloplasty; mmHg, millimeters of mercury; NonBD-BV, non-bulldog breed dogs that underwent a balloon valvuloplasty procedure; PG, pressure gradient; RV, right ventricle

## **Chapter 4**

### **Discussion**

#### *Aim 1: Comparison of French and English bulldog populations*

There was an increased male sex predisposition in the bulldog cases diagnosed with PS at the OSU VTH (87.5% of EB and 76.9% of FB). This is higher than the reported incidence in most published studies,<sup>2, 26</sup> although it more closely matches the increased male predisposition seen in at least one other report.<sup>24</sup> There was an increase in clinical signs reported in the FB group when compared to the EB group, which could reflect potential differences in the severity of the trans-pulmonic valve PG, PS morphology, presence of concurrent congenital defects, the mild trend toward an older age at presentation of the FB group, RV function, behavioral characteristics between the breeds, or be related to the small sample size. A R2A coronary artery anomaly was observed in 6 of the 8 EB that were evaluated with definitive coronary artery imaging, all of which were male. This provides further evidence supporting a sex-linked heritable cause of this coronary artery anomaly in this breed. Published case reports<sup>1, 79</sup> on coronary artery anomalies in 7 EB have shown that 5 were male. A R2A coronary artery was diagnosed in 1 mixed breed dog in the general population of dogs imaged at the OSU VTH. No coronary artery anomalies were observed in the population of FB seen at the OSU VTH, suggesting that the risk of this specific anomalous coronary artery anatomy may not be higher in FB than the general population of dogs with PS. The increased prevalence of the R2A anomaly in the EB provides further support that definitive imaging of the coronary vasculature is important in the pre-procedural evaluation.

CT imaging of the coronary vasculature was found to be feasible in all dogs providing rapid, definitive, safe, and minimally invasive evaluation. One of the EB was diagnosed with

concurrent pneumonia based on CT images, underwent bronchoscopy under the same general anesthetic procedure, recovered with oxygen support, and was discharged the following day. In one case of CT imaging in a FB, the BV procedure was performed immediately after the CT imaging scan under the same general anesthetic event without complications. Generally, the preference of all clinicians was to perform the CT at least 24 hours prior to BV procedures to decrease amount of contrast medium administered in a short period of time as well as to decrease the duration of anesthesia. General anesthesia was performed for all of the studies to facilitate the breath-hold maneuver used to minimize respiratory motion, but there is a potential that diagnostic quality images of the coronary artery anatomy could be performed with sedation rather than general anesthesia (unpublished data). Potential drawbacks of CT imaging are related to increased risk of anesthetic complications, contrast administration, as well as the added cost of the CT scan. Angiocardiography, specifically of the left-sided of the heart also carries many of the same risks. When owners were provided with the choice between a left-sided angiogram requiring an incision for arterial vascular access versus CT imaging, all owners elected CT imaging; therefore, not all of the BD underwent all of the imaging modalities. Left-sided angiograms were more commonly performed prior to acquisition of the current CT equipment at the OSU VTH.

Of the EB with PS evaluated at the OSU VTH, more were diagnosed with a R2A anomaly than were diagnosed with normal coronary artery vasculature. This could reflect the tertiary referral nature of the OSU VTH with many veterinary cardiologists referring EB patients for definitive CT coronary imaging in cases of PS. Alternatively, the prevalence of severe PS with a concurrent R2A coronary artery anatomy in EB could provide further evidence supporting the etiological hypothesis that the course of the anomalous circumpulmonary coronary artery

creates embryologic constriction of the RV outflow tract and secondary valvular PS. Interestingly, the dogs diagnosed with the R2A coronary artery anomaly were asymptomatic on presentation, but the majority developed significant clinical signs of right-sided cardiac disease at early ages. Follow-up on 5 of the EB showed that 4 had been euthanized due to refractory R-CHF at a mean age of 26.5 months (range 8-36 months). The 1 EB alive at the end of the study period was 27 months old and asymptomatic for significant cardiac disease. The 2 EB without a R2A anomaly underwent BV procedures with normal balloon diameter to pulmonary annulus ratios (1.2-1.5) and did well during the procedure with promising short-term results (both currently remain asymptomatic 6 and 18 months post-BV procedures). Conservative BV procedures in cases with a R2A anomaly were discussed with all owners, but none were performed. Based upon the increased development of clinical signs and reduced survival identified in this subset of patients, conservative BV procedures, as has been reported in veterinary literature<sup>79, 83</sup> may be more strongly considered. Ultimately, further research is needed to determine the ideal case management in this population of patients.

There was a significant difference observed between the valve morphology in the EB and the FB with PS. The FB population tended to have more markedly dysplastic valve leaflets when compared to the EB population with no or mild evidence of commissural fusion. The EB tended to have more evidence of annular hypoplasia than the FB. However, the classification of annular hypoplasia differed based on the imaging modality with more FB classified with annular hypoplasia with angiographic measurements than with CT measurements. There is no current gold standard identified for annulus measurements in veterinary medicine, but with the increased resolution and post-processing capabilities of cardiac-gated CT, it may provide more accurate measurement of annulus dimensions than echocardiography and angiography. Potential reasons

for the discrepancy in measurements and classification could reflect increased measurement error with the dysplastic valve morphology due to increased difficulty visualizing the location of the pulmonic valve leaflet hinge points, small sample size, or the potential that many dogs were borderline for annular hypoplasia. When the BD population was compared to the NonBD population, there was a significant difference in the degree of annular hypoplasia seen with the BD patients. There were also statistically significant differences in the mean aortic valve and pulmonic valve annulus measurements observed with CT imaging which might reflect body conformation/patient size.

*Aim 2: Comparison of bulldog and non-bulldog populations*

When evaluating the entire population of dogs with PS that presented to the OSU VTH, the BD group was evaluated at an earlier age with no significant differences in the observed PS severity or the presence of concurrent congenital cardiac defects. The presence and distribution of concurrent congenital cardiac defects was similar to the published veterinary literature.<sup>2, 22, 45</sup> There was a significant difference in the diagnosis of anomalous coronary artery anatomy between the groups, which might reflect case selection bias and/or increased incidence in the EB breed. Evaluation of the breeds that were diagnosed with PS at the OSU VTH revealed larger numbers of pit bulls and Chihuahuas as compared to other veterinary reports, which might reflect geographical differences not reported in other cases series.

There was a difference in the subjective analysis of pulmonic valve leaflet thickening observed in the BD group when compared to the NonBD group consistent with previously published literature on PS morphology.<sup>2, 24</sup> In people, the dysplastic morphology is most commonly seen as a component of syndromes that affect other connective tissue such as



Noonan's, cardiofaciocutaneous, Leopard, and Costello syndromes.<sup>95</sup> Many of the syndromes that present with dysplastic PS morphology involve genetic defects impacting the mitogen activated protein kinase (RAS-MAPK) pathway, with genetic mutations in genes such as PTPN11. The characteristic phenotype seen with Noonan's syndrome often includes a short stature, abnormal chest conformation, broad or webbed neck, developmental delays, and congenital cardiac disease with PS commonly observed. Very mild cases do not impact reproduction or lifespan in affected individuals. A prior veterinary report<sup>2</sup> has also speculated that the phenotypic appearance that has been selectively bred for in the BD may carry with it a genetic predisposition to abnormalities in the RAS-MAPK pathway and a higher genetic predisposition to PS with a dysplastic morphology. Further genetic analysis is needed to evaluate this potential hypothesis in the BD breeds.

Overall there was no significant difference in average heart rate observed on presentation ECG recordings. Interestingly, Mobitz type 1 second degree AV block was observed more commonly in the BD group, potentially reflecting increased vagal tone in BD breeds. Anecdotally, this was also a more common finding with overnight telemetry monitoring performed post-BV procedures. RV enlargement ECG patterns were also more commonly observed in the BD group and criteria for RA enlargement, while rare overall, was more commonly seen in the NonBD breeds.

Atenolol was variably administered with many cases in both groups receiving this medication prior to initial presentation, prior to BV procedure, or post BV. The use of atenolol may have influenced some of the echocardiographic measurements. The lack of standardization with atenolol dosing made direct evaluation of its effects on procedural success and survival not

possible as part of this study. Dogs that presented with signs of R-CHF were treated with diuretic medications and ACE inhibitors.

Routine transthoracic echocardiography revealed a significant difference in pulmonic annulus measurements, with the overall population mean for PA:Ao and Ao:PA ratios in the BD group fitting with a diagnosis of annular hypoplasia. Systolic RV measurements were performed and compared between the groups and the FAC was significantly lower in the BD group. The published 95% confidence interval range for FAC in a population of normal dogs was 45.04-47.96%, with a range of 32.83- 62.25%.<sup>71</sup> FAC values less than 35% are considered abnormal in people.<sup>72</sup> The increased FAC of  $50.6 \pm 14.9\%$  in the NonBD group might reflect loading conditions, use of beta-blocker medications leading to increased diastolic filling (preload), or reflect hyperdynamic systolic function in this population of dogs. The mean FAC of  $38.4 \pm 11.2\%$  in the BD group was at the low end of the normal range.<sup>71</sup> A statistically significant difference was not observed with the other measured markers of RV systolic performance (TAPSE and TV S'), although there was a trend with lower mean TAPSE and S' measurements in the BD group. FAC was more easily measured in this retrospective data set as the required images were more routinely acquired in the echocardiographic studies than TAPSE and TV S'. Images required for the measurement of TAPSE and TV S' were not recorded in all cases while RV dimensions could be traced on the majority of left apical images obtained on the retrospective data sets. The published 95% confidence interval range for TAPSE measurements in normal dogs was 11.40- 15.53mm, with a range of 8.53- 25.0mm.<sup>71</sup> TAPSE values less than 16mm are considered abnormal in people.<sup>72</sup> The mean TAPSE measurements in the BD group tended to be lower than in the NonBD group, and the mean TAPSE measurement was lower in both groups when compared to the 95% confidence interval reported for normal dogs. Also

fitting this observed trend was the mean TV S' measurement in the BD group, which was lower than in the NonBD group. The published 95% confidence interval range for TV S' measurements in a population of normal dogs was 12.54- 14.31cm/sec, with a range of 6.83- 26.13cm/sec.<sup>71</sup> TV S' values less than 15cm/sec are considered abnormal in people.<sup>72</sup> The mean TV S' values in both groups of dogs were lower than the 95% confidence interval reported for the normal population of dogs.

Overall, the fewer number of dogs with measurements of TAPSE and TV S' may have resulted in the lack of significant differences between the groups. The study in normal dogs with reference values revealed a significant influence of body weight in the measurement parameters as well as a moderate positive correlation of FAC with heart rate.<sup>71</sup> Allometric scaling of the RV systolic measurements in this study was not performed. Overall there was a trend for decreased systolic function in the dogs with severe PS, but many of these dogs were within the reported normal reference ranges, potentially reflecting the differing ages, PS severity seen within the groups, influence of cardiac medications, as well as concurrent cardiac defects.

There was a significantly different ( $P = 0.0129$ ) mean RA maximum chamber dimension measured from the right parasternal 4 chamber long axis imaging window between the groups with the BD-BV group measuring 2.88cm and the NonBD-BV group measuring 2.28cm. This could reflect more severe dysfunction and remodeling in the BD-BV group or could reflect the influence of a few dogs in the BD-BV group that presented with evidence of R-CHF. There was no significant difference in mean LV systolic function as assessed solely by LV fractional shortening, which was within normal reference ranges. A single linear measurement of LV

systolic function is a significant oversimplification and more definitive assessment of the RV impact on LV systolic function was not a primary aim of this study.

There was a significant difference observed in some of the measurements of RV wall thickness and chamber dimensions. Measured values of wall thickness and chamber dimensions were different based on the imaging window and view (long-axis versus short-axis). There was a significant difference in the diastolic diameter of the interventricular septum as well as a trend toward differences in systolic dimensions of the interventricular septum. These changes to the interventricular septum provide potential evidence of increased hypertrophy in the BD group. The interventricular septal margins were the most easily identified and measured in all dogs, likely reflecting the ability to visualize complete margins in standard echocardiographic studies.<sup>90</sup> There were significant differences in the long axis diastolic and systolic chamber dimensions between the groups with the BD group having evidence of increased chamber dimensions in both phases of the cardiac cycle. This may reflect increased preload and decreased systolic function in the BD groups. The feasibility to obtain a well delineated RV free wall margin on the right parasternal imaging windows was particularly challenging and could be reflected in the variability seen with the measurement ranges. Unfortunately in veterinary medicine there are no standardized guidelines for where RV measurements should be performed. This highlights the need for prospective consideration of right chamber imaging and data collection at the time of the echocardiographic study if variables of systolic function, chamber dimensions, and wall thickness measurements are desired. Considerable concentric hypertrophy occurs in cases of severe PS, which leads to impairment of diastolic function. Indices of diastolic function were not evaluated in this study. Assessment of RV diastolic function in normal and dogs with PS would be a future direction to pursue.

There was a significant difference in the reported balloon catheter diameter to pulmonic valve annulus ratio with increased “oversizing” observed in the BD group as compared to the NonBD group. This likely reflects the increased tendency for annular hypoplasia in the BD group as well as measurement variability in the groups. The barrel-chested BD conformation as well as respiratory artifact can make obtaining echocardiographic images challenging in individual patients and visualizing the pulmonary leaflet hinge points was also difficult in the cases with valvular dysplasia/type B morphology.

Transesophageal echocardiography is frequently performed during general anesthesia prior to BV at the OSU VTH and pulmonic annular measurements are now routinely collected and compared to the pre-procedural transthoracic echocardiographic and intra-operative angiographic measurements to guide appropriate balloon catheter diameter selection. There was no significant difference between the groups when paired measurements performed in the same dog were compared with a mean difference of approximately 1.0 to 1.4 mm between imaging modalities. Overall the difference between the measurements was not significant enough to impact the selection of balloon catheter size. At the OSU VTH preference is given to the imaging modality that most clearly delineates the pulmonic annulus with the angiographic diameter often used as the gold standard.

### *Aim 3: Balloon valvuloplasty procedural success*

No significant difference was observed with the mean pre-BV trans-pulmonic PG, invasive RV pressure measurement, or 24-hour post BV PG between the BD-BV and NonBD-BV groups. The mean one-month trans-pulmonic PG was significantly different with the BD group having a higher residual gradient (i.e. severe category) than the NonBD group (i.e. at the

low end of the moderate category). There was an overall trend for the PG to increase from the 24-hour measurement to the one-month measurement in the BD group as well as a decrease observed in the 24-hour gradient to the one-month measurement in the NonBD group. The continued decrease in the NonBD group may reflect greater damage to the pulmonic leaflets during BV in dogs with more commissural fusion and subsequent edema and hemorrhage within the tissue that resolved over the following weeks.<sup>69</sup> The goal of balloon catheter inflation during the BV procedure is to break down the level of stenosis and as such the continued decrease in the PG may reflect a more successful inflation and treatment of the stenosis in dogs with commissural fusion and less leaflet thickening. Using objective measurements to determine procedural success (e.g. decrease in trans-pulmonic PG by 50% from pre-procedural measurements and/or reduction in the PG to < 64mmHg), NonBD (31/42 dogs, 73.8%) were significantly more likely to meet the criteria than BD (4/12 dogs, 33.3%). Smaller numbers of dogs were available for follow-up (range in NonBD-BV of 13- 162 months and range in BD-BV of 6- 35 months), but the overall percentage of success was relatively unchanged between the groups at the last reported follow up in the individual patients.

One FB that underwent a second BV procedure and 5 NonBD dogs that underwent more than 1 BV procedure (1 NonBD had 3 BV procedures: a miniature schnauzer diagnosed with a large perimembranous bi-directional shunting VSD, alive with minimal clinical signs 101 months post initial BV procedure). In all cases, the reason for performing a second BV procedure was due to continued evidence of severe PS with trans-pulmonic PG above 100mmHg. In cases with multiple BV procedures, success was only observed in 1 of the 6 patients based on objective classification parameters and in this patient the trans-pulmonic PG remained slightly over 100mmHg. Although this was a very small group of patients, there was not a substantial benefit

observed with multiple BV procedures when using objective data as a success benchmark. However, in all cases the trans-pulmonic PG did see a decrease with subsequent procedures, so the overall benefit is uncertain.

Balloon catheter inflations were performed successfully in all patients, although the initial procedure was aborted prior to balloon inflation in 2 NonBD cases due to malignant ventricular arrhythmias and a second attempt was performed successfully in both dogs at a later time. There was a single death observed in the time period of the BV procedure (a FB that underwent cardiopulmonary arrest and died during recovery from general anesthesia). The overall procedural mortality in the dogs that underwent BV at the OSU VTH was 1.7%, with the procedural mortality in the BD group being 6.3%, since the dog that died was a FB. The overall case numbers seen at the OSU VTH with clinical signs of R-CHF prior to BV procedure is very low (2 BD and 2 NonBD), so more investigation with larger numbers of cases is necessary to determine if interventional procedures provide a survival benefit. The data obtained in this study continues to support the recommendation for performance of BV procedures prior to the onset of clinical signs in NonBD-BV dogs.

### *Aim 3: Bulldog and non-bulldog survival*

Four populations of dogs diagnosed with severe PS were used for further survival analysis of the groups: the BD group with BV procedures, the BD group without BV procedures, the NonBD group with BV procedures, and the NonBD group without BV procedures. When the four populations were compared all together, statistically significant differences in all-cause mortality and cardiac mortality were observed: there was a survival benefit observed in NonBD breeds that underwent BV procedures compared to NonBD that did not undergo BV procedures

consistent with previous veterinary reports.<sup>2</sup> When all of the BD and NonBD cases were combined into 2 populations (those that had BV performed and those that did not), there was no difference in all-cause mortality, but a significant difference in cardiac mortality providing additional evidence that BV procedures reduce cardiac dysfunction in dogs with severe PS. There was no significant difference observed between all-cause mortality and cardiac mortality when the dogs (BD versus NonBD) that did not undergo BV procedures were compared.

No significant difference was observed between the BD that did or did not undergo BV procedures. When the BV populations were evaluated (BD compared to NonBD) there was no significant difference in all-cause mortality, but there was a significantly higher difference in cardiac mortality with the BD breeds more likely to die from cardiac causes. These results provide evidence that BV procedures were less successful in this BD population with little survival benefit. This surprising result may also reflect the small sample size in the BD groups. There was a significant number of dogs that were censored because they were still alive and clinically doing well in the BD-BV population. Continued follow up in these populations as well as further case recruitment is needed to fully evaluate these results and determine the best treatment option for the bulldog population with severe PS.

Study limitations include the retrospective nature of this study, small and unequal populations sizes when comparing the BD cases, variable imaging modalities used with the BD breeds to diagnose coronary artery anatomy, lack of inter-observer variability and normal control group for the echocardiographic measurements, qualitative classification of leaflet morphology rather than a quantitative measurement, non-standardized use of cardiac medications in the patient populations, and variable length of patient follow-up.



## **Chapter 5**

### **Conclusions**

In the population of French and English bulldogs presenting to the OSU VTH, there was an increased predisposition to markedly dysplastic pulmonary valves and a majority of cases displaying normal to mild annular hypoplasia. This was different than the NonBD populations that were diagnosed with and treated for severe PS. None of the French bulldogs that underwent definitive coronary artery imaging had evidence of coronary artery anomalies, but there were 6 out of 8 cases of EB with PS that were definitively diagnosed with a R2A coronary artery anomaly. The dogs with a R2A anomaly did not undergo BV procedures and had a poor mean long-term prognosis of 26 months. There was a significant difference observed between objective classification of procedural success at the 1-month post-BV echocardiographic recheck as well as with more long-term follow up between the BD and NonBD breeds. This might reflect that BV procedures performed with current balloon catheters or percutaneous BV procedures in general are not the ideal method for treatment of severe PS in the BD breeds. RV systolic function as assessed by FAC was decreased in the BD breeds when compared to the NonBD breeds and may either be a cause or a reflection of the decreased procedural success in the BD breeds.

<sup>a</sup>Mac 5500 HD, GE Healthcare, Wauwatosa, WI, USA.

<sup>b</sup>Vivid 7, General Electric Medical Systems, Waukesha, WI, USA.

<sup>c</sup>iE33, Philips, Philips Healthcare, Andover, MA, USA.

<sup>d</sup>Toshiba America Medical Systems, Inc., Tustin, CA, USA.

<sup>e</sup>Isovue 370, Bracco Diagnostics, Princeton, NJ, USA.

<sup>f</sup>eFilm, version 3.4, Merge Healthcare, Heartland, WI, USA.

<sup>g</sup>Vitreia Workstation, software version 6.3.2, Vital Image, Inc., Minnetonka, MN, USA.

<sup>h</sup>Prism 6, Graph Pad Software Inc., San Diego, CA, USA.

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