UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

A RETROSPECTIVE REVIEW OF AORTIC STENOSIS IN 274 DOGS IN SCOTLAND: CLINICAL PRESENTATION AND DIAGNOSIS.

ALICE CRISTINA VICTORINO MACHADO

CONSTITUIÇÃO DO JÚRI

Doutor José Paulo Pacheco Sales Luís

Doutor José Henrique Duarte Correia

Doutor José Manuel Antunes Ferreira da Silva

ORIENTADOR

Dr. Mark Bayliss

CO-ORIENTADOR

Doutor José Henrique Duarte Correia

2018

LISBOA

UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

A RETROSPECTIVE REVIEW OF AORTIC STENOSIS IN 274 DOGS IN SCOTLAND: CLINICAL PRESENTATION AND DIAGNOSIS.

ALICE CRISTINA VICTORINO MACHADO

DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

CONSTITUIÇÃO DO JÚRI

Doutor José Paulo Pacheco Sales Luís

Doutor José Henrique Duarte Correia

Doutor José Manuel Antunes Ferreira da Silva

ORIENTADOR

Dr. Mark Bayliss

CO-ORIENTADOR

Doutor José Henrique Duarte Correia

2018

LISBOA

A Francisco Victorino, que não partiu inteiramente, cuja lembrança me conduz pelo caminho do coração e cujo exemplo me eleva acima de todas as tribulações.

Ao Kaiser, por ter sido o melhor amigo durante 16 anos, por ter ouvido, percebido e acarinhado como só um cão consegue.

Aos meus pais, Filipe e Ni, por tudo.

Acknowledgements

Firstly, I want to thank Craig Devine for leading the way in helping me choose this theme. You have given me the tools, methods, and confidence to succeed. You have helped me find the ability to create. Thank you for the work and time you dedicated to this study. Thank you for teaching me and giving the best answers to my questions. Your help has been precious, and without it, this thesis would not exist.

I am grateful to all at Ardene House Vets for helping me become a (better) veterinarian. I want to thank Mark Bayliss, my traineeship supervisor; Alison Cheshire, Anne, Lili, Alison Clarke, Chantal, Ian, Fergus, Marcel, Malcolm, and the nursing team. I will treasure all that you have taught me, and of course, our morning cups of tea. Thank you for this wonderful experience.

I want to thank Val for making me feel at home in Scotland, for her friendship and kind advice.

I am also grateful for the help of Professor José Correia, for the knowledge that he passed onto me as a teacher, for his advice, for the insight he has given me as a supervisor and for the support in the redaction of this dissertation. Thank you for making me a better student and vet.

A special thank you to Dr. Telmo Nunes, for his support in the statistical analysis of this study.

On a personal note, I want to thank my dad and mom, Filipe and Ni, who have fought bravely and steadily so their children could be the best version of themselves. Your love has never failed me; it has raised me to be kind, passionate and dedicated. From my child years, I can distinctly remember my parents helping me with homework, taking interest in my achievements and, ultimately, they have given me this opportunity. There was not one single moment in my life when I could not count on you, I would be lost without your nurturing and guidance.

There is a special place in my heart for my friends who started this journey with me in the Azores Islands and have stood by my side ever since. I am proud to say no one knows me better than them. I feel blessed that we have such a unique bond. I would be discouraged without their example and backbone support. In particular, I want to thank Costa and Tony who taught me what true strength does not exist without weakness or without each other.

I am so thankful to Ivo who is the living proof that love conquers all. Thank you, for the sacrifice so that I can live up to my dreams. You are my cornerstone, flexible and resilient. Thank you for choosing to come with me on this adventure, for growing and changing with me, for your affection, and patience. For staying awake while I was finishing this dissertation.

I want to thank my avô Chico, I wish you could be here to see this. I want to thank my auntie, and cousin, Micas and Vitor, for the support and love; my brother, Alex, for being a role model.

Last but not least, I am thankful to my dog, my cat; and the remaining family, friends, and teachers who have been an essential part of this journey. You have given me roots, wings and a deep sense of fulfilment. Thank you for being where my heart is.

Abstract

In the dog, aortic stenosis (AS) is classified as a congenital heart defect; however, its nature is progressive and some authors believe it to be acquired. In reviews, it has been reported as the most common congenital cardiac defect in Europe. Anatomically, it is classified as sub-valvular, valvular or supra-valvular. Boxers, Newfoundland dogs, Golden Retrievers, German Shepherds, are among the breeds considered to be predisposed to some forms of this defect. The present retrospective review encompasses 274 dogs with aortic stenosis, examined and diagnosed by a mobile cardiology referral practice in Scotland in a period of 11 years. Diagnostic classification, signalment, clinical, electrocardiographic, and echocardiographic features were obtained from records. A statistical analysis was performed in order to characterize the sample and to assess relationships between variables. The majority of the dogs in the sample were Boxers (40.51%), followed by Golden Retrievers (12.77%), Labrador Retrievers (6.20%) and German Shepherd Dogs (4.74%). Contrarily to current information, the anatomical classification of AS in this study was mostly valvular (47.08%), followed by sub-valvular fixed lesions (30.66%). There were 40.51% dogs with clinical signs at presentation, the most frequent motive for consultation was a recently detected heart murmur (46.72%), and the most common clinical sign at presentation was intolerance to exercise (27.44%), followed by syncope (24.45%). There were slightly more males (56.57%) than females in the sample and a relationship between haemodynamic severity and gender was established, as males were more severely affected than females. The mean age at presentation was three years, and the age of dogs in the sample ranged from one month to 14 years of age. Although it is a congenital defect, its progressive nature could contribute to this age distribution. Most dogs had murmurs on auscultation and murmur intensity was associated with haemodynamic severity. The presence of echocardiographic findings, such as post-stenotic dilation (2.55%), concentric left ventricular hypertrophy (23.44%), aortic valve leaflet thickening (39.11%), and aortic regurgitation (63.14%) were related with severity. The presence of concurrent heart disease was common in the sample. Concomitant congenital defects found were pulmonic stenosis (4.74%), tricuspid valve dysplasia (17.42%) and mitral valve dysplasia (40.15%). Finally, we emphasize that a scientific consensus for diagnosis guidelines of aortic stenosis is much warranted.

Keywords: Congenital heart defects, aortic stenosis, diagnosis, echocardiography, dog.

Resumo

No cão, a estenose aórtica (EA) é classificada como um defeito cardíaco de origem congénita; contudo a sua natureza é progressiva e alguns autores consideram-na uma doença adquirida. Na Europa, é o defeito cardíaco congénito mais frequente. Quanto à localização anatómica das lesões, a EA é classificada como sub-valvular, valvular or supra-valvular. Cães das raças Boxer, Terranova, Golden Retriever e Pastor Alemão estão entre as que são consideradas como predispostas a alguns tipos espefícicos desta malformação.

Este estudo retrospectivo incluiu 274 cães com estenose aórtica, examinados e diagnosticados por uma unidade móvel de referência em cardiologia na Escócia, durante um período de 11 anos. Variáveis como classificação em termos de diagnóstico, identificação do paciente, sinais clínicos, electrocardiográficos e ecocardigráficos foram obtidos a partir de relatórios. A análise estatística foi realizada com o fim de caracterizar a amostra e avaliar a relação entre variáveis. A maioria dos cães da amostra eram de raça Boxer (40.51%), Golden Retriever (12.77%), Labrador (6.20%) e Pastor Alemão (4.74%). Em contraste com dados de outros estudos, em termos de classificação anatómica de lesões, a EA valvular representou a maioria dos casos (47.08%), enquanto que a estenose subvalvular fixa apenas foi representada por 30.66% dos casos. Em termos da apresentação clínica, 40.51% dos cães apresentavam sinais, sendo que o mais frequente foi intolerância ao exercício (27.44%), seguido por síncope (24.45%). O estímulo iatrotrópico mais frequente foi a detecção de sopros em clínicas de primeira opinião (46.72%). A amostra apresenta um número ligeiramente maior de machos (56.57%) em relação a fêmeas, e uma relação entre o estadio da doença e o género foi estabelecida. A média da idade à apresentação foi de três anos, e neste contexto, a idade da amostra variou entre um mês e 14 anos. Embora se trate de um defeito congénito, a sua natureza progressiva pode justificar esta distribuição etária.

A maioria dos cães apresentou um sopro à ausculatação, o qual foi relacionado com a estadio da doença. A presença de achados ecocardiográficos como a dilatação pós-estenótica (2.55%), hipertrofia concêntrica do ventrículo esquerdo (23.44%), espessamento dos folhetos valvulares da válvula aórtica (39.11%) e regurgitação aótica (63.14%) apresentaram também uma relação com o estadio da doença.

A presença de doença concomintante na amostra foi frequente. A frequência de defeitos cardíacos congénitos associados na amostra por ordem crescente foi: estenose pulmonar (4.74%), displasia da válvula tricúspide (17.52%) e displasia da válvula mitral (40.15%). Por fim, enfatizamos que um novo consenso científico para directrizes de diagnóstico é relevante e necessário.

Palavras chave: defeitos cardíacos congénitos, estenose aórtica, diagnóstico, ecocardiografia, cão.

Table of Contents

Index of figures

Index of tables

Index of charts

List of abbreviations and symbols

AoSA- Aortoseptal angle

AS – Aortic stenosis

AVLT - Aortic valve leaflet thickening

BAV– Bicuspid aortic valve

CHD – Congenital heart disease

CHF – Congestive heart failure

CLVH – Concentric left ventricular hypertrophy

ECG – Echocardiogram

EKG – Electrocardiogram

EKG – Electrocardiogram

EOAi – Effective Orifice Area Index

LVOT – Left ventricular outflow tract

LVOT:Ao – Left ventricular outflow tract to aortic orifice

LVOTO – Left ventricular outflow tract obstruction

LVOTV – Left ventricular outflow tract velocity

LVOTVmax – Peak left ventricular outflow tract velocity

MMVD – Myxomatous mitral valve degeneration

MVD – Mitral valve dysplasia

NFD – Newfoundland dog

OR – Odds ratio

PDA – Patent ductus arteriosus

PG - Pressure gradient

PS – Pulmonic stenosis

PTSD – Post-stenotic dilation

SubAS – Sub-valvular aortic stenosis

SupAS- Supra-valvular aortic stenosis

TGF-β – Transforming growth factor beta

VAS – Valvular aortic stenosis

VSD – Ventricular septal defect

Brief Description of the Traineeship

The Ardene House Veterinary Practice

The Ardene House is a mixed veterinary practice situated in Aberdeen, Northeast of Scotland. The origins of the practice can be traced back to 1943 when it was mostly dedicated to the care of horses. Currently, the practice consists of a branch at Bridge of Don, a suburb of Aberdeen situated north of the city centre, and a veterinary hospital at Kingswells, to the east of the city centre, the home to farm and equine veterinarians, in addition to featuring small animal facilities.

Whilst the Bridge of Don offers companion animal care to the local community, it houses fully functional and equipped premises to support most routine interventions and consultations (including the access to basic laboratorial analysis and a substantial pharmaceutical inventory), in addition to an appropriate structure for planning surgical procedures and specialist referrals in Kingswells, receiving the respective post-operative patient follow ups.

The branch in Kingswells is Aberdeenshire's only small animal hospital standard veterinary practice as regulated by the RCVS. The facilities allow veterinary surgeons to carry out medical and surgical care, in-house laboratory diagnostics, digital radiography, ultrasonography, and video-endoscopy. The hospital hosts two extensive surgical operating theatres, a dental suite, a pharmacy, separate intensive care and isolation units, and two individual cattery and kennel wards for hospitalization.

Every department has its own team of vets, nurses and support staff. Presently there are 19 vets and 10 nurses working with Ardene House, in addition to nurses in training from colleges throughout Scotland. The practice hosts a specialist referral service in cardiology, pain management, dermatology, ophthalmology and animal behavioural therapy.

The out of hours' service is provided by Vets Now, ensuring the presence of a specialist veterinarian and a nurse responsible for the in-patients, emergency and critical care.

Curricular traineeship

The curricular internship as part of the 11th semester of the integrated master's degree in Veterinary Medicine was based at the Kingswells Animal Hospital and occurred in the period between October 17, 2016 and February 17, 2017. It consisted of clinical rotations between consults, surgical and non-surgical fields. It was undertaken under the supervision of Mark Bayliss BVMS MRCVS, with a cumulative duration of 506 hours.

My schedule consisted of a Monday to Friday working week, from 08:00 a.m. to 4:00 p.m. In most days, I was involved in surgical and non-surgical procedures (requiring general

anaesthesia) in the mornings, whereas the afternoons were mainly concerned with consultations, follow-ups, and care to hospitalized patients. On a weekly basis, I had the opportunity of seeing practice under specialist care in pain management, where I was exposed to cases of refractory pain, rehabilitation after trauma, chronic and neurologic pain management, where the use of acupuncture and pharmacological treatment was used in combination. I was briefly engaged in ophthalmology appointments, mainly concerning the screening of hereditary eye disease in dogs, and dermatology.

Main activity

Primarily in consults, I met with clients and proceeded with a systematic observation and physical examination of the patient. This first contact with the patient included the anamnesis, approaching and restraining the patient, making initial observations of general appearance, body condition, posture, gait and hydration status; evaluating vital signs (temperature, heart rate, pulse quality and respiratory rate), perfusion indicators (such as mucous membrane colour and capillary refill time) and a thorough examination by organ systems. In a consultation environment, I discussed differential diagnosis, methods of diagnosis and treatment options (medical and surgical) with the veterinarian. On occasion, I had contact with exotic species such as rabbits, rats, chinchillas, guinea pigs and a python.

I participated in administration of injectable medication and vaccines, accessing medicine choice, doses and treatment options, post-operative examination of wounds, examination of ear canals with otoscope, examination of eyes with use of direct and indirect ophthalmoscopy, use of the Schirmer's test and the fluorescein test on eye examination, use of auscultation for accessing and grading heart murmurs.

When working with methods of diagnosis, hospitalized patients and minor interventions/procedures, I performed a set of tasks including the admission of pets with the nursing team before surgery, going through consent forms with owners, restraining patients, physical examination with weight check, microchip verification, measuring blood pressure, taking blood samples (recurring to the cephalic and jugular veins), placing and removing IV catheters. In a laboratorial context, I processed blood samples (full blood profile and electrolytes), examined urine samples (using dipstick urinalysis test, microscopic visualization, and identification of sediment), microscopic preparation and observation of fine needle aspirate samples, skin and ear swabs, bronchial samples and blood smears.

I carried out minor interventions and procedures as follows: draining of facial abscesses, draining of aural abscesses (with suture of a draining tube), thoracentesis of two cats and a dog in heart failure, microchip insertion, urinary catheterisation in male dogs and cats, anal gland flush, dental scale and polish, hair and nail clipping, male cat castration and ear canal flushing.

I gave my assistance in: nose biopsy for lupus diagnosis, ACTH stimulation test, blood glucose curve measurements, bile acid stimulation test, TSH stimulation test in dogs, treatment of *Dirofilaria immitis*, head muscle biopsy (masticatory muscle myositis), palate foreign body removal, use of contact lens bandage (in cases of keratitis) and management of chronic illness such as pneumonia, heart and kidney failure, and complications associated to neoplasia.

In the imaging area, I was daily involved in radiology: positioning the patient, adjusting kV and mA per body weight and exposed body part, ensuring collimation of the beam; aiding in the diagnosis of skeletal lesions and indicators of heart disease, thoracic and abdominal defects. Moreover, I participated very frequently in ultrasound abdominal scans, and many echocardiography scans with the cardiology specialist (along with electrocardiograms). Additionally, I was exposed briefly to rhinoscopy and endoscopy and aided in the diagnosis of larynx paralysis. Similarly, I was involved in contrast radiology with iodine in search of urinary tract defects.

Furthermore, I was exposed to patients in critical condition after road traffic accidents, high-rise syndrome, and dog attack; to the convulsing epileptic patient, and poisoned patients (rodenticide, in other cases onions and chocolate), as well as cases of ingestion of foreign bodies (floor tiles, diamond earrings, and socks).

During hospitalization, I participated in taking pets to kennels and ensuring their comfort (setting up their beds, providing them with food and water, taking them for walks, setting up heat lamps and blankets, maintaining a clean and calm environment), monitoring health status (including in critical care), administration of medication and updating day care sheets.

In surgical procedures requiring general anaesthetic I administrated pre-anaesthesia medication (acepromazine and methadone or butorphanol and medetomidine), pain relief, and the inducing agent (propofol). I participated in chamber anaesthesia of aggressive/exotic pets (sevoflurane). I positioned endotracheal tubes, clipped and prepared the surgical field using aseptic technique, opened sterile instrument sets and drapes for surgeons, carried the patients into theatre, monitored vital parameters during anaesthesia (heart rate, respiratory rate and blood pressure). I assumed the role of assistant surgeon when required – for example in TTA (tibial tubercle advancement), patellar ridge insertion or exploratory laparotomy. I was also included in theatre for surgical operations such as enterotomy (obstruction of the small intestine by foreign body), cystotomy for cystoliths removal, cystotomy with mass removal, prostatic cyst biopsy, surgical intervention after dog attack, surgical dew claw removal, bitch spay as consequence of pyometra, eye enucleation, umbilical hernia repair, mammary mass removal, and retained teeth removal. As first surgeon, I performed some lumpectomies, bitch spays and dog castrations (achieving autonomy in these procedures). I monitored patients as they woke up from anaesthesia and returned them to kennels.

I carried out euthanasia (sodium pentobarbital) of a few dogs, a chinchilla, and further assisted during euthanasia in the presence of owners.

A. Literature Review

1. Introduction

The choice of this dissertation's theme was intuitive and the direct result of the experience I acquired at the Ardene House Practice, where most Thursdays I was in close contact with small animal cardiology, under the supervision and guidance of a specialist. Our sessions were based on a practical approach to cases, supported by detailed scientific knowledge; comprising each step necessary to reach an evidence-based diagnosis of cardiovascular illness and covering the comprehensive use of complementary diagnosis methods such as echocardiography, electrocardiography, and Holter. This approach frequently ensured an immediate chance to respond with medical treatment or surgical referral. Follow-up consultations were quite common and provided a link between diagnosis and prognosis, establishing an in-depth insight of disease process and evolution. On this note, I assisted in consultations of patients with aortic stenosis included in this study.

This review is born as an attempt to further contribute to knowledge of aortic stenosis; it is a dissertation comprising a bibliographic review and a retrospective clinical study.

The primary chapters are hence aimed at the left ventricle outflow tract anatomy, physiology, and events occurring during formation of the heart relevant for a better appreciation of the morphogenesis of aortic stenosis. The following chapters explain the intricacies of clinical epidemiology, aetiology and pathology of this illness, and further resume the most recent data on practical clinical features aimed at diagnostic method, prevention, and management options.

The second part of this study characterizes 274 cases of dogs affected with aortic stenosis and Scotland and analyses the relationship between signalment, clinical features, and associated heart disease of patients at the time of presentation and/or diagnosis of aortic stenosis.

2. The Left Ventricular Outflow Tract

2.1 Gross functional anatomy

Eighty percent of the total volume of blood within the dog's body passes through the heart each minute (Dyce & Wensing, 2010).

The apex of the heart is formed by the left ventricle that constitutes its largest structure. Morphologically, its shape is conical; in section, it is circular (Bezuidenhout, 2013; Kienle & Kittleson, 1998). The left ventricle receives oxygenated blood from the lungs, by way of the pulmonary veins and the left atrium. Its purpose is to pump it through the high-resistance systemic circulation, ensuing the transportation of numerous essential nutrients required at a cellular level (Stephenson, 2013).

The myocardium of the left ventricle has three to four times the thickness of the right ventricle, in conformity with the greater work it must perform with each contraction (Bezuidenhout, 2013; Goshal, 1975).

As the left atrium forms the dorsocaudal part of the heart, it receives the pulmonary veins that enter via five or six openings to this chamber, situated caudodorsally and craniodorsally (Bezuidenhout, 2013; Dyce & Wensing, 2010).The left atrioventricular ostium is the opening between the left atrium and ventricle, and it is closed by the mitral valve that is formed by two cusps: a septal cusp that arises from the fibrous ring, broader than the coadjutant parietal cusp that comes from the remainder of the ring associated with the outer wall of the ventricle. (Bezuidenhout, 2013; Dyce & Wensing, 2010).

Blood enters the atrium given the pressure within the veins exceeds that within the heart, thus during the stage of ventricular relaxation the blood flows directly into the ventricles, through the open atrioventricular ostium. The papillary muscles attach to the interventricular valves do not serve to open the valves, which are opened passively by the blood-flow (similar to the opening of a door by a gust of wind) (Klaus-Dieter, McCarthy, Fricke & Richter, 2007).

A division of the left ventricle into an inflow and outflow region is physiologically established by the septal leaflet of the mitral valve (Kienle & Kittleson, 1998). The inflow region reaches from the mitral valve annulus to the apex of the heart, whilst the outflow tract is set out at the apex and extends to the aortic valve, situated cranially to the septal leaflet of the mitral valve, at the level of the heart base (Kienle & Kittleson, 1998). Hence, the left ventricular outflow tract (LVOT) extends from the free edges of the septal leaflet of the mitral valve to the semilunar attachments of the aortic valve leaflets (Freedom, Yoo, Russell, Perrin, & Williams, 2005). It is outlined medially by the muscular interventricular septum and by the craniolateral portions of the left ventricular free wall (Freedom *et al.*, 2005; R. Kienle & Kittleson, 1998).

The septal leaflet of the mitral valve is continuous at its superior extent with the noncoronary leaflets of the aortic valve. It is a fibrous continuity, arising from the fibrous body and termed the intervalvar septum (Hill & Iaizzo, 2015).

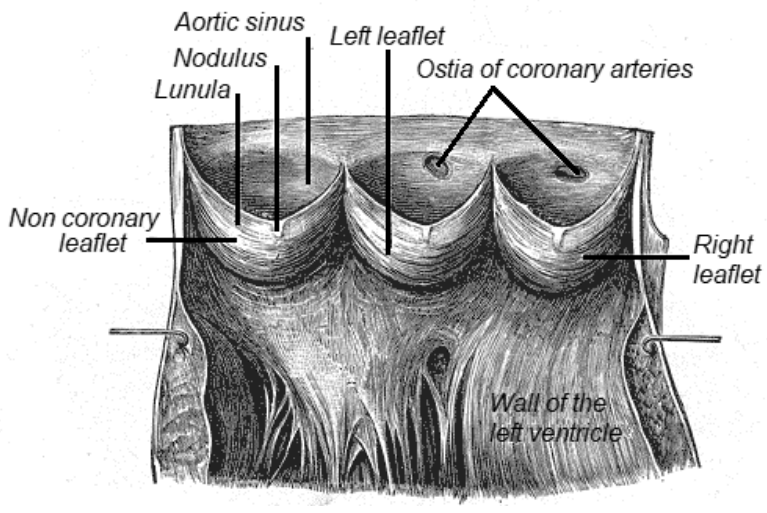
In systole, the mitral valve is maintained in its closed position by the chordae tendineae and the papillary muscles, so that the cusps are not everted into the atrium by reversal of blood flow (Klaus-Dieter, McCarthy, Fricke & Richter, 2007). This construction is essential because the blood leaving the ventricle through the aorta is under approximately four times more pressure than that which leaves the right ventricle through the pulmonary trunk (Bezuidenhout, 2013; Klaus-Dieter *et al.*, 2007).

The aortic ostium is situated near the centre of the heart base, connecting the left ventricle with the ascending aorta. The aortic valve closes the aortic ostium and is formed by the fibrous annulus, the root of the aorta, and three aortic valve cusps (Goshal, 1975; Kienle & Kittleson, 1998).

The fibrous annulus encircles the orifice and constitutes a scaffold that anchors the aortic valve cusps. The fibrous skeleton of the heart supports all the four valves of the heart. However, the arterial fibrous ring that encircles the aorta, the aortic fibrous ring, is the most developed and its collagenous matrix possesses yellow fibres that provide the ascending aorta with elasticity. Furthermore, the projections between the attachments of the semilunar valvulae are composed in part of hyaline cartilage (Bezuidenhout, 2013; Dyce & Wensing, 2010).

The root of the aorta protrudes slightly behind each valve cusp to form the sinuses of Valsalva, within two of the sinuses lie the ostia of the right and left coronary arteries (Figure 1) The function of the sinuses is to prevent the covering of the coronary ostia by the aortic valve cusps during systole. (Kienle & Kittleson, 1998). The coronary arteries that spring from the sinuses supply the heart with blood, receiving about 15% of the output of the left ventricle (Dyce & Wensing, 2010).

Figure 1. Anatomy of the human aortic valve (adapted from: Gray, 1918).



The coronary or aortic valve cusps or leaflets are named in association to the position of the coronary ostia, thus the cusp associated with the ostium of the right coronary artery is the right

cusp of the aortic valve, the same for the left. The cusp that is not associated with a coronary artery is the noncoronary cusp of the aortic valve (Kienle & Kittleson, 1998). Each cusp of the aortic valve, near the centre of its concave free border, holds a nodule, guaranteeing the coaptation of the leaflets. Extending from the nodules in the direction of the free edges of the cusps, are the lunulae, representing the contact surface between adjacent cusps when the valve is closed and they are important to warrant this motion (Goshal, 1975).

The aortic valve is closed during ventricular relaxation as the pressure within the aorta exceeds that within the left ventricle. As the contraction develops, blood forces the semilunar valve to open and the aorta expands with this sudden input. The aortic valve functions strictly passively. The free border of each cusp is projected into the lumen of the aorta and flutters with the physiological direction of the bloodstream. With reversal of blood flow, the cusp is expanded by the inflow of blood; the free borders contact one another and close the valvular orifice (Klaus-Dieter, McCarthy, Fricke & Richter, 2007).

The aorta is a thick-walled and highly elastic vessel from which the large systemic arteries have their origin directly. For descriptive purposes, it is divided into an ascending and a descending aorta, separated by the transverse arch. The ascending aorta at its origin is slightly expanded to form the bulb of the aorta, concealed between the atria. This initial part is approximately two centimetres in size, and attaches to the fibrous base of the heart, located within the pericardium and rises immediately behind the right auricle. As it emerges from the left ventricle it angles dorsally and cranially. Once the aorta rises above the plane of the main pulmonary artery, it curves backwards as the transverse segment. The remainder of the aortic arch to its terminal iliac branches is the descending aorta. The descending aorta is further divided into a thoracic part and an abdominal part, supplying the whole of the organism with blood (Bezuidenhout, 2013; Dyce & Wensing, 2010; Goshal, 1975; Kienle & Kittleson, 1998).

2.2 Embryology

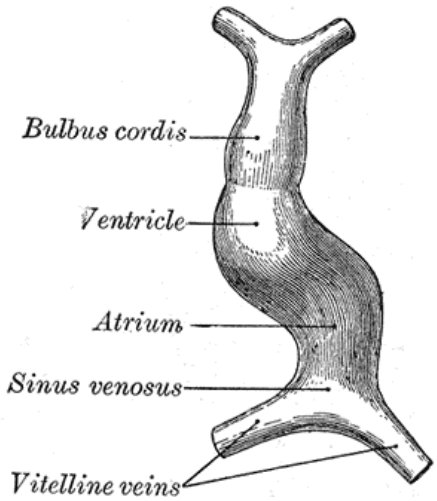
2.3.1. Heart tube and Cardiac Loop Formation

The heart one of the first organs to form in dogs and it stems from a series of morphogenetic interactions, involving cells from several embryonic origins (Hyun & Lavulo, 2006). The mesodermal tissues that give rise to the heart first become evident when the embryo is under a process named gastrulation, that occurs in the dog around the 16th day after fertilization. The primary plate of cells that originate the heart consists of myocardial cells intermingled with a plexus of endothelial strands, derived from the cardiac crescent at the cranial border of the embryo (McGeady, Quinn, Fitzpatrick, & Ryan, 2006; Moorman, 2003; Srivastava & Olson, 2000).

Soon after their specification, cardiac muscle cells converge along the ventral midline of the embryo to form a beating tube. This ensures the presence of a circulatory system, and on days 20 to 21 coordinated heartbeats can be detected via ultrasound (McGeady *et al.*, 2006; Moorman, 2003). Thus, unlike any other organ system in the embryo, soon before the attainment of the definitive cardiac and vascular structures as seen on the mature animal, the cardiovascular system presents concurrent function and formation for the embryo to survive (Srivastava & Olson, 2000).

Subsequently to gastrulation, the heart tube takes the shape of a “Y”, and then the migration of cells, from a secondary heart field, form the outflow tract and a primordium of the right ventricle, pronouncing the elongation of the primary heart tube (Moorman, 2003) (Figure 2).

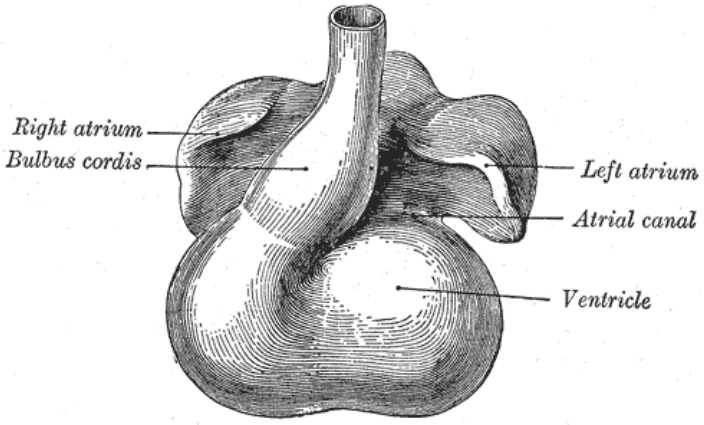
Figure 2. Tubular heart (Gray,1918)



The linear heart tube forms alternate dilations and indentations along its longitudinal axis. In posterior-anterior order, the expanded portions of the cardiac tube are the sinus venosus (where the veins open into the cardiac tube), the atrium, the ventricle, the bulbus cordis and the truncus arteriosus, there the outlet into the aorta is found, thus the heart is divided into

atrial and ventricular components along with an outflow tract. However, the process of how these chamber identities are established is not known (Hytell, 2010; Srivastava & Olson, 2000). With further development, marked transformations occur in all parts of the tube as to produce left and right structures. The ventricles are derived from the bulboventricular part of the heart tube, the stem of the “Y” shaped heart. Because this portion of the heart tube outgrows the pericardial cavity, and given the two ends are fixed, it is compelled to adapt to the available pericardial space, bending cranially and to the right, forcing the tube to twist. After this stage of looping the outward appearance of the primitive heart tube evokes its future four chambered anatomy (Kienle & Kittleson, 1998; Moorman, 2003). Figure 3.

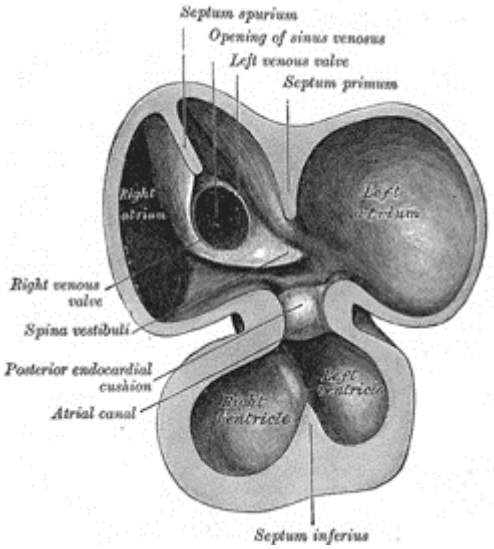
Figure3. Primitive heart resembling its mature, four chambered anatomy (Grey, 1918).



2.3.2. Internal formation of the heart chambers

The partitioning of the heart into four chambers is a continuous process and involves the formation of different septa in parallel (Hytell, 2010; Kienle & Kittleson, 1998). Figure 4.

Figure 4. Heart septation (Gray, 1918).

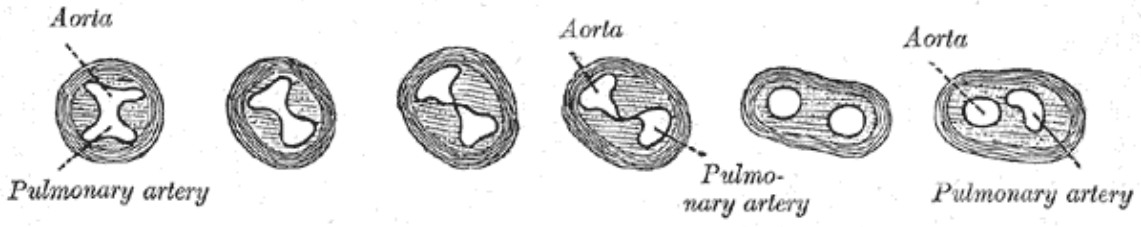


The sinus venosus reduces in size and becomes the coronary sinus at the level of the right atrium. The atrial division occurs because of the development of two distinct septa, the *septum primum* and the *septum secundum* that eventually fuse and to form the interatrial septum.
The atrioventricular septum originates in proliferative mesoderm, via the endocardial cushions that grow actively (Kienle & Kittleson, 1998).
Paired endocardial cushions also develop within the bulbus cordis and truncus arteriosus; they ultimately fuse to form the aorticopulmonary septum, which has a slight spiral, progressively rotating distally in a clockwise manner. A continuous septum divides the bulbus cordis and truncus arteriosus into the aorta and main pulmonary artery that twists around it. The interventricular septum grows passively as a result of the caudal expansion of the ventricle combined with elongation along the midline (Hytell, 2010; Kienle & Kittleson, 1998).
The left ventricular outflow tract is formed during the development of the aorticopulmonary septum, the ventricular septum and the septal leaflet of the mitral valve (Kienle & Kittleson, 1998).

2.3.3. Cardiac valve formation

The correct formation and function of the cardiac valves demand a multitude of cell and tissue interactions where any divergences can result in severe developmental defects. These interactions initiate the early specification and assembly of the various components of the cardiovascular system and the LVOT is no exception (Hyun & Lavulo, 2006).
The septation of the primitive heart into distinct chambers is achieved through cardiac cushions of extracellular matrix, that also form a rudimentary basis of atrioventricular valves. Reciprocal signalling between the endocardial and myocardial cell layers in the cushion region, mediated in part by TGF-β (transforming growth factor-β) family members, induce a transformation of endocardial cells into mesenchymal cells. These migrate into the cushions and differentiate into fibrous tissue of the valves (Moorman, 2003; Srivastava & Olson, 2000).
The origins of semilunar valves are small cushions of subendocardial tissue that develop as swellings at the outlet of the truncus arteriosus into the ventral aortae as of the division of the truncus. Once the aorticopulmonary septum is complete, the swellings originate three primitive cusps in each of the aortic and pulmonary outlets. Figure 5. The valves attain their final shape by a process of excavation on the upper surface, the sinuses of Valsava (Hytell, 2010; Kienle & Kittleson, 1998).

Figure 5. Transverse sections through the aortic bulb to show the growth of the aortic septum (Gray, 1918).



3. Aortic stenosis

3.1 Definition

Aortic stenosis is defined as a congenital obstructive malformation of the heart. It has been reported in the dog, cat, cow, sheep, horse, pig, and man (King, Flint, & Anderson, 1988; Lofland *et al.*, 2001; O'grady, Holmberg, Miller, & Cockshutt, 1989; Robinson & Maxie, 1993; Stepien & Bonagura, 1991). Anatomically, aortic stenosis is classified as valvular (VAS), sub-valvular (SubAS) or supra-valvular (SupAS). Functionally, regarding the characteristics of the obstruction, it is categorized as either fixed or dynamic in sub-valvular cases (Kienle, 1998).

3.1.2 Sub-valvular aortic stenosis

The most common type of aortic stenosis is of the sub-valvular form. In the early 1990's this type of stenosis constituted more than 95% of the identified cases (Kienle, 1998).
In the dog, the lesion usually occurs in the sub-valvular position where the dimensions of the restrictive orifice are static or fixed by the anatomic characteristics of the lesion. The severity is not altered from beat to beat and does not change as systole progresses. In cases of labile obstruction, however, the severity is altered by changes in heart rate or inotropic state and usually varies as systole progresses. (Kienle, 1998) Regarding the dynamic form of SubAs, there are few reports of specific cases (Fernández del Palacio, Bayón, Bernal, Cerón, & Navarro, 1998).

3.1.3 Valvular aortic stenosis

In the dog, the valvular type of lesion is considered to be infrequently encountered (Beijerink, Oyama, & Bonagura, 2017; O'grady *et al.*, 1989).
Bull Terriers seem to be predisposed to valvular aortic stenosis, in which the leaflets are thickened and the aortic valve annulus is mildly hypoplastic. Mild AS caused by a bicuspid valve (BAV) occurs on rare occasions (Visser & Scansen, 2013). In a study, BAV was found as an isolated defect causing valvular aortic stenosis and once more concomitantly with sub-aortic stenosis (Oliveira *et al.*, 2011). Quadricuspid aortic valve defects seem to be fairly more common in dogs throughout the veterinary literature and have been depicted as insufficient with mild degrees of stenosis severity (Serres, Chetboul, Carlos Sampedrano, Gouni, & Pouchelon, 2008).

3.1.4 Supra-valvular aortic stenosis

The supra-valvular lesion is very rare in dogs (O'grady *et al.*, 1989). In supra-valvular AS, a narrowing occurs at the level of the aortic arch, and immediately above or at the sinuses of Valsalva (French *et al.*, 2000).

3.2 Clinical Epidemiology

3.2.1. Congenital heart disease

CHD has a calculated prevalence of 0.56%-0.85% in dogs presented for veterinary examination (Buchanan, 1999; Detweiler & Patterson, 1965). Of the dogs presented for cardiovascular examination in Italy, it has been suggested that 21% of patients have CHD. (Oliveira *et al.*, 2011). However, the precise occurrence of these malformations is difficult to determine and could be considered greater than the published percentages. Some CHD are underdiagnosed for they do not cause audible cardiac murmurs, may lead to perinatal death, and regional differences in breed prevalence affect the frequency of successful diagnosis (Buchanan, 1999; Oliveira *et al.*, 2011). The complex nature of the differences found between breeds and countries (and often within regions of a given country) indicate that gene pools play a crucial role in the aetiology of AS, thus the interpretation of this data should be careful (Buchanan, 1999).

A recent study in the U.S.A. , including mixed-breed dogs, reported a prevalence of CHD equivalent to 0.13%, an obviously lower percentage in comparison to previous studies that encompassed purebred dogs (these dogs were screened by shelter veterinarians and subsequently evaluated by cardiologists) (Schrope, 2015). This is supported by more studies where purebred dogs also had a significantly higher probability of presenting CHD when compared to mongrel dogs (Oliveira *et al.*, 2011).

In respect to aortic stenosis, several studies have revealed that purebred dogs also present a greater probability of expressing this condition (Bellumori, Famula, Bannasch, Belanger, & Oberbauer, 2013; Buchanan, 1999; Detweiler & Patterson, 1965; Oliveira *et al.*, 2011).

Despite differences in prevalence, the most common cardiac malformations have remained largely unchanged for the last half-century. Patent ductus arteriosus (PDA), sub-aortic stenosis (SubAS) and pulmonic stenosis (PS) have topped the list of most studies (Aherne & Beijerink, 2013; Buchanan, 1999; Oliveira *et al.*, 2011). Most of the existing studies on the prevalence and incidence of AS have been achieved in university hospitals, comprising a considerable number of cases, significant for statistical evaluation.

3.2.2. Aortic stenosis

Canine sub-aortic stenosis is possibly the most commonly encountered congenital heart defect observed in dogs, though it has ranked second in frequency studies (Bonagura & Lehmkuhl, 1999). In Europe, aortic stenosis has been reported as the most common congenital cardiac deformity representing 31.5% and 35% of all CHD cases (Baumgartner & Glaus, 2004; Tidholm, 1997). Nevertheless, a study held in Italy, combining SubAS and VAS accounted for the second most frequent congenital pathology with an incidence of 27%. PS was the most

common malformation (Oliveira *et al.*, 2011). A recent study in California suggests that the prevalence of dogs with aortic stenosis in 0.49%(Belanger, Bellumori, Bannasch, Famula, & Oberbauer, 2017). In the U.S.A, aortic stenosis has been reported to be the second to third most frequently encountered congenital cardiac abnormality in the dog, after patent ductus arteriosus and pulmonic stenosis (Patterson, 1968; Schrope, 2015). In Australia, a study showed that SubAS is a congenital deformity second only to PDA in prevalence (Aherne & Beijerink, 2013).

3.2.3. Breed

The first cases of sub-aortic stenosis resulting from a sub-valvular fibrous connecting tissue ring were observed in 1952 (Patterson & Detweiler, 1963).

In 1968, Patterson described that aortic stenosis occurs most frequently in the following breeds: the Newfoundland, Golden Retriever, German Shepherd and Boxer. In 1978, an increased incidence in the Bouvier des Flandres, Rottweiler and Bull Terrier breeds was remarked by the same author. At this stage, the frequency of occurrence of sub-aortic stenosis in the Golden Retriever and Newfoundland Dog strongly suggested an inherited basis. (Patterson, 1968, 1976).

Other individuals or small groups of breeds included in studies were the German Shorthaired Pointer (Mulvihill & Priester, 1973), Dogue de Bordeaux, Samoyed, the Mastiff, Pug, Labrador, Shar Pei, Pitt Bull, Cocker, Old English Sheep Dog, Border Collie , Bernese Mountain Dog, American Straffordshire Terrier, Dachshund, Dalmatian, English Setter, Great Pyrenees, Giant Schnauzer, Hovawart, Alaskan Malamute, Standard Poodle, Wire Haired Fox Terrier, Keeshond, English Bull Dog, and the Kuvasz (Baumgartner & Glaus, 2004; Kienle, Thomas, & Pion, 1994); along with mixed breed dogs and breeds not specified in some studies due to the low prevalence (Aherne & Beijerink, 2013; Kienle *et al.*, 1994).

In a recent study held in America, the Irish Terrier was considered one of the four breeds with the most cases of congenital aortic stenosis (Bellumori *et al.*, 2013). Individuals of Bichon Maltais, Miniature Pinscher, Bichon Frisé, Chzechoslovakian Wolfdog, Hairless Chinese Crested Dog, West Highland White Terrier and Yorkshire Terrier (Kander, Paslawska, & Staszczyk, 2015) are also conveyed in the literature, though these breeds have not been typically described as affected by this congenital disorder.

The Boxer breed is reported as being at an increased risk of developing congenital heart disease, with SubAS being the most frequent finding (Buchanan, 1999). A study concerning Boxers concluded 17.8% of Boxer dogs were affected by CHD; and SubAS accounted for 85% of the cases, making it the most usual form of CHD. The remaining cases were assigned to pulmonic stenosis (Claudio Bussadori, Quintavalla, & Capelli, 2001).

The Black Russian Terrier has been reported of having SubAS with both fixed and dynamic components, in association with mitral dysplasia (Pikula *et al.*, 2005).

3.2.4 Gender

Some surveys suggest that males are more affected by aortic stenosis in comparison with females. In a study that only included mixed-breed dogs, sub-valvular stenosis was more frequent in males, accounting for 69% of the percentage of affected dogs (Schrope, 2015).
Specific male predisposition has been found also in other studies involving purebred dogs, for 62% SubAS and 70% when VAS was discriminated. Supra-valvular aortic stenosis is considered to be rare, consequently, it is not prudent to draw conclusions on prevalence (Oliveira *et al.*, 2011). In the boxer breed, males also seem to be predisposed to SubAS (Buchanan, 1999). It has also been found that the association of AS and PS is more common in male dogs (Kander *et al.*, 2015). Other studies have failed to find an obvious predilection for males in gender distribution (Aherne & Beijerink, 2013; Tidholm, 1997).

3.2.5. Associated congenital heart defects

The incidence of complex congenital heart defects has been reported as 7-15% (Oliveira *et al.*, 2011; Tidholm, 1997). Concomitantly with sub-aortic stenosis, several CHD have been observed, being PS the most common defect followed by PDA, mitral dysplasia, ventral septal defect (VSD), *situs inversus*, aortic stenosis, aortic root hypoplasia, persistent left cranial *vena cava*, bicuspid aorta, quadricuspid aorta, tricuspid dysplasia, double-chambered right ventricle and one case of supra-valvular aortic stenosis (Aherne & Beijerink, 2013; Oliveira *et al.*, 2011; Tidholm, 1997). The relevant concurrence of atrial septal defect and mitral dysplasia have also been remarked particularly in the Boxer breed (Chetboul *et al.*, 2006).
More specifically in the case of co-occurrence of congenital aortic stenosis and pulmonic stenosis, it represented a 7.6%-26.4% of dogs with CHD (Kander *et al.*, 2015; Oliveira *et al.*, 2011). Multiple heart defects were also associated with VAS, there are reports with the following associated cardiovascular malformations: pulmonic stenosis, aortic root hypoplasia, BAV, and PDA (Aherne & Beijerink, 2013; Oliveira *et al.*, 2011).

3.3 Aetiology

3.2.1 Teratology

Great debate surrounds the embryological origin for all forms of aortic stenosis. Whilst it is classified as a congenital heart disease, the cause of the malformation is yet to be determined. Different forms of aortic stenosis occur in several anatomical forms. Each of which most probably has a peculiar embryologic basis (Kienle, 1998).

Any abnormality in the development of the structures of the LVOT may result in AS. It has been hypothesized that persistent embryonic endocardial tissue may retain its proliferative capacity and chondrogenic potential for some time after birth, from which can derive the fibrocartilaginous ring. It may be associated with the malformation of the proximal truncus septum where it joins the conus septum, within the conotruncal septum. It is worth to mention that the appearance of cartilage in the ring could be attributed to embryonical connective tissue in the heart (reffering to the aortic annulus of the fibrous skeleton) being located in a site contiguous to the sub-aortic region (Kienle, 1998; Pyle, Patterson, & Chacko, 1976).

Counter-intuitively, it could be that CHD is not due to any mutation in structural proteins, for example there, would be no gene encoding “the atrial septum”. The genetic mutations are mainly in genes encoding transcription factors. These interact with other factors signalling molecules and receptors and, ultimately, structural proteins, so that abnormal development in the heart may be due to changes in one or more of the factors in these pathways (Dukes-McEwan, 2006; Hyun & Lavulo, 2006; Hyun & Park, 2010).

The TGF-β signalling pathways, occur with the binding of TGF- β family members to receptors in the cell membrane and nucleus. They act as transcription factors and participate in the regulation of target gene expression. The TGF-β are multifunctional peptides controlling proliferation, differentiation and cardiac morphogenic signal induction, as they play a part in the septation and valvulogenesis of the outflow tract. In addition, other pathways and molecules have been implicated in the regulation of valve development. The hyaluronic acid pathways are of prime importance among these, for they control cell proliferation and apoptosis of endocardial cushion cells, maintaining the optimum cell number for valve formation. The involvement of these genetic pathways in the origin of CHD has been studied in rat models. The use of comparative genetics is also deemed as a helpful tool in the search of candidate genes for CHD in dogs, and equally for understanding the disease process. Progress in the elucidation of congenital heart disease in domestic animals necessarily involves dialogue and collaboration between cardiologists, developmental biologists, pathologists, and geneticists (Hyun & Lavulo, 2006; Parker, Meurs, & Ostrander, 2006).

Valvular aortic stenosis is likely related to the faulty development of the endocardial cushions in the truncoconal septum, from which the semilunar valve leaflets originate (Kienle & Kittleson, 1998).

It is thought that the rapid development of cardiorespiratory components, mainly the transformations that occur particularly between day 16 and 22, suggest that this phase of development is one requiring the most attention to understand embryonic malformations. (Martins *et al.*, 2016).

3.2.2 Genetic predisposition and mode of inheritance

Many genetic aetiologic factors responsible for human CHD have been identified, although few of them have been discovered for CHD in pets. A number of studies support the concept that identifiable genetic factors contribute to the development of the embryonic heart and relate to CHD (Buchanan, 1999; Hyun & Lavulo, 2006; Hyun & Park, 2010; Ohad, Avrahami, Waner, & David, 2013; Srivastava & Olson, 2000; Tidholm, 1997).

The observation that AS displays a breed predilection strongly suggests a heritable basis in many cases. Breeding studies in different breeds have established a genetic basis for the perpetuation of SubAS (Chetboul *et al.*, 2006; Ohad *et al.*, 2013; Patterson & Detweiler, 1963; Stern, Meurs, Nelson, Lahmers, & Lehmkuhl, 2012).

The mode of inheritance does not obey to simple Mendelian transmission. Some genes could establish additive or modifying effects and conceive a discrete phenotype once a Mendelian trait has been inherited, including variability in the penetrance of that characteristic (Bonagura & Lehmkuhl, 1999; Ohad *et al.*, 2013; Stern *et al.*, 2014). It has been hypothesised, relying on genetic studies of non-cardiac disease in dogs, that AS can be a breed-specific single-trait, signifying every breed has a distinct genetic aetiology but exhibits a comparable phenotypic result in similarity to other breeds (Hyun & Park, 2010). Patterns of inheritance are consistent with a polygenic threshold model, in which multiple genes act additively, and the incidence and degree of severity increasing with the dose of genes predisposing to the defect (Freedom *et al.*, 2005).

SubAS has been most extensively examined in the Newfoundland dog (NFD). Breeding studies in the NFD established a genetic basis for the perpetuation of SubAS. The mode of transmission is compatible with an autosomal dominant trait with modifying genes, or a polygenic or monogenic co-dominant with partial to full penetrance mechanism (Pyle *et al.*, 1976; Reist-Marti *et al.*, 2012). An autosomal dominant abnormality present in the gene PICALM has been related to SubAS and provided evidence that equivocally affected individuals could pass SubAS to their progeny. With prospects of facilitating the reduction of SubAS disease in NFD, it was suggested to test for the presence of this PICALM insertion. (Joshua A. Stern *et al.*, 2014). Nevertheless, this information requires confirmation in the Newfoundland breed (Drögemüller *et al.*, 2015).

The Golden Retriever and the Boxer breeds are over-represented in the prevalence of SubAS, indicating a possible genetic aetiology; however, this is not well documented in the literature (Bussadori, Amberger, Le Bobinnec, & Lombard, 2000; Stern *et al.*, 2012). Compared to other breeds, Golden Retrievers have an increased odds ratio (OR) of 6.8 for diagnosis of SubAS (Buchanan, 1999). A study attempted to find a mode of inheritance and although simple autosomal dominant and X-linked recessive mechanisms were excluded based on the families of participants, the information was scarce to conclusively determine a mode of inheritance (Stern *et al.*, 2012). The characterisation of SubAS in the Golden Retriever suggested a similar phenotype to other breeds exhibiting an extensive range of aortic velocities and supportive echocardiographic findings (Stern *et al.*, 2012).

The Dogue de Bordeaux appears to be highly predisposed to AS and, in the last few years, an increasing number of these dogs has been identified in Denmark (Höllmer, Willesen, Jensen, & Koch, 2008; Ohad *et al.*, 2013).

A study conducted in Israel concluded that the most probable mode of inheritance in the Dogue de Bordeaux appeared to be autosomal recessive, and, inclusively, added the possibility of both sub-aortic stenosis and aortic stenosis were likely of single gene transmission for both defects, contrasting to each one alone. However, a different allele could be responsible for the disease in previously studied populations. The mode of inheritance was correlated with the presence of a subclinical state associated with a normal phenotype and with the small number of affected and suspected dogs. Generation “skipping” was compatible with an autosomal recessive mode of inheritance because heterozygous carrier dogs have a normal phenotype. However, a study including more dogs is required to confirm this information (Ohad *et al.*, 2013).

A study using Bull Terriers with polycystic kidney disease revealed an increased prevalence of LVOTO, with both VAS and SubAS. These dogs were at an increased risk of cardiac abnormalities. The significance of this suggests the existence of a hereditary basis for these findings (O’Leary, Mackay, Taplin, & Atwell, 2005).

Recent advances in canine genomics made available a dense genome map, providing veterinary biology with a foothold in comparative medicine, allowing advances that already have been made in human medicine to be applied to companion animals, facilitating the identification of hereditary disease genes in dogs (Parker *et al.*, 2006). Ideally, in the near future, the identification of various mutations responsible for AS will allow a complete description of inheritance patterns. Clinicians and scientists are urged to collaborate in this area of comparative cardiovascular medicine (Dukes-McEwan, 2006; Stern *et al.*, 2012).

3.3.3 Inherited versus acquired

There has been much discussion as to whether SubAS is inherited, acquired or both (Reist-Marti *et al.*, 2012) The lesion producing the typically fixed obstruction in the left ventricular

outflow tract seemingly changes and evolves in the first months after birth (Freedom *et al.*, 2005; Hyun & Park, 2010; Javard, Bélanger, Côté, Beauchamp, & Pibarot, 2014). Some authors claim that SubAS does not consist of a true congenital defect, but rather one that develops post-natally. Supporting this statement is the evidence that pups with less than three weeks of age rarely show lesions of SubAS and that moderate to severe lesions are found predominantly in dogs over six months of age. Hence, structural lesions are either not present at birth or are not fully developed at birth. In addition, the lesions characteristic of SubAS have not been documented in foetal life(Freedom *et al.*, 2005; Pyle *et al.*, 1976).

However, severe SubAS has been observed in neonates, in which case it would be considered a congenital defect (Pikula *et al.*, 2005). SubAS has also been found in a dog as early as 8 weeks, and this information suggests that very young and asymptomatic puppies may suffer from a severe complex form of SubAS (Fernández del Palacio *et al.*, 1998). The evidence of complex heart defects in dogs also suggests a reason as to why the fixed form of SubAS could be considered congenital (Freedom *et al.*, 2005).

In Man, the nature of fixed SubAS is well recognized and a four-stage aetiological process has been proposed for its development. The fist predisposing factor is the presence of subtle morphological abnormalities within the outflow tract. These lead to the second contributor, an elevation of septal sheer stress, by significant changes in fluid dynamic forces. The elevation of septal sheer stress triggers the third factor, namely the genetic predisposition and is exacerbated by a fourth factor, as cellular proliferation and biochemistry adjust in response to the abnormal septal sheer stress. The result is the fixed SubAS (Cape, Vanauker, Sigfússon, Tacy, & del Nido, 1997).

Similarly, in dogs, it is also currently hypothesized that the etiology of the fixed forms of SubAS has both congenital and acquired aspects (Beijerink *et al.*, 2017; Freedom *et al.*, 2005; Quintavalla, Guazzetti, Mavropoulou, & Bussadori, 2010; Reist-Marti *et al.*, 2012).

3.3.4 Progressive Nature

The LVOTV obstruction caused by SubAS often does become progressively more severe during the developmental period (Pyle *et al.*, 1976).

A study detected the progression of SubAS in a dog by classifying pressure gradient (with continuous wave Doppler) across the stenotic ring and CLVH during a period of 32 months. This reinforced the notion that, regardless of the age of the dog, the lesions continue to progress over time (Nakayama, Wakao, Ishikawa, & Takahashi, 1996). However, it has only been documented in a few individuals and is not considered to be a common clinical course. It is uncertain at what age an obstruction becomes fully developed. In any case, it is probably inappropriate to “clear” dogs for SubAS before they are fully grown (Kienle, 1998; Kienle *et al.*, 1994).

3.4 Pathology

3.4.1 Anatomical Pathology and Pathogenesis

The classic portrayal of SubAS is that of a discrete fibrous ridge that encircles the LVOT, totally or in part, immediately under the aortic valve (Detweiler & Patterson, 1965). The lesion usually consists of a ridge, ring or complex network of fibrous or fibromuscular tissue located just below the aortic valve which extends across or encircles the LVOT (Muna, Ferrans, Pierce, & Roberts, 1978). Pathologic studies in breeding colonies have shown, however, that a range in severity of the lesion exists; the mildest form of which is clinically silent (Kienle *et al.*, 1994; O'grady *et al.*, 1989; Pyle *et al.*, 1976).

Pyle *et al.* defined the lesions with post-mortem studies in Newfoundland puppies. In the mildest form (grade 1), the lesions were made of minute, salient nodules of thickened endocardium on the interventricular septum, below the cusps of the aortic valve. Similar lesions could be appreciated on the ventral surface of the aortic valve cusps in some puppies. Grade 1 was only recognized in dogs between 3 and 12 weeks of age in this study. (Pyle *et al.*, 1976)

Grade 2 lesions consisted of a fine ridge of whitish thickened endocardium in the LVOT below the aortic valve, encircling it partially. It seemed that this tissue had arisen at the base of the anterior mitral valve leaflet and traversed across the interventricular septum for a variable distance (Pyle *et al.*, 1976).

In grade 3, the most severe form, a fibrous band or collar completely encircled the LVOT directly underneath the aortic valve. The ridge was raised up 1 to 2 mm beyond the endocardial surface and reached around the totality of the LVOT, including the base of the anterior mitral valve leaflet. Grade 3 lesions were mainly identified in puppies older than 6 months of age. In these cases, the surfaces of the aortic valve were also thickened. As the lesion severity was confined to definite age ranges. This author postulated the same could progressively worsen over time, at least for the first 6 months of life (Pyle *et al.*, 1976).

Histologically, the ultrastructure of the lesions was characterized by the presence of large, uni- and multinucleated, rounded connective tissue cells that resembled chondrocytes. The chondrocyte-like cells contained numerous cisterns of rough-surfaced endoplasmic reticulum and prominent Golgi complexes and they were surrounded by thick, concentrically arranged layers of basement membrane like material. This disposition of the connective tissue differs in people and in dogs (Muna *et al.*, 1978). The stenotic ring can further be described as consisting of loosely arranged reticular fibres, mucopolisaccharide ground substance, and elastic fibres (Beijerink *et al.*, 2017). In advanced lesions, discrete bundles of collagen and cartilage can be found (Muna *et al.*, 1978; Pyle *et al.*, 1976).

In dogs with dynamic SubAS, the pathological findings differ from the classical description. The anterior mitral valve leaflet is thickened in apposition to a septal plaque of endocardial fibrosis, where the mitral leaflet impacts the interventricular septum (Fernández del Palacio *et al.*,

1998). Instead of a fibrous collar, the septum is uniformly hypertrophied or a broad fibromuscular ridge, arising from the base of the interventricular septum, protrudes into the LVOT. Malformed, malpositioned, or misaligned papillary muscles, thickened *chordae tendinae*, and elongated or distorted mitral leaflets contribute to the development of obstruction (Buoscio, Sisson, Zachary, & Luethy, 1994).

Associated findings on examination include: concentric hypertrophy of the left ventricle, left atrial enlargement, myocardial failure, concomitant mitral insufficiency, and post-stenotic dilation. Mild malformations of the mitral valve that are usually not functionally important are also common in dogs with SubAS (Kienle, 1998).

Histologically, accompanying these changes, dogs with elevated pressure gradients further demonstrate extensive lesions of the intramural coronary arteries and arterioles, characterized by intimal smooth muscle proliferation accompanied with degeneration, luminal narrowing, and medial degeneration (Flickinger & Patterson, 1967; Muna *et al.*, 1978).

3.4.2 Physiopathology

Independent of the nature of the obstruction, the principal hemodynamic consequence of aortic stenosis is an increased resistance to the left ventricular systolic outflow (Kienle, 1998).

According to Ohm's law, $resistance = \frac{pressure}{blood\ flow}$, an increase in resistance causes an increase in the pressure gradient (PG) across the stenotic region, a decrease in flow through the region, or both in combination (Kienle, 1998).

To maintain blood flow, the heart is required to generate a greater pressure to accelerate blood through the smaller area associated with the *vena contracta*. The *vena contracta* is the site where the functional flow area is minimal and where the flow jet attains a maximal velocity. (Brown & Smith, 2002) The pressure gradient across the narrowing increases in inverse proportion to the decrease in the size of the stenotic ring (resistance). And, provided blood flow is constant, the velocity of flow through the stenotic region also increases in inverse proportion to the size of the stenotic ring (Kienle, 1998).

The left ventricle outflow tract velocity (LVOTV) and pressure gradient have a constant relationship, defined by the modified Bernoulli's equation $pressure\ gradient = 4V^2$. Bernoulli's equation describes the conservation of energy between two sites on the same streamline within a flowing liquid such as blood (Brown & Smith, 2002). Either the pressure or the LVOTV can be used to detect abnormal hemodynamic measurements associated with AS (Kienle, 1998).

Owing to the increase in left ventricular systolic wall stress, cardiac muscle mass is stimulated. (Bonagura & Lehmkuhl, 1999; Kienle, 1998). Concentric left ventricular hypertrophy (CLVH) develops in dogs with fixed or dynamic SubAS and VAS (Beijerink *et al.*, 2017). Its thickness is believed to be related to the severity of the stenosis, however severe hypertrophy produces

an increase in ventricular stiffness. This, in turn, may reduce the ability of the ventricle to fill properly, reducing the end-diastolic volume (Kienle, 1998).

Left atrial hypertrophy develops as a consequence of the elevated left atrial pressure needed to fill the stiff, hypertrophied left ventricle, due to a decrease in left ventricular compliance. (Bonagura & Lehmkuhl, 1999; Kienle, 1998). In a study, 62% of the animals had atrial dilation indicating that the majority of dogs with clinical signs had adapted to an increase in cardiac workload (Falk, Jönsson, & Pedersen, 2004).

Secondary to the forceful striking of the walls of the ascending aorta, the aortic arch vessels, and brachicephalic trunk, occurs a dilation. This post-stenotic dilation is often greater with severe obstructions, and can range from trivial to severe; however, this relationship is variable (Beijerink *et al.*, 2017; Bonagura & Lehmkuhl, 1999; Kienle, 1998).

Dogs with elevated pressure gradients demonstrate coronary lesions and, presumably, they may be precipitated by the increased left ventricular systolic pressure, increased systolic wall tension, and abnormal coronary blood flow associated with the sub-aortic region (Beijerink *et al.*, 2017).

Over time, ischaemic events in the myocardium may lead to increasing amounts of fibrous tissue as a response to chronic injury. In dogs, myocardial fibrosis seems to increase with age. It is proposed that the development of vascular lesions is related to systolic occlusion of the intramyocardial arteries, due to increased wall tension without a corresponding increase in intra-arterial pressure. Ischemia may result from a reduced density of capillaries within the hypertrophied myocardium, reduced left circumflex coronary artery blood flow and systolic reversal of the intramyocardial coronary blood flow. The subendocardium and papillary muscles are the most vulnerable to ischemia (Flickinger & Patterson, 1967; MacDonald, 2006). Consequently, small injuries to the myocardium close to the conduction system might be lethal, contributing to factors leading to sudden death (Falk *et al.*, 2004). Although the cause of sudden death has not yet been determined, it has been associated with malignant ventricular arrhythmias (Beijerink *et al.*, 2017; Hyun & Park, 2010).

Consequently to the pathogenesis of these defects, systolic function could be maintained within relatively normal standards; however, there may be a diastolic dysfunction when there is chronic, severe, concentric hypertrophy (MacDonald, 2006).

Mild degrees of regurgitation (AR) are recognized with frequency as a complication of AS. It can be related to the involvement of the valve leaflets with the fibrous ring, secondary to trauma created by jet lesions, leading to thickening of the valve, and impaired mobility of the aortic valve leaflets. Isolated, congenital AR is rare. It may also develop as a complication of balloon catheter dilation, bacterial endocarditis, and with tetralogy of Fallot (Bonagura & Lehmkuhl, 1999; Kienle, 1998).

It is suspected that turbulent blood flow also sufficiently damages the endothelium of the aortic valve to predispose the site to bacterial colonisation (Roth, 1994). The frequency of infective

endocarditis in animals with discrete sub-aortic stenosis is not known, because it is uncommonly reported (Muna, Ferrans, Pierce, & Roberts, 1978). The first molecular identification of *B. henselae* and *B. koehlerae*, two zoonotic *Bartonella* species, from valves of dogs with canine infective endocarditis, suggested their role in the pathogenesis of this disease. *Bartonella* DNA was detected in aortic valve tissue from two Boxer dogs with moderate and severe SubAS. These dogs were littermates and housemates (Ohad, Morick, Avidor, & Harrus, 2010).

When consecutive left-sided congestive heart failure develops in patients with AS, it is likely due to slowly developing myocardial factors or complicating factors such as myocardial failure, increased ventricular stiffness, aortic regurgitation, moderate-to-severe mitral regurgitation, atrial fibrillation, or a combination of these factors. The elevated end-diastolic pressure resulting from decreased ventricular compliance is generally only mild and not high enough to produce pulmonary oedema (Bonagura & Lehmkuhl, 1999; Kienle, 1998).

The exercise-induced effects of a sudden increase in left ventricular systolic pressure on ventricular baroreceptor activity are also speculated to be the mechanisms behind exertional syncope and sudden death. Severe hypotension could result from exercise-induced increases in left ventricular pressure, activation of left ventricular mechanoreceptors, inappropriate bradycardia and peripheral arterial vasodilation in the face of the obstruction, resulting in a vagal manoeuvre. To further explain, the Bezold-Jarisch reflex promotes reflex bradycardia, vasodilation, and hypotension after the stimulation by stretch of inhibitory cardiac sensory receptors with nonmyelinated vagal afferent pathways (Bonagura & Lehmkuhl, 1999; Grech & Ramsdale, 1991; Mark, 1983). Some investigators contend malignant ventricular tachyarrhythmias may be noted in a few individuals that collapse, occurring secondary to myocardial ischemia, fibrosis and sympathetic activation (MacDonald, 2006).

3.5 Clinical presentation

3.5.1 History

While collecting the anamnesis it is vital to note the breed, as CHD have an alleged genetic basis and most forms of AS show breed predilections. It is of relevance to review the available records of the progenitors and any siblings that may be available. Stunted growth in comparison with siblings is uncommon (Beijerink *et al.*, 2017; Swift, 1996).

The clinical diagnosis is usually motivated by the recognition of an incidental cardiac murmur during a routine examination or health clinic consult. A dog presents an incidentally detected heart murmur if it is an unexpected discovery during consultation that was not initially focused on the cardiovascular system. Most owners report a normal and apparently healthy individual when presenting the dog for routine examination (Côté *et al.*, 2015; Kienle, 1998; Pyle, 2000). Clinical signs that entertain the potential for aortic stenosis comprise exertional collapse or syncope, rear limb weakness, or, rarely, clinical signs related to congestive heart failure (such as coughing). Sudden death without premonitory signs is of frequent occurrence (Kienle *et al.*, 1994). In puppies, the presence of clinical signs can vary: mild-to-moderate AS can be present in moderately affected individuals and absent in severely affected individuals. However, in puppies with mild AS, clinical signs are minimal (Beijerink *et al.*, 2017; Fernández del Palacio *et al.*, 1998).

3.5.2 Physical Examination

A careful cardiac examination that emphasizes cardiac auscultation is cost-effective and expedient; it is preferred method for the initial identification of CHD in dogs. A cardiac murmur is the characteristic feature of CHD; however, a number or murmurs are undetected in clinical practice. Intractability, panting and rapid heart rates, cardiac rotation, and closely spaced auscultatory areas, typical of puppies pose challenges to auscultation (Beijerink *et al.*, 2017; Kienle, 1998).

Affected dogs can be asymptomatic and have a low-intensity ejection murmur that can be easily confused with the possibility of benign (innocent) systolic murmurs (Pyle, 2000). Nonpathological, soft murmurs, are common in puppies; nevertheless physiological reasons for murmurs, such as fever, infection and anaemia should be excluded, because to define a heart murmur as innocent requires the exclusion of all potential pathological aetiology for the murmur and these should be absent by six months of age (Côté *et al.*, 2015; O'grady *et al.*, 1989).

The most prominent clinical finding of AS is a crescendo-decrescendo murmur, located at the left heart base, or over the right cranial thorax. This murmur originates from the turbulence of blood flow caused by LVOT lesions and varies in intensity, duration, and frequency

components according to the degree of pathological change (*i.e*. the degree of haemodynamic obstruction, heart rate and left ventricular performance) (Kvart *et al.*, 1998).

The murmur is most intense in the sub-aortic region (about the 4th intercostal space) and tends to radiate towards the left apex, up the carotid arteries, on either side of the trachea and even to the calvarium. Frequently the systolic murmur is equally loud at the right cranial thorax (heart base), presumably from radiation into the ascending aorta or from true VAS (Bonagura & Lehmkuhl, 1999; Kienle, 1998).

As previously mentioned, SubAS most likely develops in the postnatal period, thus the murmur may become increasingly prominent during the first months of life. As the obstruction associated with SubAS develops during the first 3 to 8 weeks of life, this progression is significant regarding the detection of murmurs in pups of breeds known to be at risk for SubAS (Bonagura & Lehmkuhl, 1999). The clear majority of dogs with SubAS that are older than 3 months of age will have a heart murmur. However, it may be difficult to attribute the origin of the murmur to SubAS in some cases, because the lesion may not be fully developed and, consequently, the peak LOVTV may be within normal limits. Some dogs may plateau at this stage and are only destined to have mild SubAS. Only dogs that develop severe SubAS can definitely be detected by clinical examination (Kienle, 1998).

In mild cases of AS, the physical examination is unremarkable, other than for the presence of a systolic ejection murmur, usually loudest in the left basilar region, and represent a diagnostic challenge. On auscultation, either no murmur or a grade I-II/VI systolic murmur is heard best in the left third to fifth intercostal spaces, just above the costochondral junction (Pyle, 2000).

In contrast, a moderate to loud systolic murmur or a murmur with a palpable thrill can be indicative of AS in a young animal. Dogs with moderate to severe AS have loud systolic ejection murmurs, heard best in the left third to fifth intercostal spaces and just above the costochondral junction, the lower right second to fifth intercostal spaces and at the thoracic inlet over the carotid arteries. There is often a precordial thrill over the region of maximum intensity (O'grady *et al.*, 1989; Pyle, 2000).

In some cases, a to-and-fro murmur of aortic stenosis/aortic regurgitation is evident. There is a soft diastolic murmur secondary to mild-to-moderate aortic insufficiency, also heard in the left basilar region. These murmurs can be somewhat similar in timing and point of maximal intensity; and therefore, difficult to distinguish. More often, AR is inaudible but can be documented by Doppler echocardiography (Bonagura & Lehmkuhl, 1999; R. Kienle, 1998).

Because of the increased resistance, peak left ventricular ejection is delayed, resulting in a late-rising, variably diminished arterial pulse, hypokinetic (decreased pressure) or and with a tardy delayed peak (*pulsus parvus et tardus*) (Beijerink *et al.*, 2017).

With increasing AS severity, as the flow becomes more unpredictable, the underlying murmur becomes more complex. A study based on the complexity of the phonocardiography signal

has proven this method to be helpful in the differentiation of physical murmurs and murmurs caused by mild AS (Ahlstrom *et al.*, 2008).

Other clinical findings in severe AS include rhythm disturbances and prominent left ventricular apical impulse due to left ventricle hypertrophy (O'grady *et al.*, 1989).

3.6 Diagnosis Methods

The diagnostic workup of the patient with suspected CHD centres today on echocardiography with Doppler studies. Additional examinations include radiography, electrocardiography and clinical laboratory tests. Cardiac catheterization with angiography is still a method for diagnosis, but is rarely undertaken except during catheter therapies.

AS is a problematic disorder for it is very difficult to diagnose in mildly affected dogs and difficult to treat when it is severe. In mild cases of AS, because it is clinically insignificant, however potentially heritable, there is no gold standard for diagnosis.

The gold standard for diagnosis of SubAS is the demonstration of lesions on post-mortem examination. However, the best pre-mortem non-invasive diagnosis of AS is typically based on an estimation of increased aortic velocity by continuous-wave Doppler echocardiography measurement. Other supportive echocardiographic findings of AS may be useful in diagnosis (O'grady *et al.*, 1989; Stern *et al.*, 2012).

New ways of diagnosing disease based on molecular tests, genetic testing and counselling, and development of breed strategies that optimize health over conformation are all topic of deep discussion in the dog world (Parker *et al.*, 2006).

3.6.2 Electrocardiogram

In the absence of a cardiac arrhythmia, the value of multi-lead electrocardiogram (EKG) is lower in the era of Doppler echocardiography. Nevertheless, EKG can often identify moderate to severe atrial or ventricular enlargement, or conduction disturbances that may indicate the presence of AS (Beijerink *et al.*, 2017)

Either left axis deviation or increased R-wave amplitude in lead II with a normal frontal axis can be revealed due to left ventricular enlargement. Left ventricular hypertrophy and myocardial ischemia are also responsible for widening of the QRS complex. S-T segment depression can also occur in lead II as a result of endocardial ischemia and injury to the myocardium; however, its significance in the dog with SubAS remains uncertain (Davainis, Meurs, & Wright, 2004). Holter monitor studies often demonstrate exercise-induced ischemia or ventricular extrasystoles. These changes could be related to the severity of the disease. A normal ECG does not exclude a diagnosis of AS (Bonagura & Lehmkuhl, 1999; O'grady *et al.*, 1989; Pyle, 2000).

3.6.1. Echocardiography

The echocardiogram is the optimal method for identifying and quantitating moderate to severe AS in the dog. The findings that form the basis for diagnosis include: a narrowed LVOT, left ventricular concentric hypertrophy and increased blood flow velocity or pressure gradient within the LVOT. These features can be identified using two-dimensional, M-mode and Doppler echocardiography (Oyama & Thomas, 2002).

3.6.1.1. Doppler ultrasound

Normal blood flow proceeds in a streamlined way, in the presence of aortic stenosis the flow becomes turbulent and is characterized by fluid whirlpools, stirring in all directions, within the vessel. Doppler ultrasound allows the measurement of velocity, direction, and nature of blood flow (Oyama & Sisson, 2001).

For the diagnosis of aortic stenosis, spectral Doppler technology is coupled to routine echocardiography and allows one to measure the velocity of blood flow across the stenotic orifice. As the stenosis progresses, the velocity of blood flow increases (O'grady *et al.*, 1989). The modified Bernoulli equation is used as the tool to examine hydrostatic pressure within individual cardiac chambers and is used to quantify disease severity. Maximal velocity of flow is measured and can be converted to units of pressure (mmHg) via this equation:

$$PG = 4\,x\,LVOTVmax^2$$

Thus, measurement of LVOTVmax or left ventricular systolic pressure gradient (PG) is commonly used as the principal diagnostic means to assess the severity of AS and establish a prognosis (Javard *et al.*, 2014). The flow velocity, however, is dependent on the flow rate and this represents a limitation to the use of this method (Brown & Smith, 2002).

3.6.1.2. Left Ventricular Outflow tract velocity and Pressure gradient

The definite diagnosis of AS typically involves Doppler echocardiography, but clear criteria for SUBAS screening in dogs is controversial, there is no clear consensus among the cardiologists regarding the velocity limits that accurately predict liability for AS (Kienle *et al.*, 1994; Stern *et al.*, 2012).

In the normal dog, the mean Doppler-derived velocity across the aortic valve is has been reported as 1.18 m/s (Yuill & O'Grady, 1991).

In mild cases of LVOTO, subtle changes in the velocity spectra are detected. These include resting maximal velocities of between 1.7 and 2.2 m/sec, associated with spectral dispersion, and mild aortic regurgitation. When these relatively low values are selected as normal limits, the Doppler examination should be sensitive for the detection of mild SubAS. However, the lower the normal velocity limit, the greater the chance that normal dogs with functional murmurs

will be diagnosed as affected. When the slightly higher velocity limits of normal are chosen (such as those recommended in Europe) the diagnosis is likely to be more specific but less sensitive (Bonagura, 2001; Bonagura & Lehmkuhl, 1999; Bussadori *et al.*, 2000).

A study in boxer dogs attempted to minimize this effect, by deducing the usefulness of contrast echocardiography, using microbubble-based sonographic contrast agents, used as enhancers of the subcostal Doppler signals, in which plain doppler signals are difficult to obtain (Höglund *et al.*, 2007).

On the other hand, in cases of moderate to severe obstruction, changes in velocity spectra are dramatic (above 3.5m/s) and maximal Doppler gradients of greater than 50mmHg are considered moderate, and above 80mmHg (4.5m/s) are severe. When peak velocities are found in the LVOT, the values can be then transformed into estimated PG with the simplified Bernoulli's equation. These are the recommended guidelines in Europe (Bussadori *et al.*, 2000).

Aortic insufficiency is also a common finding in AS, and the regurgitant jet is usually subjectively classified in severity and it is detected by Colour-flow Doppler. With this technique the spectral Doppler information is superimposed over the 2D image as a series of colours corresponding to blood-flow direction and velocity (Oyama & Sisson, 2001). Mitral regurgitation may also be detected by doppler when there is concurrent mitral valve dysplasia or involvement of the septal mitral valve leaflet in the sub-aortic ridge (Bonagura & Lehmkuhl, 1999).

3.6.1.3. Anatomic (two-dimensional) assessment.

It is important to additionally document and classify any recognized pathoanatomical abnormality of the recordings for the differentiation between types of LVOTO. (Bussadori *et al.*, 2000). For this purpose, two-dimensional echocardiography enables one to visualize the internal anatomy of the heart (O'grady *et al.*, 1989).

The left ventriculogram outlines a the ventricular cavity and illustrates the presence of sub-valvular obstruction, post-stenotic dilation, reduced LV orifice area, concentric LV hypertrophy, aortic leaflet structural changes (such as thickening), and intercurrent complications like mitral regurgitation, and prominence of the left coronary artery branches (Beijerink *et al.*, 2017).

3.6.1.4. EOAi and AoSA

The pressure gradient and blood flow velocity are used as indexes of lesion severity. However, because they are affected by both lesion severity (resistance) and flow, they are less accurate than determinations of the lesion's cross-sectional area of resistance (Beijerink *et al.*, 2017; M. C. Belanger, Côté, & Beauchamp, 2014; Javard *et al.*, 2014).

The aortoseptal angle has been associated with AS in dogs, becoming steeper as severity increases, and it is hypothesized that it could represent a factor for the progression of SAS in Boxers and in Golden Retrievers (Belanger *et al.*, 2014; Quintavalla *et al.*, 2010).

The direct measurement of the stenotic region via the LVOT:Ao (Left ventricular outflow tract to aortic orifice) ratio is unaffected by flow and should be accurate across a wide range of cardiac outputs (Oyama & Thomas, 2002).

The EOAi (effective orifice surface area indexed to body surface area) is an accurate indicator of AS in dogs and it was considered to be a superior in predicting the occurrence of adverse events in the SAS dogs when compared with currently used echocardiographic parameters such as LVOTVmax and transstenotic gradients. The EOA represents the cross-sectional area occupied by the flow at the level of the *vena contracta* (Bélanger, Di Fruscia, Dumesnil, & Pibarot, 2001).

A study using EOAi and Doppler in combination associated a high likelihood of progression of AS in Golden Retrievers puppies, in fact, the same puppy could have a higher probability of satisfying criteria for AS in the adult stage. When both of these measurements were used in combination, the sensitivity could be slightly higher, however, the usefulness of EOAi in puppies as an early marker for SubAS is rather unclear (Javard *et al.*, 2014).

3.6.3 Radiology

Thoracic Radiographs are typically normal in mildly affected individuals. Regarding aortic stenosis, the great majority of affected dogs have no abnormalities on survey radiographs (O'grady *et al.*, 1989).

In moderate to severely affected dogs the radiographic identification of left ventricular and atrial enlargement, dilation of the great vessels, and pulmonary circulation changes may be possible (Beijerink *et al.*, 2017).

In severely affected dogs, apparent mild cardiomegaly as a result of left ventricular concentric hypertrophy may also show on radiographs. Another finding in severely affected dogs is enlargement of the aortic root, with widening of the mediastinum on the dorsoventral view, or loss of the caudal waist of the cardiac silhouette on the lateral view. Unless pulmonary oedema and venous congestion are present, pulmonary circulation is normal and no indication is found on radiographs. When moderate to severe left atrial enlargement is present, intercurrent mitral regurgitation should be suspected (Bonagura & Lehmkuhl, 1999; Kienle, 1998).

The results of radiography should be considered as only one part of the diagnostic assessment in order to avoid placing unwarranted emphasis on the perceived size or shape of the cardiac silhouette and associated structures. Even in the hands of experienced clinicians, survey radiography is seen as an inaccurate method of diagnosing AS (Lamb, Boswood, Volkman, & Connolly, 2001).

3.6.4 Cardiac catheterization

In the 1960's, cardiac catheterization was the gold standard method of diagnosis used to identify and quantitate SubAS (O'grady *et al.*, 1989).

Most catheterization procedures involve hemodynamic measurements, mainly intracardiac and intravascular pressures, along with angiography and some other forms of catheter based interventions. Due to anaesthetic effects, hemodynamic measurements, especially blood pressures and cardiac output, are markedly depressed compared to the awake, or lightly tranquilized states. Doppler has a great advantage over catherization in that it doesn't require the use of general anaesthetic or potentially harmful contrast agents, or the insertion and manipulation of intracardiac catheters (O'grady *et al.*, 1989). Cardiac catheterization measurements will not correspond to Doppler-derived measurements, unless they are performed under the same conditions, as dogs under general anaesthesia show depressed pressure gradients, as a result of diminished flow (reduced stroke volume) (Lehmkuhl, Bonagura, Jones, & Stepien, 1995).

Today, cardiac catheterization is mainly used only in procedures designed to repair or palliate AS, including the example of balloon valvuloplasty (Shen *et al.*, 2017).

3.7. Prognosis

Some forms of AS indicate that many dogs with mild disease can be expected to have a normal life expectancy in the face of this congenital heart defect, even without any treatment. If the stenosis is mild, dogs will experience no decrease in lifespan, these dogs only rarely exhibit clinical symptoms (Beijerink *et al.*, 2017; O'grady *et al.*, 1989).

However, the severity of aortic stenosis can range from trivial to life-threatening, and clinical signs may manifest anytime from the neonatal period to relatively late in life. Whenever mild-to-moderate SubAS has been documented in a young dog, no prognosis should be given, because the obstruction often does ultimately become more severe (Kienle, 1998).

Dogs with minimal ventricular hypertrophy, mild ventricular outflow obstruction, and a maximal doppler pressure gradient of less than 75mmHg, are likely to be relatively normal pets. Dogs with Doppler gradients exceeding 100 to 125mmGg are prone to develop complications or sudden death (Bonagura & Lehmkuhl, 1999).

If the velocity of blood flow across the stenotic orifice fails to exceed five meters per second by the time a dog reaches maturity, it has been suggested that these dogs will likely experience no reduction in longevity or quality of life (O'grady *et al.*, 1989).

Moderate stenosis can be well tolerated, although severe functional consequences can occur, including CHF, cardiac arrhythmia or sudden cardiac death. Complications usually occur in dogs with mild-to-moderate obstructions, probably because these dogs live long enough to develop secondary complications. Mitral regurgitation, mitral stenosis, aortic regurgitation, aortic valve endocarditis, and atrial fibrillation cause additional complicating factors increasing morbidity and mortality (Beijerink *et al.*, 2017).

Severe SubAS is a discouraging condition, since most dogs either die suddenly, develop congestive heart failure or have symptoms that degrade their quality of life. It has a poor prognosis. Sudden death usually occurs in the first three years of life (median age of sudden death is 14.4 months), mainly but not exclusively in dogs with severe obstructions – gradient greater than 80 mmHg. Up to 70% of severely affected dogs die suddenly during this time (Kienle *et al.*, 1994). There is indication that when the Doppler velocity across the stenotic orifice reaches six to seven meters per second, the dog can be expected to succumb to the disorder in the very near future (O'grady *et al.*, 1989).

Congestive heart failure is particularly likely in dogs with intercurrent mitral valve malformation and regurgitation or severe aortic regurgitation. Dogs born with moderate to severe mitral valve dysplasia that develop moderate-to severe SubAS usually develop severe left congestive heart failure within the first three to six months of age and die young (Beijerink *et al.*, 2017; Kienle, 1998).

3.8. Prevention

Dogs with genetic coding for SubAS may clearly remain unidentified using currently approved screening methods, especially when dogs less than six months of age are involved. Because the lesion may be clinically silent in dogs less than three months of age, dogs less than this age cannot be classified as free of the disease based on any type of clinical examination except for necropsy. This makes genetic counselling difficult in dogs of breeds known to be at increased risk for SubAS when a soft systolic murmur of unknown or questionable origin is identified, for even a normal physical examination does not equate with genetic normalcy (R. Kienle, 1998)

The complexity and doubt surrounding the mode of inheritance of many cardiac defects deter the counselling of owners and breeders who want to use specific dogs for breeding. Even with careful attention to pedigree, and the result of breeding trials, it can be very difficult to influence the overall prevalence of AS in the larger population. Progress in this regard hinges on the development of economical genetic tests that can detect the presence of genetic mutations responsible for specific malformations. (Beijerink *et al.*, 2017)

In 1991, nearly half of all dogs referred to the Royal School of Veterinary Study in Edinburgh with sub-aortic stenosis were Boxers. Because of this, a scheme to control breeding has been in operation in the United Kingdom since 1990. The scheme used Doppler echocardiography to confirm the diagnosis after the detection of a murmur higher than grade II/IV (velocities above 2.0m/s were considered abnormal). Because of the high prevalence of murmurs in the breed, using dogs without irregularities at auscultation would severely limit the gene pool available for breeding. Early breeding data suggested the program was working well (Swift, 1996).

3.9. Management options

3.9.1. Clinical approach

Therapy is targeted at preventing sudden death, reducing exercise intolerance or syncopal episodes. Medical therapy for CHF or atrial fibrillation can be beneficial in some dogs. Therapy usually is unnecessary in mildly affected individuals, and the efficacy of treatment is undetermined in cases of moderate-to-severe aortic stenosis. Dogs with moderate-to-severe SubAS should have restricted exercise. In dogs with LOVTO, prophylaxis for bacterial endocarditis should be used prudently and in appropriate circumstances, such as in periods of anticipated bacteraemia. The use of antibiotics prior and after surgery or dentistry have been recommended to minimize the risk of aortic valve infection (Beijerink *et al.*, 2017; Bonagura & Lehmkuhl, 1999; Kienle, 1998; MacDonald, 2006).

3.9.2 Medical Therapy

The foundation of therapy has been the use of beta-adrenergic blocking agents, such as atenolol, to dogs with a history of syncope or documented exercise intolerance, to reduce the likelihood of ventricular arrhythmia. Beta-blockers reduce myocardial oxygen consumption, increase coronary perfusion secondary to the negative chronotropic and inotropic effects (decreasing heart rate and contractility), and protect the diseased myocardium against arrhythmic effects of sympathetic surges (catecholamine activity) (MacDonald, 2006).
In the presence of CHF, medical therapy with diuretics such as digoxin, furosemide, dietary sodium restriction, and nitrates can be initiated. Low doses of angiotensin-converting enzyme inhibitors may be prescribed. Cautionary use of arterial vasodilator agents is advised in the setting of fixed outflow tract obstruction. None of these medical strategies has been evaluated in placebo-controlled trials (Beijerink *et al.*, 2017; Bonagura & Lehmkuhl, 1999).

3.9.3 Surgery

The pressure gradient can be reduced by aggressive balloon catheter dilation (at the risk of increasing aortic regurgitation), or by surgical resection of the sub-valvular ring during cardiopulmonary bypass. Unfortunately, neither has been proven to effectively reduce morbidity or mortality in the majority of treated dogs, as this benefit attenuates over time. Surgical excision of the fibrotic ring and septal myectomy under cardiopulmonary bypass effectively reduce the systolic pressure gradient but do not alter the survival time compared with dogs treated with atenolol (Orton *et al.*, 2000). Likewise, Balloon valvuloplasty moderately reduces the systolic pressure gradient but does not alter survival compared with atenolol (Meurs, Lehmkuhl, & Bonagura, 2005),

A novel procedure combining cutting balloon and high-pressure balloon valvuloplasty has been studied as a possible treatment for dogs with severe SubAS. It is, nonetheless, unknown if this approach results in the effective reduction of the PG over time (Kleman *et al.*, 2012). Recently, the amplitude of AoSA has been suggested as useful to select candidates for treatment with this technique, especially regarding the determination of the patient's long term ability to maintain a persistent reduction in systolic left ventricular to aortic PG (Shen *et al.*, 2017). The motive surgeries seem to have limited impact on the complete resolution of the obstructions is associated with shear stress on the basalmost part of the interventricular septum immediately apical to the aortic valve, as a result of irregular blood flow caused by abnormal septal angles and possibly other anatomical variations (Shen *et al.*, 2017).

Most surgical procedures entail great risks of life-threatening complications, such as fatal arrhythmia, development of aortic valve endocarditis, rupture of the aortic annulus, and avulsion of the brachiocephalic artery during balloon withdrawal. The procedures are usually limited in availability and are prohibitively expensive to be deemed as adequate and practical options. The inability of the current surgical approach in preventing sudden death is indicative that effective treatment requires more than the reduction of the pressure gradient(Beijerink *et al.*, 2017).

B. Retrospective clinical study in aortic stenosis in dogs.

1.Objective

This retrospective study was designed to investigate and characterize AS in dogs presented to Border's Cardiology Referral, in a period of 11 years. The aim of the present study is to assess the distribution of the different forms of AS, to determine the main epidemiological features, including the relationship between signalment, clinical features, and associated heart disease of patients at the time of presentation and/or diagnosis of aortic stenosis.

2.Materials and Methods

The medical records and diagnostic studies of all dogs with suspected or confirmed aortic stenosis presented to a second opinion, mobile cardiology referral service in Scotland, were reviewed retrospectively. The data used in these analyses were obtained by searching through the Border's Cardiology Ltd. electronic records from 08/09/2005 to 21/12/2016. This period of time was used due to the availability of records. The search was conducted by the use of "aortic stenosis" as a keyword. For this keyword, a database was created.
Patient records contained fields comprising relevant history, clinical signs, clinical diagnosis (including electrocardiography and echocardiography), and other comments.

2.1. Signalment

The entire medical record for each dog was interrogated. Demographic data from these records were extracted from the database and tabulated. Data obtained from the medical records included patient identity, clinical diagnosis, breed, sex, age at presentation, presenting complaint, clinical signs (syncope, intolerance to exercise, congestive heart failure, dyspnoea or cough); pulse quality; presence or absence, location and intensity of murmurs (graded from I/VI to VI/VI): electrocardiogram findings (indication of enlargement or other irregularities), echocardiographic findings (presence or absence of aortic valve leaflet thickening, presence or absence of post-stenotic dilation, presence of absence of concentric left ventricular hypertrophy, presence or absence of aortic regurgitation) indicators of severity (LVOTV or PG across the stenotic region) and presence of concurrent congenital defects.

2.2 Diagnostic assessment

The examinations were performed by an RCVS Diplomate in Veterinary Cardiology. However, a few cases were diagnosed by a veterinarian with an RCVS Certificate in Veterinary Cardiology, during the period of 2009-2012. All dogs had been evaluated with auscultation

and echocardiographic examination, with continuous-wave Doppler measurements of aortic velocity and/or pressure gradient. EKGs were simultaneously obtained. Radiographs were inconsistently present in the reports for they were only performed as indicated.

Many echocardiograms were reviewed by the examiner to ensure consistency of diagnosis and each record was screened for accuracy. When more than one report was available for one dog, the report with the highest LVOTVmax and peak pressure gradient was considered.

Due to inconsistencies concerning the definition of various AS, particular criteria were used for disease classification in this study. Affected dogs were classified as diagnosed with valvular, sub-valvular, supra-valvular stenosis or as unaffected. Cases of equivocal diagnosis and unclassified diagnosis (when there were no evident lesions on echocardiography or the records were incomplete but the velocity was above 2.25m/s) were also included in the study. The diagnosis of sub-aortic stenosis (SubAS) was only definitive if a discrete fibrous ridge was identified on two-dimensional echocardiography or if the stenosis was immediately below the valve, but associated with the valve apparatus.

In terms of severity, patients were divided into four groups, depending on blood flow velocity through the aortic valve or pressure gradient as equivocal, mild, moderate and severe. Table 1.

Table 1. Severity according to velocity

Diagnosis	Maximum LVOTV	Peak PG
Equivocal *	1.7-2.25m/sec	From 12 to 20 mmHg
Mild	2.25-3.50m/sec	from 20 to 49 mmHg
Moderate	3.50-4.50m/sec	from 50 to 80 mmHg
Severe	over 4.50m/sec	above 80 mmHg

*Equivocal cases were only considered when they had concomitant morphologic abnormalities on 2D or aortic regurgitation.

2.3. Inclusion criteria

A dog was eligible for inclusion in this study when records of direct imaging of the obstructive lesions and/or turbulent aortic flow with a peak velocity of 1.7m/s or greater by continuous wave Doppler echocardiography (estimated by the modified Bernoulli's equation).

Dogs with LVOTV between 1.71 and 2.25m/s were considered equivocal if no obstructive lesions were evident. In cases where the LVOTV was above 2.25m/s without evident structural abnormalities or regurgitation on echocardiography, and in cases of incomplete records that fitted within the previous clause, the dogs were included in the study under the designation of

unclassified, because, although it was not possible to determine the anatomical lesion that originated the stenosis, the velocity was above the cut-off value.

2.4 Exclusion criteria

Of the 406 likely cases of AS firstly identified, 132 were excluded as they did not meet the diagnosis requirements, or had incomplete medical records. 274 remained with equivocal or confirmed diagnosis of AS.

Dogs were classified as unaffected when maximal LVOTV was <1.7m/s and had no concurrent structural abnormalities or evidence of aortic regurgitation.

Any record that referred to suspected AS, a presumptive diagnosis or that included a diagnosis that was unconfirmed were excluded from the study However, in cases when the examiner could not determine if the patient was affected or not the examination was “equivocal”, for this purpose, a category with LOVTV between 1.7 and 2.25m/s without additional malformations was created.

It is also relevant to note that dogs sedated on examination were included in the present study, However, the degree of the obstruction’s severity could be slightly underestimated. Dogs with a previous history of endocarditis were excluded.

3.Statistical analysis

Data processing and statistics were performed resorting to R® Statistical Software for Windows, version 3.4.2 (R Core Team, 2017), and Microsoft Excel 2016®.

The Pearson’s Chi-squared Test was used to determine whether there was a significant difference between the expected frequencies and the observed frequencies between qualitative variables, thus evaluating their association. The Fisher’s exact test was used to assess this association when expected frequencies were less than five. For a value of $P < 0.05$ the null hypothesis was rejected, and the association was considered significant.

Qualitative data are expressed in absolute frequency and in percentage, quantitative data are expressed in means and standard deviation.

4.Results

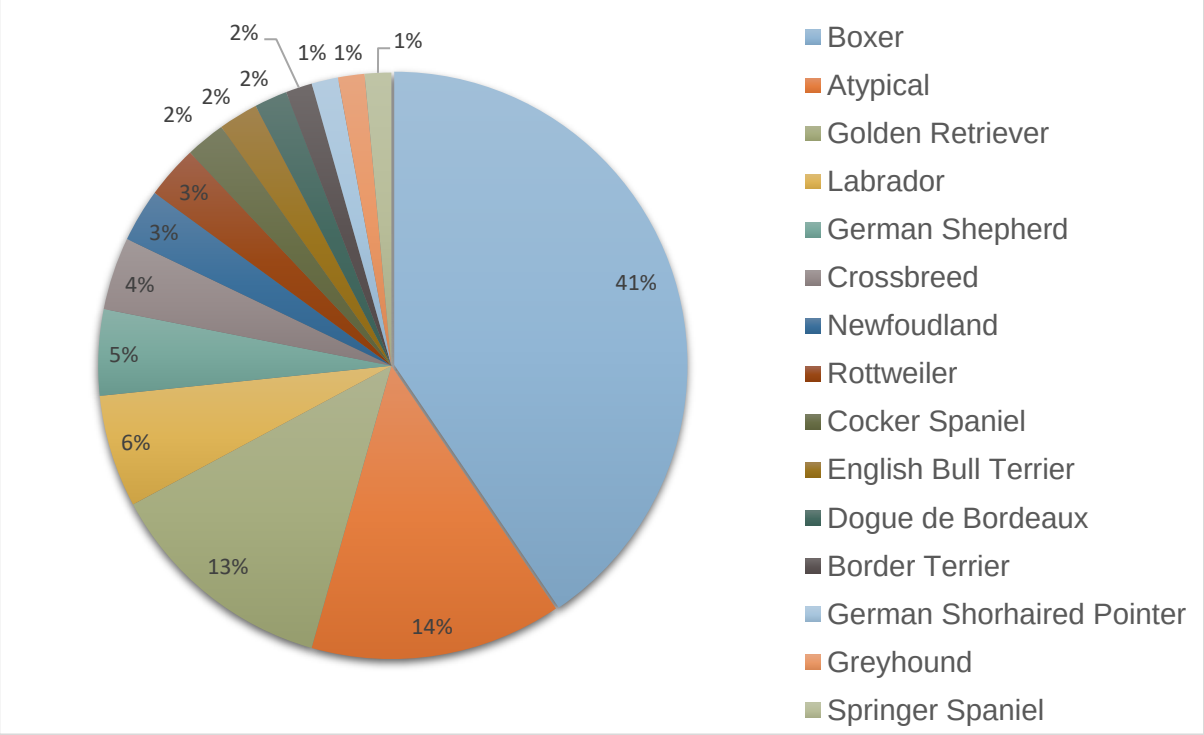
4.1. Sample Characteristics

The present retrospective study considered a total of 274 dogs (100%) with AS for evaluation.

4.1.1 Breed

A total of 41 breeds are represented in this study, in addition to crossbred dogs. However, only breeds represented with a minimum of four dogs were characterized in the analysis.
Almost half of the all the dogs in the sample were Boxers (111- 40.51%), followed by breeds with less than four representatives (38 - 13.87%), Golden Retriever (35 - 12.77%), Labrador (17 - 6.20%), German Shepherd Dog (13 - 4.74%), Crossbreed dogs (11 - 4.01%), New Foundland Dog (8 - 2.92%), Rottweiler (8 -2.92%), Cocker Spaniel (6 - 2.19%), English Bull Terrier (6 - 2.19%), Dogue de Bordeaux (5 - 1.82%), German Shorthaired Pointer (4 - 1.46%), Border Terrier (4 - 1.46), Springer Spaniel (4 - 1.46%), Greyhound (4 - 1.46%). Chart 1

Chart 1. Distribution of breeds represented by more than four individuals.



Breeds with less than four representatives were the Alaskan Malamute (1) , Bernese Mountain Dog (1), Bichon Frise (2), Black Russian Terrier (1), Border Collie (1), Bull Mastiff (1), Cairn Terrier (1), Flat Coated Retriever (1), French Bulldog (1), Great Dane (2), Irish Setter (2), Irish Terrier (1), English Bulldog (2), Leonberger (1), Lhasa Apso (1), Munster (1), Northern Inuit Dog (1), Löwchen (1), Rhodesian Ridgeback (1), Schnauzer (1), Siberian Husky(1),

Staffordshire Bull Terrier (1), Hungarian Viszla (1), West Highland White Terrier (2), Wheaten Terrier (1), Yorkshire Terrier (1), and Old English Sheepdog (3).

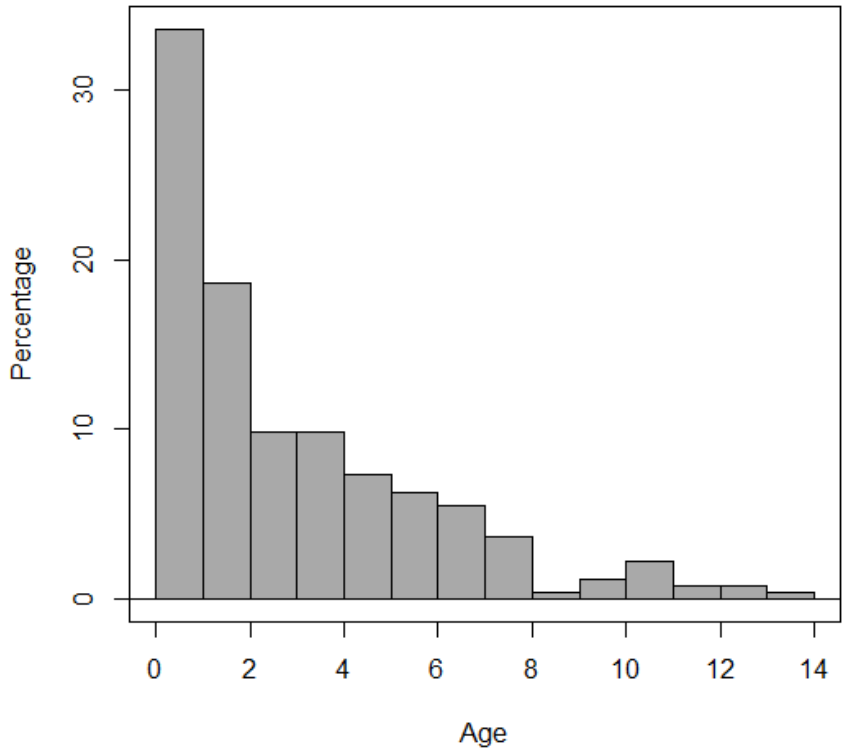
4.1.2. Gender

In the sample, a slightly higher number of males was present: 155 (56.57%) whilst there were 119 females (43.43%) also counted.

4.1.3. Age at presentation

Dogs in this study ranged from 1 month to 14 years of age at time of evaluation, of these 92 (33.57 %) were puppies under one year of age, and 182 (66.43 %) were adults (above one year of age). Chart 2. The mean age at presentation was 3 years and 2 months and the sample deviation was of 3 years.

Chart 2. Distribution of age at presentation



4.2. Diagnostic and clinical characteristics

4.2.1. Clinical significance

In the sample, 40.51% of dogs (111) had clinical signs at presentation, the remaining 59.49% (163) were asymptomatic. The motive for the first consultation was most frequently a recently detected heart murmur 128 dogs (46.72%), followed by syncope (53 - 19.34%), heart murmur investigation 33 - (12.04%) and intolerance to exercise (12 - 4.38 %). An additional 21 (7.66%) were follow up consultations. The frequency of clinical signs in symptomatic dogs was topped by intolerance to exercise (76 - 27.44%), syncope (67 - 24.45%), dyspnoea or cough (31 - 11.31%), congestive signs (15 - 5.47%) and lastly, failure to thrive (6 - 2.2%). Table 2.

Table 2. Distribution of clinical signs at presentation

Clinical signs at presentation	**N**	**%**
Intolerance to exercise	76	27.44
Syncope	67	24.45
Dyspnoea or cough	31	11.31
Congestive signs	15	5.47
Failure to thrive	6	2.2

4.2.2 Physical examination

The most usual finding on physical examination was the presence of a murmur on cardiac auscultation, as 272 cases (99.27%) exhibited a left heart base murmur, (182 - 66.42%) were similarly heard over the right hemithorax, and (168 - 61.54%) over the thoracic inlets. In table 3, the frequency of the distribution of heart murmurs according to intensity is shown.

Pulse quality in the sample is characterized as normal in (245 - 89.42%), reduced in (16 - 5.84%) and poor in (13 - 4.74%).

Table 3. Distribution of murmur intensity on auscultation

Murmur intensity	N	%
Silent	2	0.73
I/VI	3	1.10
II/VI	120	43.96
III/VI	67	24.54
IV/VI	43	15.75
V/VI	37	13.55
VI/VI	1	0.37

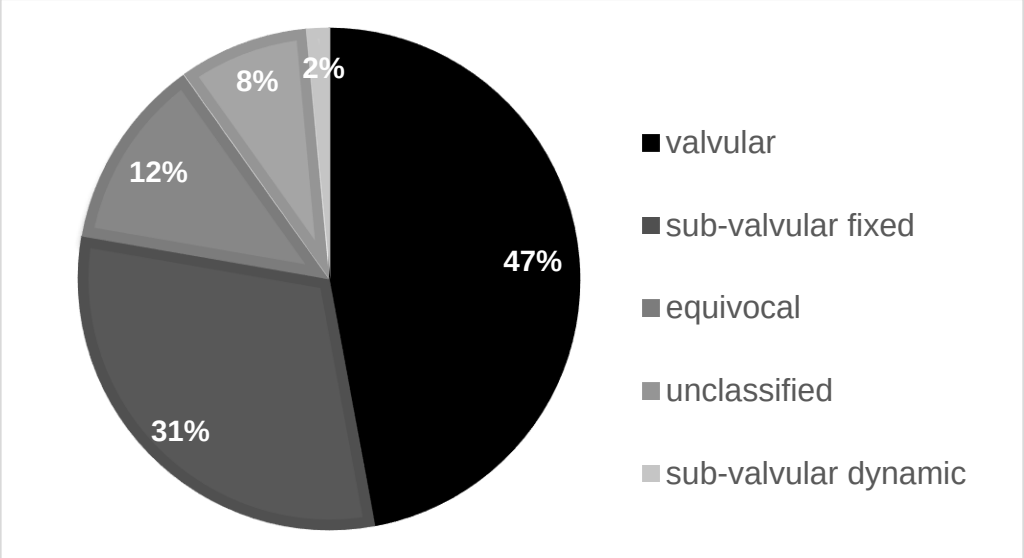
4.2.3. Electrocardiogram

The EKG was normal in 165 dogs (70.82%); however, (68 - 29.18%) had changes considered to be pathological, and 36 dogs (15.45%) qualified as having signs of heart enlargement.

4.2.4. Anatomical classification

The anatomical classification of AS was valvular in 129 cases (47.08%), 84 cases (30.66%) were sub-valvular fixed, (4 - 1.46%) sub-valvular dynamic, (34 - 12.41%) equivocal and (23 - 8.39%) unclassified diagnosis. Chart 3.

Chart 3. Distribution by anatomical classification



4.2.5. Echocardiographic findings

The frequency of findings obtained on echocardiography, in ascending order, were: post-stenotic dilation (7 - 2.55%), concentric left ventricular hypertrophy (64 - 23.44%), aortic valve leaflet thickening (106 - 39.11%) and aortic regurgitation (173 - 63.14%).

4.2.6. Indicators of severity (LVOTV and PG)

The left ventricular outflow tract velocity ranged from 1.71 to 9.00m/s with a mean of 3.05± 1.32 m/s. The severity of the stenosis, according to ejection velocity was classified as: equivocal in 34 cases (12.41%), mild in (154 - 56.20%), moderate in (36 - 13.14%) and severe in (50 - 18.25%).

4.2.7. Concurrent Heart Disease

In the sample, 161 dogs had evidence of concurrent heart disease (58.76%). Concomitant congenital heart disease found in this study by ascending order are pulmonic stenosis in 13 dogs (4.74%), tricuspid valve dysplasia in 48 dogs (17.52%) and mitral valve dysplasia in 110 (40.15%). Table 4.

Table 4. Associated congenital defect distribution

Associated congenital Heart Defects	N	%
Mitral valve dysplasia	110	40.15
Tricuspid valve dysplasia	48	17.52
Pulmonic stenosis	13	4.74
Ventricular septal defect	7	2.55
Patent Ductus Arteriosus	4	1.46
Persistent Left Cranial Vena Cava	3	1.09
Double Chambered Right Ventricle	2	0.73
Mitral valve stenosis	2	0.73
Atrial Septal Defect	1	0.36
Coronary Artery Malformation	1	0.36

Other intercurrent heart diseases found were mainly: mitral valve regurgitation (124 - 45.26%), tricuspid valve regurgitation (30 - 10.45%), and myxomatous mitral valve degeneration (22 - 8.06%). Table 5.

Table 5. Concurrent heart disease distribution

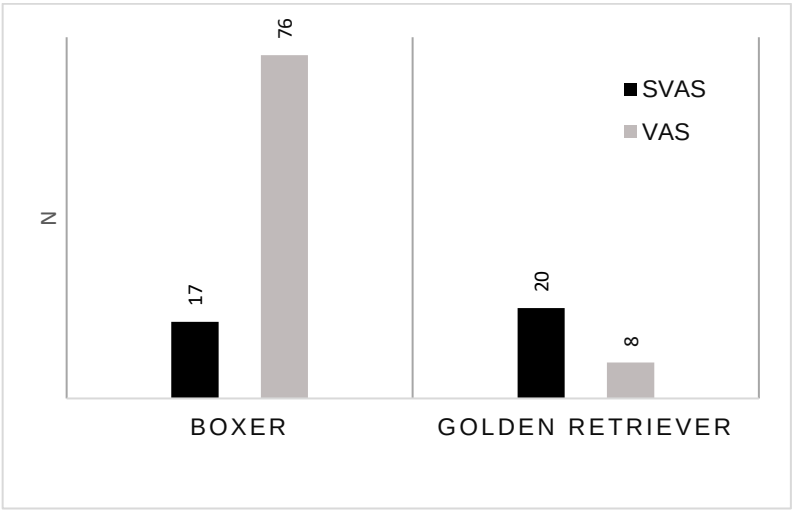
Concurrent heart disease	N	%
Mitral valve regurgitation	124	45.26
Tricuspid valve regurgitation	30	10.45
Myxomatous mitral valve degeneration	22	8.06
Eccentric left ventricular hypertrophy	4	1.46
Arrhythmogenic cardiomyopathy of the Boxer	3	1.09
Heart base mass	2	0.73
Interventricular septum aneurysm	1	0.36
Right ventricular concentric hypertrophy	1	0.36

4.3. Significant relationships between variables and diagnostic features.

4.3.1. Breed and Severity

In this category, dogs of different breeds were divided in two groups according to the anatomical location of the defect (valvular or sub-valvular cases). The equivocal and unclassified dogs were excluded from this analysis. In the Boxer breed, a predominance of valvular aortic stenosis 76 (82.11%) was found ($P = 7 \times 10^{-8}$). The Golden Retriever revealed a predominance of subvalvular aortic stenosis 20 (71.42%) ($P = 3 \times 10^{-4}$). For the remaining breeds, this association was not significant (P>0.05). Chart 4.

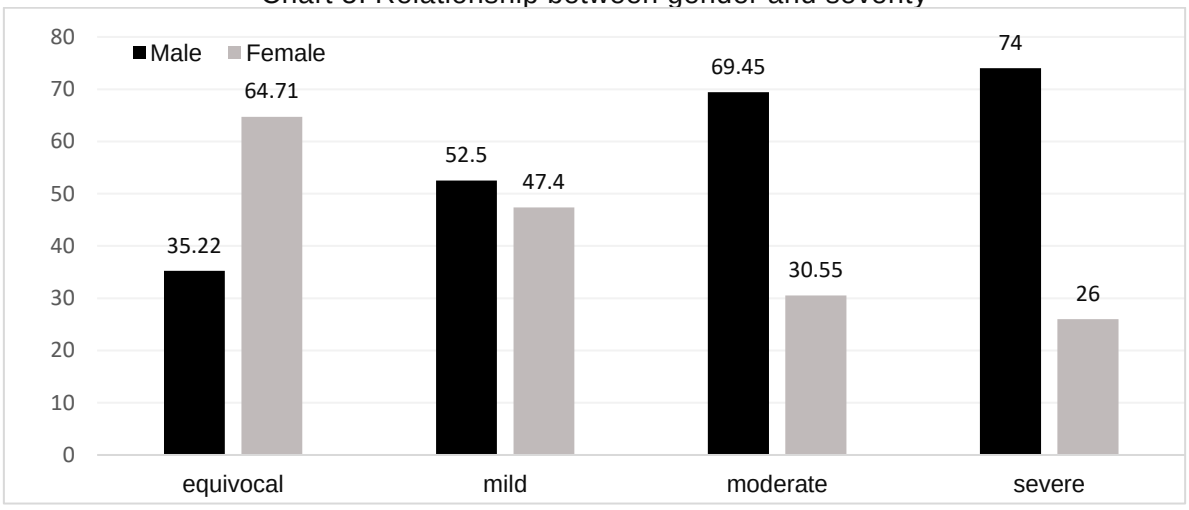
Chart 4. Relationship between breed and anatomical classification of AS



4.3.2. Gender and severity

A significant relationship between gender and severity was established. Whilst females represented the majority in the equivocal category, males surpassed females in the subsequent categories with an increasing difference in percentage (P=0.001). Chart 5.

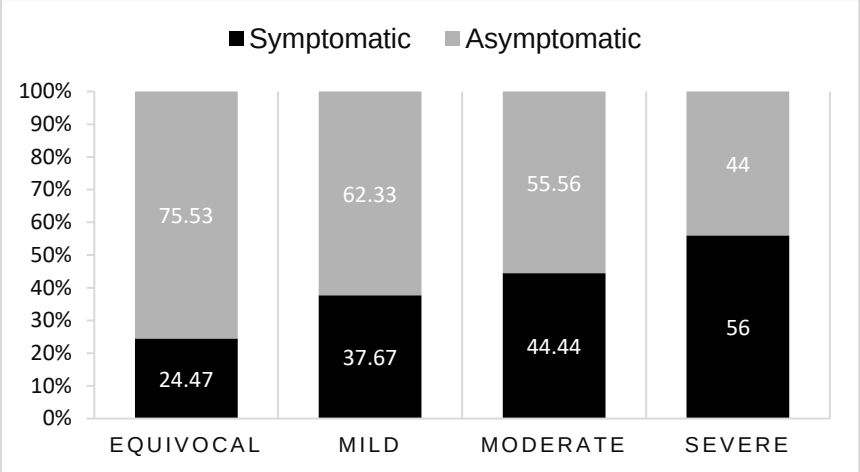
Chart 5. Relationship between gender and severity



4.3.3. Presence of clinical signs and severity

The presence of clinical signs increased with severity, yet only in severe cases did the percentage of symptomatic dogs exceed the percentage of silent dogs (P=0.03). Chart 6.

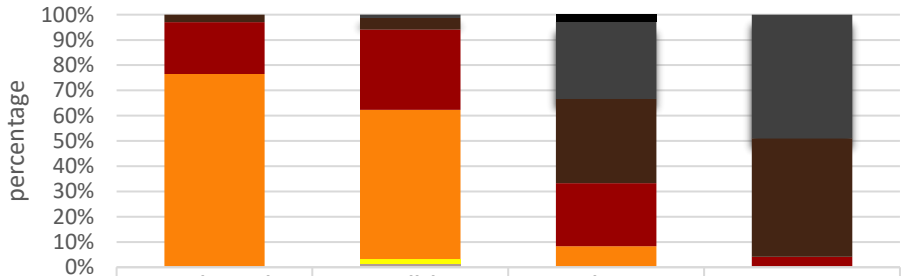
Chart 6. Relationship between presence of clinical signs and severity



4.3.4. Murmur intensity and severity

Murmur intensity increased with severity. In equivocal cases, the intensity of the murmur was most often a II/VI grade; in mild cases, a II/VI grade; in moderate cases, a IV/VI grade, and in severe cases, a V/VI grade ($P<2.2x10^{-16}$). Chart 7.

Chart 7. Relationship between murmur intensity and severity

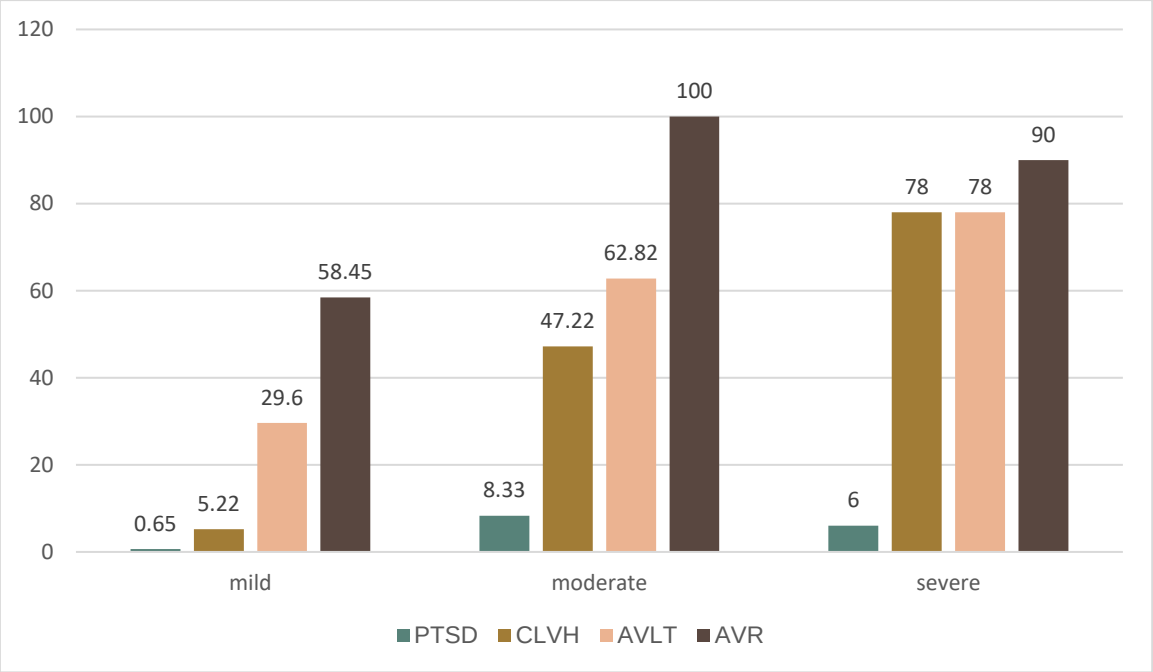


	Equivocal	Mild	Moderate	Severe
VI/VI	0	0	1	0
V/VI	0	2	11	24
IV/VI	1	7	12	23
III/VI	7	49	9	2
II/VI	26	91	3	0
I/VI	0	3	0	0
Silent	0	2	0	0

4.3.4. Presence of echocardiographic findings and severity

The relationship between severity and concentric left ventricular hypertrophy, aortic valve leaflet thickening, aortic valve regurgitation (P<2.2x10-16) and post-stenotic dilation (P=0.01) were significative. Chart 8.

Chart 8. Relationship between echocardiographic findings and severity



5.Discussion

5.1. Review framework and limitations

This study retrieved data from records of dogs examined by a mobile cardiology referral company. The background of these cases was not restricted to a specific Scottish region. On the contrary, the geographical coverage of the practice was extensive. It is likely, nonetheless, that this study rather underestimates the manifestation of aortic stenosis, for it is a second opinion practice and the cases that reach Border's Cardiology for examination have been filtered by first opinion practices and are dependent on the owner's acceptance of referral.

It is also important to note that differences in breed distribution in Scotland and the detection of murmurs in an early stage are factors that influenced the variables of the study.

The retrospective nature of this study is a limitation, as the records used were not designed specifically for this purpose and there were inconsistencies in the availability of data, leading the ruling out of 132 cases. To counteract these effects, when information differed in reports, the examiner reviewed the respective patient's echocardiographic records. It was not possible, in spite of this, for the examiner to review the echocardiographic records of patients presented prior to 2008. In these cases, the tabulated information originated solely from reports.

The retrospective nature of the study allowed for a substantial number of cases of aortic stenosis to be examined. This would be difficult to achieve in a prospective study due to the low prevalence of this defect in the general population.

The diagnosis of aortic stenosis was based mainly upon LVOTV or PG obtained using spectral Doppler. Because it is a technique that is flow-dependent, it is not appreciable how sympathetic activity and high cardiac output may have increased the ejection velocity across the stenotic LVOT. In the Boxer, a study has found that the effects of emotional stress seem to be a major determinant of change in velocity and recommended that dogs should be given enough time to acclimate to an environment and physical restraint prior to examination (Pradelli *et al.*, 2014). The current study was not able to recollect data relating to the animal's overall emotional status on examination, as this could also contribute to bias in the inclusion of dogs.

In this context, authors suggest that higher cut-offs could be considered for specific breeds, such as the Boxer and the Golden Retriever (Pradelli *et al.*, 2014; Stern *et al.*, 2012). Yet, in this study, these cut-offs were not applied.

In the absence of a gold-standard antemortem method of diagnostic for SubAS, and bearing in mind the factors that impact changes in velocity, some dogs included in the equivocal category could actually represent a mildly affected state of SubAS and vice-versa. Thus, it is probable that this study slightly overestimates the frequency, including as it does, the milder malformations. It makes this study to be more sensitive yet relatively less specific and could allow normal dogs to pass as mildly affected (Bonagura, 2001). In contrast, mildly sedated

dogs were included in this study leading to the underestimation of the velocity and consequently severity in a few cases. However, this factor is less likely to influence velocity than emotional stress.

The records obtained are from a time period of 11 years. Within this period, the recommended guidelines for the diagnosis of aortic stenosis have altered, such that the cut-off for determining presence of AS by aortic velocity has changed over time. This factor could also contribute to introduce bias in the diagnosis of equivocal cases represented in this study such that some of the cases that would fall in the equivocal range may not have been consistently documented.

Aortic stenosis is a complex, multifaceted disease and the determination of clinical severity is not as simple as choosing the optimal hemodynamic index. However, the introduction of stenosis indices including AoSA and iEOA in echo reports may provide helpful and useful information for future guidelines on the echocardiographic diagnosis of aortic stenosis (Bonagura, 2001).

5.2. Signalment

In the present sample, Boxers and Golden Retrievers are overrepresented. Other breeds found that had been reported in previous studies are the German Shepherd Dog, the Labrador Retriever, the German Shorthaired Pointer, the English Bull Terrier, the Rottweiler, the Great Dane, the Black Russian Terrier, the English Bull Dog, the Border Collie, the Irish Setter, the Irish Terrier, the Bull Terrier, the West Highland White Terrier, the Yorkshire Terrier, the Newfoundland Dog and the Cocker Spaniel. Crossbreeds in the study were also included and represented four percent of the totality of patients.

Breeds represented as having aortic stenosis in this study that have not previously been described in other studies are: the Border Terrier, the Alaskan Malamute, the Cairn Terrier, the Greyhound, the Springer Spaniel, the Leonberger, the Lhasa Apso, the Bernese Mountain dog, the Rhodesian Ridgeback, the Hungarian Vizsla, the Old English Sheep Dog, the Siberian Husky, the Wheaten Terrier, the Flat-coated Retriever, the Munster, the Northern Inuit Dog, the Bull Mastiff and the Löwchen. The Greyhound is a breed that has increased left ventricular cavity dimensions and septal wall thickness that differs remarkedly from that of other breeds(Snyder, Sato, & Atkins, 1995). We consider that this factor could lead to the overestimation of the LVOTV.

The sample in this study's age ranges from one month to 14 years of age. This supports the current notion that structural lesions may not be present at birth in SubAS (Pyle, Patterson, & Chacko, 1976). However, five dogs as young as eight weeks old were diagnosed with severe forms of both valvular and sub-valvular aortic stenosis, three of which were clinically silent although they presented with heart base murmurs. This adds to the concept that young pups may be affected by severe forms of AS (Freedom *et al.*, 2005). The majority of affected dogs

in this study (153 - 55.83%) were under three years of age, which is compatible with the early onset of AS. It is also worth to mention that it was not possible to evaluate the origin of the stenosis, for many cases were only identified at later periods in life and one would not expect to find congenital heart disease in older dogs. There is much debate as to whether AS is a congenital or an acquired malformation. Because this study is based on diagnosis with the LVOTV, we cannot guarantee that other acquired pathological processes in the left ventricular outflow tract did not contribute to some cases in this study, as well as the presence of emotional arousal or other conditioning factors that could lead to an increase of the LVOTV. The study failed to find a relationship between age at presentation and severity of the anatomical location of the lesions. It can be seen the average time of presentation was around three years of age, and this could be influenced by the later diagnosis of asymptomatic and mild cases. Ideally, in the future, we would expect defects to be identified at earlier ages. In contrast to other studies, no relationship between age at presentation and severity of diagnosis was evident.

This study encompasses slightly more males than females. Such findings have been reported in previous studies. However, when associating the gender with severity we reached a novelty result, as males seemed to be more severely affected than females, and females tended to manifest equivocal velocities more often. This suggests an association of progression with gender that has not been described before.

5.3. Clinical Features

Most dogs were clinically silent at the time of presentation, and the motive that moved most owners to seek a cardiology referral was a recently detected heart murmur in first opinion practice. In the present study, murmur intensity was related to severity of the disease and it indicates that cardiac auscultation in the first opinion practice is a useful tool for screening for this heart defect. The equivocal and milder cases however, had lower intensity heart murmurs and these could be harder to detect; for this reason, it is important to systematize careful cardiac examination in first opinion practices. Conversely, moderate-to-severe cases manifested obvious murmurs and could be identified and referred more clearly. The location of maximum intensity of the murmur was in most cases at the left heart base and, in some cases, it also radiated to the right hemithorax and the thoracic inlets, in agreement with information of previous studies (Aherne & Beijerink, 2013; Baumgartner & Glaus, 2004; Buchanan, 1999; Oliveira *et al.*, 2011; Tidholm, 1997). The pulse quality was only somewhat reduced in a small percentage of mild to moderately affected patients; it was poor in moderate to severely affected patients, in a similar way.

The most common clinical sign reported by owners was intolerance to exercise, followed by syncope. Syncope was reported even in equivocal and mild cases of stenosis, and, given the high number of concurrent heart disease in this investigation, it cannot be linked to aortic

stenosis alone. In this study, many dogs had abnormal findings on EKG and this could also point to possible conduction disturbances caused by underlying illness.
The presence of clinical signs increased with severity, even so, only in severe cases did the presence of clinical signs exceed the percentage of silent cases, reinforcing the concept that clinical signs are not a reliable indication of the presence of aortic stenosis.

4.4 Diagnostic characteristics

The EKG was normal in most cases, and the abnormalities found were not always attributed to aortic stenosis, expectedly, because 58.70% of dogs had additional cardiac disease. Only 72% of patients with CLVH were detected with this method, suggesting that it is, in fact, a less sensitive method of detection of this complication compared with echocardiography.
The major discrepancy found between this and other studies concerns the anatomical location of lesions. The most frequent location of lesions in the sample was valvular, followed by sub-valvular. This contrasted with all previous studies that observed sub-valvular lesions to be more frequent and found valvular lesions to be uncommon when observed at all (Aherne & Beijerink, 2013; Tidholm, 1997).
This finding could be accounted for in the specific criteria used for anatomical classification. Such criteria consisted of the classification of subvalvular aortic stenosis only if there was evidence of a sub-stenotic vena contracta. In some studies, mild cases of AS are reported as SubAS in the absence of sub-valvular changes. Instead, the cases in this study without 2D abnormalities of the LVOTV were considered unclassified or equivocal. VAS was diagnosed based on echocardiographic findings such as aortic valve leaflet thickening or aortic regurgitation, along with the Doppler-derived evidence of the vena contracta occurring situated at this level. This approach has pros and cons for in milder cases of subvalvular aortic stenosis because the specificity of echocardiography is lesser than in post-mortem diagnosis of SubAS.
Opining about the anatomical location changes over time: in older studies, VAS was inconsistently found, whereas currently there has been a growing emphasis on the importance of valvular cases. Further functional classification of SubAS, revealed that only a few cases were dynamic in comparison to the fixed form of the disease.
Two of the dogs with VAS had bicuspid aortic valves (a Springer Spaniel and a Munster). It is also of interest to mention that a case of supra-valvular aortic stenosis was found in the original reports, but had to be excluded due to the absence of a LVOTV or a PG record.
The haemodynamic severity distribution in the sample presented a majority of mild cases (154 - 56.20%) rather than severe (50 - 18.25%) or moderate cases (36 - 13.14%). This study did not identify a significant association of these and breeds in the sample. However, a factor contributing to the high number of mild cases could be due to the inclusion of milder cases of

AS. In fact, a sizeable quantity of the sample was comprised by individuals of the Boxer breed and these have been reported to have a higher LVOTV in other studies (Claudio Bussadori *et al.*, 2001; Linde & Koch, 2006)

The hemodynamic severity is only one attribute of the disease, other abnormalities found in echocardiography were the presence of post-stenotic dilation, concentric left ventricular hypertrophy, aortic valve leaflet thickening and aortic regurgitation. These were positively related to increased hemodynamic severity. A previous study showed that severity of stenosis correlates well with the cumulative presence of these echocardiographic characteristics, and the present study may add to that concept (Stern *et al.*, 2012).

As to the presence of concurrent congenital heart disease tricuspid valve dysplasia, mitral valve dysplasia, and pulmonic stenosis were the most common complex congenital defects associated with aortic stenosis, in this sample. The high prevalence of mitral valve dysplasia is also an atypical finding and it could be linked to the retrospective nature of this study. As structural changes in the mitral valve (namely MMVD) are quite common in dogs, we were not able to assess whether the presence of other pathological processes could be responsible for the reported lesions, especially in older dogs. In this study, MVD and TVD were considered separate congenital defects; in light of this, atrioventricular valve dysplasia is represented by its respective individual percentages. The presence of further intercurrent heart disease included MMVD, Ahrrythmogenic cardiomyopathy of the Boxer, heart base tumours. The influence these concurrent illnesses have in changes of LOVTVmax across the stenotic gradient is unclear and, their consideration emphasizes the need of careful echocardiographic examinations as a prerequisite for diagnosis.

6.Conclusion

The importance of the present study ties in with the sizable number of cases it encompasses. Some results of this study are in accordance with previous studies, with slight differences. The existence of breed and sex tendencies were as observed previously by other authors.

In this study, anatomical location of the lesions disagreed with the predispositions described by other studies. Another novelty finding in this study was the relationship between severity and gender. The relatively high percentage of complex congenital heart defects and intercurrent heart disease denotes the importance of precise and comprehensive examinations in cases of aortic stenosis. Also, one must bear in mind the dual causality of AS, congenital and acquired, which may explain the diagnosis of AS later in life.

In the future, guidelines for the diagnosis and characterization of AS, including the EOAi and AoSA could provide an improved consistency of results between different examiners, contributing to a consensus in AS screening and evaluation.

References

Aherne, M., & Beijerink, N. J. (2013). Congenital heart disease : a case series of 135 dogs. *Australian Veterinary Practitioner*, *43*(3), 466–470.

Ahlstrom, C., Höglund, K., Hult, P., Häggström, J., Kvart, C., & Ask, P. (2008). Assessing aortic stenosis using sample entropy of the phonocardiographic signal in dogs. *IEEE Transactions on Biomedical Engineering*, *55*(8), 2107–2109. https://doi.org/10.1109/TBME.2008.923767

Baumgartner, C., & Glaus, T. M. (2004). Erworbene herzerkrankungen beim hund: Eine retrospektive analyse. *Schweizer Archiv Fur Tierheilkunde*, *146*(9), 423–430.

Beijerink, N. J., Oyama, M. A., & Bonagura, J. D. (2017). Congenital Heart Disease. In S. J. Ettinger, E. Côté, & E. C. Feldman (Eds.), *Textbook of Veterinary Internal Medicine* (8th ed., pp. 2952–3032). Elsevier Saunders.

Belanger, J. M., Bellumori, T. P., Bannasch, D. L., Famula, T. R., & Oberbauer, A. M. (2017). Correlation of neuter status and expression of heritable disorders. *Canine Genetics and Epidemiology*.

Belanger, M. C., Côté, E., & Beauchamp, G. (2014). Association between Aortoseptal Angle in Golden Retriever Puppies and Subaortic Stenosis in Adulthood. *Journal of Veterinary Internal Medicine*, *28*(5), 1498–1503.

Bélanger, M. C., Di Fruscia, R., Dumesnil, J. G., & Pibarot, P. (2001). Usefulness of the indexed effective orifice area in the assessment of subaortic stenosis in the dog. *Journal of Veterinary Internal Medicine / American College of Veterinary Internal Medicine*, *15*(5), 430–437.

Bellumori, T. P., Famula, T. R., Bannasch, D. L., Belanger, J. M., & Oberbauer, A. M. (2013). Prevalence of inherited disorders among mixed-breed and purebred dogs: 27,254 cases (1995–2010). *Journal of the American Veterinary Medical Association*, *242*(11), 1549–1555.

Bezuidenhout, A. (2013). The Heart and Arteries. In H. Evans & A. de Lahunta (Eds.), *Miller's Anatomy of the Dog* (4th ed., pp. 428–442). Elsevier Saunders.

Bonagura, J. D. (2001). Problems in the canine left ventricular outflow tract. *Journal of Veterinary Internal Medicine*, *15*(5), 427–429.

Bonagura, J. D., & Lehmkuhl, L. B. (1999). Congenital Heart Disease. In P. Fox, D. D. Sisson, & S. Moise (Eds.), *Textbook of Feline and Canine Cardiology, Principles and Clinical Practice* (2nd ed., pp. 471–536).

Brown, D. J., & Smith, F. W. K. (2002). Stenosis hemodynamics: from physical principles to clinical indices. *Journal of Veterinary Internal Medicine / American College of Veterinary Internal Medicine*, *16*(6), 650–7.

Buchanan, J. (1999). Prevalence of Cardiovascular Disorders. In P. Fox, D. Sisson, & N. S. Moise (Eds.), *Textbook of Feline and Canine Cardiology, Principles and Clinical Practice* (2nd ed., pp. 457–470). W.B. Saunders Company.

Buoscio, D. A., Sisson, D., Zachary, J. F., & Luethy, M. (1994). Clinical and pathological characterization of an unusual form of subvalvular aortic stenosis in four Golden Retriever puppies. *Journal of the American Animal Hospital Association*, *30*(2), 100–110.

Bussadori, C., Amberger, C., Le Bobinnec, G., & Lombard, C. W. (2000). Guidelines for the echocardiographic studies of suspected subaortic and pulmonic stenosis. *Journal of Veterinary Cardiology*, *2*(2), 15–22.

Bussadori, C., Quintavalla, C., & Capelli, A. (2001). Prevalence of Congenital Heart Disease in Boxers in Italy. *Congenital Heart Disease*, *3*(2).

Cape, E. G., Vanauker, M. D., Sigfússon, G., Tacy, T. a, & del Nido, P. J. (1997). Potential role of mechanical stress in the etiology of pediatric heart disease: septal shear stress in subaortic stenosis. *Journal of the American College of Cardiology*, *30*(1), 247–254.

Chetboul, V., Trollé, J. M., Nicolle, A., Carlos Sampedrano, C., Gouni, V., Laforge, H., … Pouchelon, J. L. (2006). Congenital heart diseases in the Boxer dog: A retrospective study of 105 cases (1998-2005). *Journal of Veterinary Medicine Series A: Physiology Pathology Clinical Medicine*, *53*(7), 346–351.

Côté, E., Edwards, N. J., Ettinger, S. J., Luis Fuentes, V., Macdonald, K. A., Scansen, B. A., … Abbott, J. A. (2015). Management of incidentally detected heart murmurs in dogs and cats. *Journal of Veterinary Cardiology*, *17*(4), 245–261.

Davainis, G. M., Meurs, K. M., & Wright, N. a. (2004). The relationship of resting S-T segment depression to the severity of subvalvular aortic stenosis and the presence of ventricular premature complexes in the dog. *Journal of the American Animal Hospital Association*, *40*(1), 20–23.

Detweiler, D. K., & Patterson, D. F. (1965). The Prevalence and Types of Cardiovascular Disease in Dogs. *Annals of the New York Academy of Sciences*, *127*(1), 481–516.

Drögemüller, M., Jagannathan, V., Dolf, G., Butenhoff, K., Kottmann-Berger, S., Wess, G., & Leeb, T. (2015). A single codon insertion in the PICALM gene is not associated with subvalvular aortic stenosis in Newfoundland dogs. *Human Genetics*, *134*(1), 127–129.

Dukes-McEwan, J. (2006). A foundation for future research: the developmental genetics of congenital heart disease in animals. *The Veterinary Journal*, *171*(2), 195–197.

Dyce, K. M., & Wensing, C. J. D. (2010). The Cardiovascular System. In K. M. Dyce, W. O. Sack, & C. J. D. Wensing (Eds.), *Textbook of Veterinary Anatomy* (4th ed., pp. 223–238). Elsevier Saunders.

Falk, T., Jönsson, L., & Pedersen, H. D. (2004). Intramyocardial arterial narrowing in dogs with subaortic stenosis. *The Journal of Small Animal Practice*, *45*(9), 448–453.

Fernández del Palacio, M. J., Bayón, A., Bernal, L. J., Cerón, J. J., & Navarro, J. a. (1998). Clinical and pathological findings of severe subvalvular aortic stenosis and mitral dysplasia in a rottweiler puppy. *The Journal of Small Animal Practice*, *39*(10), 481–485.

Flickinger, G. L., & Patterson, D. F. (1967). Coronary lesions associated with congenital subaortic stenosis in the dog. *J Pathol Bacteriol*, *93*(1), 133–140.

Freedom, R. M., Yoo, S.-J., Russell, J., Perrin, D., & Williams, W. G. (2005). Thoughts about fixed subaortic stenosis in man and dog. *Cardiology in the Young*, *15*(2), 186–205.

French, a, Luis Fuentes, V., Dukes-McEwan, J., Darke, P. G., Martin, M., & Corcoran, B. (2000). Progression of aortic stenosis in the boxer. *The Journal of Small Animal Practice*, *41*(10), 451–456.

Goshal, N. G. (1975). Coração e Artérias do Carnívoro. In R. Guetty (Ed.), *Sisson/Grossman anatomia dos animais domésticos* (5th ed., pp. 1497–1500). Guanabara Koogan.

Gray, H. (1918). The Arteries. In *Anatomy of the Human Body*. Warren H. Lewis.

Grech, E. D., & Ramsdale, D. R. (1991). Exertional syncope in aortic stenosis: Evidence to support inappropriate left ventricular baroreceptor response. *American Heart Journal*, *121*(2 PART 1), 603–606.

Hill, A. J., & Iaizzo, P. A. (2015). Comparative cardiac anatomy. *Handbook of Cardiac Anatomy, Physiology, and Devices, Third Edition*, 89–114.

Höglund, K., Bussadori, C., Domenech, O., Häggström, J., Pradelli, D., & Kvart, C. (2007). Contrast echocardiography in Boxer dogs with and without aortic stenosis. *Journal of Veterinary Cardiology*, *9*(1), 15–24.

Höllmer, M., Willesen, J. L., Jensen, A. T., & Koch, J. (2008). Aortic stenosis in the Dogue de Bordeaux. *Journal of Small Animal Practice*, *49*(9), 432–437.

Hytell, P. (2010). Development of the blood cells, heart and vascular system. In P. Hyttel, F. Sinowatz, & M. Vejlsted (Eds.), *Essentials of domestic animal embryology* (1st ed., pp. 182–207). Elsevier Saunders.

Hyun, C., & Lavulo, L. (2006). Congenital heart diseases in small animals: Part I. Genetic pathways and potential candidate genes. *The Veterinary Journal*, *171*(2), 245–255.

Hyun, C., & Park, I. (2010). Congenital heart diseases in small animals : Part II . Potential genetic aetiologies based on human genetic studies, *171*(2006), 256–262.

Javard, R., Bélanger, M.-C., Côté, E., Beauchamp, G., & Pibarot, P. (2014). Comparison of peak flow velocity through the left ventricular outflow tract and effective orifice area indexed to body surface area in Golden Retriever puppies to predict development of subaortic stenosis in adult dogs. *Journal of the American Veterinary Medical Association*, *245*(12), 1367–1374.

Kander, M., Paslawska, U., & Staszczyk, M. (2015). Retrospective analysis of co-occurence of congenital aortic stenosis and pulmonary artery stenosis in dogs.

Kienle, R. (1998). Aortic Stenosis. In R. Kienle & M. Kittleson (Eds.), *Small Animal Cardiovascular Medicine Textbook* (pp. 261–271). St. Louis: Mosby, Inc.

Kienle, R. D., Thomas, W. P., & Pion, P. D. (1994). The Natural Clinical History of Canine Congenital Subaortic Stenosis. *Journal of Veterinary Internal Medicine*, *8*(6), 423–431.

Kienle, R., & Kittleson, M. (1998). Cardiac Embryology and Anatomy. In R. Kienle & M. Kittleson (Eds.), *Small Animal Cardiovascular Medicine Textbook* (pp. 2–87). St. Louis: Mosby, Inc.

King, J. M., Flint, T. J., & Anderson, W. I. (1988). Incomplete subaortic stenotic rings in domestic animals--a newly described congenital anomaly. *The Cornell Veterinarian*, *78*(3), 263–271.

Klaus-Dieter, B., McCarthy, P., Fricke, W., & Richter, R. (2007). Heart, coronary vessels, heart valves, cardiac conduction system. In B. Klaus-Dieter (Ed.), *Anatomy of the Dog* (pp. 46–48). Schlütersche Verlagsgesellschaft mbH & Co.

Kleman, M. E., Estrada, A. H., Maisenbacher, H. W., Prošek, R., Pogue, B., Shih, A., & Paolillo, J. A. (2012). How to perform combined cutting balloon and high pressure balloon valvuloplasty for dogs with subaortic stenosis. *Journal of Veterinary Cardiology*, *14*(2), 351–361.

Kvart, C., French, a T., Fuentes, V. L., Häggström, J., McEwan, J. D., & Schober, K. E. (1998). Analysis of murmur intensity, duration and frequency components in dogs with aortic stenosis. *The Journal of Small Animal Practice*, *39*(7), 318–324.

Lamb, C. R., Boswood, A., Volkman, A., & Connolly, D. J. (2001). Assessment of survey radiography as a method for diagnosis of congenital cardiac disease in dogs. *The*

Journal of Small Animal Practice, *42*(11), 541–5.

Lehmkuhl, L. B., Bonagura, J. D., Jones, D. E., & Stepien, R. L. (1995). Comparison of catheterization and Doppler-derived pressure gradients in a canine model of subaortic stenosis. *Journal of the American Society of Echocardiography*, *8*(5 PART 1), 611–620.

Linde, A., & Koch, J. (2006). Screening for aortic stenosis in the Boxer: Auscultatory, ECG, blood pressure and Doppler echocardiographic findings. *Journal of Veterinary Cardiology*, *8*(2), 79–86.

Lofland, G. K., McCrindle, B. W., Williams, W. G., Blackstone, E. H., Tchervenkov, C. I., Sittiwangkul, R., & Jonas, R. A. (2001). Critical aortic stenosis in the neonate: A multi-institutional study of management, outcomes, and risk factors. *The Journal of Thoracic and Cardiovascular Surgery*, *121*(1), 10–27.

MacDonald, K. A. (2006). Congenital heart diseases of puppies and kittens. *Veterinary Clinics of North America - Small Animal Practice*, *36*(3), 503–531.

Mark, A. (1983). The Bezold-Jarisch reflex revisited: clinical implications of inhibitory reflexes originating in the heart. *Journal of the American College of Cardiology*, *1*, 90–102.

Martins, A. A., Favaron, P. O., de Jesus Oliveira, L., Sch??fer, B. T., Oliveira, F. D., & Miglino, M. A. (2016). Development of the cardiorespiratory system in dogs from days 16 to 46 of pregnancy. *Reproduction in Domestic Animals*, *51*(5), 804–812.

McGeady, T. A., Quinn, P. J., Fitzpatrick, E. S., & Ryan, M. T. (2006). Establishment of the Basic Body Plan. In *Veterinary Embryology* (pp. 54–59).

Meurs, K. M., Lehmkuhl, L. B., & Bonagura, J. D. (2005). Survival times in dogs with severe subvalvular aortic stenosis treated with balloon valvuloplasty or atenolol. *Journal of the American Veterinary Medical Association*, *227*(3), 420–424.

Moorman, A. (2003). Development of the Heart: (1) Formation of the Cardiac Chambers and Arterial Trunks. *Heart*, *89*(7), 806–814.

Mulvihill, J. J., & Priester, W. A. (1973). Congenital heart disease in dogs: Epidemiologic similarities to man. *Teratology*, *7*(1), 73–77.

Muna, W. F., Ferrans, V. J., Pierce, J. E., & Roberts, W. C. (1978). Ultrastructure of the fibrous subaortic “ring” in dogs with discrete subaortic stenosis (abstract). *Lab Invest*, *39*(5), 471–482.

Muna, W. F. T., Ferrans, V. J., Pierce, J. E., & Roberts, W. C. (1978). Discrete subaortic stenosis in Newfoundland dogs: Association of infective endocarditis. *The American Journal of Cardiology*, *41*(4), 746–754.

Nakayama, T., Wakao, Y., Ishikawa, R., & Takahashi, M. (1996). Progression of subaortic stenosis detected by continuous wave Doppler echocardiography in a dog. *Journal of Veterinary Internal Medicine / American College of Veterinary Internal Medicine*, *10*(2), 97–98.

O'Grady, M. R., Holmberg, D. L., Miller, C. W., & Cockshutt, J. R. (1989). Canine congenital aortic stenosis: A review of the literature and commentary. *The Canadian Veterinary Journal. La Revue Vétérinaire Canadienne*, *30*(10), 811–5.

O'Leary, C. A., Mackay, B. M., Taplin, R. H., & Atwell, R. B. (2005). Auscultation and echocardiographic findings in Bull Terriers with and without polycystic kidney disease. *Australian Veterinary Journal*, *83*(5), 270–275.

Ohad, D. G., Avrahami, A., Waner, T., & David, L. (2013). The occurrence and suspected mode of inheritance of congenital subaortic stenosis and tricuspid valve dysplasia in Dogue de Bordeaux dogs. *Veterinary Journal*, *197*(2), 351–357.

Ohad, D. G., Morick, D., Avidor, B., & Harrus, S. (2010). Molecular detection of Bartonella henselae and Bartonella koehlerae from aortic valves of Boxer dogs with infective endocarditis. *Veterinary Microbiology*, *141*(1–2), 182–185.

Oliveira, P., Domenech, O., Silva, J., Vannini, S., Bussadori, R., & Bussadori, C. (2011). Retrospective review of congenital heart disease in 976 dogs. *Journal of Veterinary Internal Medicine*, *25*(3), 477–483.

Orton, E. C., Herndon, G. D., Boon, J. a, Gaynor, J. S., Hackett, T. B., & Monnet, E. (2000). Influence of open surgical correction on intermediate-term outcome in dogs with subvalvular aortic stenosis: 44 cases (1991-1998). *Journal of the American Veterinary Medical Association*, *216*(3), 364–367.

Oyama, M. A., & Thomas, W. P. (2002). Two-dimensional and M-mode echocardiographic predictors of disease severity in dogs with congenital subaortic stenosis. *Journal of the American Animal Hospital Association*, *38*(3), 209–215.

Oyama, M. a, & Sisson, D. D. (2001). Evaluation of canine congenital heart disease using an echocardiographic algorithm. *Journal of the American Animal Hospital Association*, *37*(6), 519–535.

Parker, H. G., Meurs, K. M., & Ostrander, E. A. (2006). Finding cardiovascular disease genes in the dog. *Journal of Veterinary Cardiology*, *8*(2), 115–127.

Patterson, D. F. (1968). Epidemiologic and Genetic Studies of Congenital Heart Disease in the Dog. *Circulation Research*, *23*(2), 171–202.

Patterson, D. F. (1976). Congenital defects of the cardiovascular system of dogs: studies in comparative cardiology. *Advances in Veterinary Science and Comparative Medicine*, *20*, 1–37.

Patterson, D. F., & Detweiler, D. K. (1963). Predominance of German shepherd and boxer breeds among dogs with congenital subaortic stenosis. *American Heart Journal*, *65*, 429–430.

Pikula, J., Pikulova, J., Bandouchova, H., Kohout, P., Najman, K., Tichy, F., & Treml, F. (2005). Subaortic stenosis and mitral dysplasia in three Black Russian Terrier puppies. *Veterinarni Medicina*, *50*(7), 321–326.

Pradelli, D., Quintavalla, C., Crosta, M. C., Mazzoni, L., Oliveira, P., Scotti, L., … Bussadori, C. (2014). The Influence of Emotional Stress on Doppler-derived Aortic Peak Velocity in Boxer Dogs. *Journal of Veterinary Internal Medicine*, *28*(6), 1724–1730.

Pyle, R. L. (2000). Interpreting low-intensity cardiac murmurs in dogs predisposed to subaortic stenosis. *Journal of the American Animal Hospital Association*, *36*(5), 379–382.

Pyle, R. L., Patterson, D. F., & Chacko, S. (1976). The genetics and pathology of discrete subaortic stenosis in the Newfoundland dog. *American Heart Journal*, *92*(3), 324–334.

Quintavalla, C., Guazzetti, S., Mavropoulou, A., & Bussadori, C. (2010). Aorto-septal angle in Boxer dogs with subaortic stenosis: An echocardiographic study. *Veterinary Journal*, *185*(3), 332–337.

Reist-Marti, S. B., Dolf, G., Leeb, T., Kottmann, S., Kietzmann, S., Butenhoff, K., & Rieder, S. (2012). Genetic evidence of subaortic stenosis in the Newfoundland dog. *Veterinary Record*, *170*(23), 597–597.

Robinson, W., & Maxie, G. (1993). The cardiovascular system. In F. K. Jubb, P. Kennedy, & N. Palmer (Eds.), *Pathology of Domestic Animals* (4th ed., pp. 1–55). Academic Press, inc.

Roth, L. (1994). Bacterial aortic valvular endocarditis associated with subaortic stenosis. *Journal of Small Animal Practice*, *35*(3), 169–172.

Schrope, D. P. (2015). Prevalence of congenital heart disease in 76,301 mixed-breed dogs and 57,025 mixed-breed cats. *Journal of Veterinary Cardiology : The Official Journal of the European Society of Veterinary Cardiology*, *17*(3), 192–202. https://doi.org/10.1016/j.jvc.2015.06.001

Serres, F., Chetboul, V., Carlos Sampedrano, C., Gouni, V., & Pouchelon, J. L. (2008).

Quadricuspid aortic valve and associated abnormalities in the dog: Report of six cases. *Journal of Veterinary Cardiology*, *10*(1), 25–31.

Shen, L., Estrada, A. H., Côté, E., Powell, M. A., Winter, B., & Lamb, K. (2017). Aortoseptal angle and pressure gradient reduction following balloon valvuloplasty in dogs with severe subaortic stenosis. *Journal of Veterinary Cardiology*, *19*(2), 144–152.

Snyder, P. S., Sato, T., & Atkins, C. E. (1995). A Comparison of Echocardiographic Indices of the Nonracing, Healthy Greyhound To Reference Values From Other Breeds. *Veterinary Radiology & Ultrasound*, *36*(5), 387–392.

Srivastava, D., & Olson, E. N. (2000). A genetic blueprint for cardiac development. *Nature*, *407*(September), 221–226.

Stephenson, R. B. (2013). Overview of Cardiovascular Function. In B. G. Klein (Ed.), *Cunningham's textbook of veterinary physiology* (5th ed., pp. 158–170). Elsevier Saunders.

Stepien, R. L., & Bonagura, J. D. (1991). Aortic stenosis: clinical findings in six cats. *Journal of Small Animal Practice*, *32*(7), 341–350.

Stern, J. A., Meurs, K. M., Nelson, O. L., Lahmers, S. M., & Lehmkuhl, L. B. (2012). Familial subvalvular aortic stenosis in golden retrievers: Inheritance and echocardiographic findings. *Journal of Small Animal Practice*, *53*(4), 213–216.

Stern, J. A., White, S. N., Lehmkuhl, L. B., Reina-Doreste, Y., Ferguson, J. L., Nascone-Yoder, N. M., & Meurs, K. M. (2014). A single codon insertion in PICALM is associated with development of familial subvalvular aortic stenosis in Newfoundland dogs. *Human Genetics*, *133*(9), 1139–1148.

Swift, S. (1996). The problem of inherited diseases. 2: Subaortic stenosis in boxers. *Journal of Small Animal Practice*, *37*(7), 351–352.

Tidholm, A. (1997). Retrospective study of congenital heart defects in 151 dogs. *Journal of Small Animal Practice*, *38*(3), 94–8.

Visser, L. C., & Scansen, B. A. (2013). Congenital bicuspid aortic valve in an English bulldog. *Journal of Veterinary Cardiology*, *15*(1), 87–92.

Yuill, C. D., & O'Grady, M. R. (1991). Doppler-derived velocity of blood flow across the cardiac valves in the normal dog. *Canadian Journal of Veterinary Research = Revue Canadienne de Recherche Veterinaire*, *55*(2), 185–192.