Thesis

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Short-Term Outcome and Clinical Data Assessment of 21 Dogs undergoing Patent Ductus Arteriosus Ligation (2017-2019)

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Table of Content

1 Introduction *3*

2 Review of literature *4*

2.1 Aetiology **4**

2.2 Pathogenesis **4**

2.3 Pathophysiology **6**

2.4 Nicoladoni-Branham sign **8**

2.5 Clinical Findings **9**

2.5.1 Left-to-right shunting PDA 11

2.5.2 Right-to-left shunting PDA 12

2.6 Clinical Management **12**

3 Materials and Methods *15*

3.1 Data Collection **15**

3.2 Anaesthesia Protocol **15**

3.3 Postoperative Care **18**

4 Results *19*

4.1 Clinical results **19**

4.2 Echocardiographic Results **22**

4.3 Results retrieved during Anaesthesia **24**

5 Discussion *26*

6 Abstract *29*

7 References *31*

1 Introduction

The Patent Ductus Arteriosus (PDA) is a very common congenital heart disease in dogs in which the prenatal ductus arteriosus Botalli fails to close shortly after birth. (Buchanan and Sci, 2001). If left untreated, this malformation leads to an over circulation of the lungs and, as a consequence, left heart dilation and often mitral regurgitation (Weirich WE, 1978). Most dogs are under 6 months of age at presentation and surgical closure of PDA is associated with a decrease in heart size and increased survival time (Saunders et al., 2014).

Hereditary features of this congenital vascular malformation as well as its treatment and outcome have been subject to various studies, however only few of them have ever described in detail the phenomenon of the occurrence or non-occurrence of the Nicoladoni-Branham sign (Branham´s sign) after successful closure of the PDA (Patterson, 1971; Bureau, Monnet and Orton, 2005). Ligating an artery proximal to an arterio-venous fistula, as Nicoladoni in 1875 and Branham in 1890 first described, results in acute bradycardia. Since then, this sign has been numerously reported and reproduced, mainly in human vascular surgery. (BURCHELL, 1958; Velez-Roa et al., 2004). The literature about such cases in dogs is, however, very limited with only one study so far dedicated to investigate the occurring bradycardia in dogs following PDA closure (De Monte *et al*., 2017).

The aim of this study was to collect as many cases as possible of dogs undergoing successful closure of PDA over the course of a 2-year period at the Clinic of the University of Veterinary Medicine Budapest, in order to investigate in a prospective way if (1) there are any correlation of a positive Branham´s sign and the age of the patient as well as (2) the tendency of left atrial remodelling in correlation with age.

2 Review of literature

2.1 Aetiology

The Ductus Arteriosus Botalli is a fetal blood vessel that originates from the sixth aortic arch, connecting the descending aorta with the main pulmonary artery. It directs the venous blood away from the collapsed fetal lungs into the systemic circulation. After parturition and with the onset of breathing, pulmonary vascular resistance falls, the flow in the duct reverses and the resulting rise in arterial oxygen tension inhibits local prostaglandin release which leads to endothelial smooth muscle contraction in the duct and its closure.

Functional closure of the ductus arteriosus normally occurs in dogs within days after birth and is followed by structural changes that occur over several months causing permanent closure (King 1999; Kittleson 1998). As a consequence, the duct should transform over time into a ligament, as Ligamentum Arteriosum. Continued patency for more than a few days results in a Patent Ductus Arteriosus (PDA). Persistence of the ductus arteriosus is the most common congenital heart defect seen in dogs, accounting for approximately 25% to 30% of congenital malformations (Patterson 1968; Buchanan and Sci, 2001; Buchanan and Patterson, 2003).

2.2 Pathogenesis

The absence of closure of the Botallo duct in dogs is characterized by distinct histological abnormalities within the ductal wall. While the ductal wall in a healthy puppy contains a loose branching pattern of circumferential smooth muscle throughout its length, the ductal wall in a dog suffering from PDA contains elastic rather than contractile smooth muscle fibres. A grading system has been established in 2001 by JW Buchanan to distinguish 6 degrees of ductus arteriosus abnormalities. It is based upon the extent of aorta-like elastic fibre in the otherwise muscular ductus wall. The author concluded that the muscle mass is failing to encircle the lumen, precluding complete stricture and occlusion of the duct. A secondary phenotypic abnormality stems from the absence of a ductus muscle inducer or promoter leading to the presence of the reciprocal non-contracting elastic wall segment. “Both the primary (hypoplasia of the DMM=ductal muscle mass) and secondary (elastic segment) abnormalities contribute to failure of the ductus to close properly” (Figure 1)

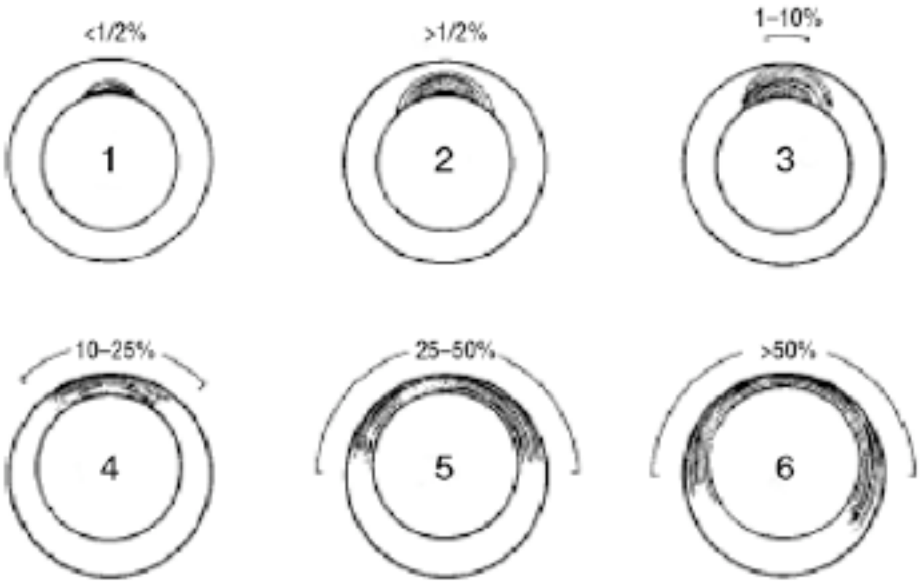

Figure 1: Morphological PDA grading system by Buchanan based on the extent of aorta-like elastic tissue in the otherwise muscular PDA wall (Buchanan and Sci, 2001)

In its mildest form, the ductus arteriosus closes at the pulmonary end, leading to a blind sack called *ductus diverticulum*. This anomaly of incomplete ductal closure could, at the time, only be detected by fluoroscopy or necropsy, nevertheless indicating that the dog possesses genes for this defect.

With the increasing genetic liability, the shape of the duct changes, leading to a tapered, funnel-shaped ductus arteriosus that remains open after the early natal period allowing the arterial blood to flow in the low-pressure pulmonary artery with varying amounts of left-to-right shunting. The most severe and least common type is the cylindrical, non-tapering duct with persistent, postnatal pulmonary hypertension (*Eisenmenger syndrome)* and bidirectional or right-to-left shunting. (Buchanan and Sci, 2001; Buchanan and Patterson, 2003).

The latest study focusing on classifying the morphology of PDA was conducted at Texas A&M University using transoesophageal echocardiography (TEE). This new approach "provided unique information regarding overall shape and measurements by displaying the pulmonary ostium of the PDA en face." The evaluation of the shape of the ampulla and pulmonary ostium demonstrates that there are a variety of shape combinations with the majority (71%) of dogs in the study identified as having an oval-shaped pulmonary ostium. This is much higher than in a previous study in which only 6% were reported to have an elliptical shaped ostium based on TEE-2D imaging. The study proposes an update to the current classification system to include additional PDA morphologies (Doocy *et al*., 2018).

In humans, a morphological classification was introduced by Krichenko in 1989 based on angiography. In this study, the configuration of the PDA varied considerably. The majority tended to have a funnel or conical shape due to ductal smooth muscle constriction at the pulmonary artery insertion, although narrowings in the middle or aortic ends were also observed as well as tubular and PDA with little or no tubular wall (Krichenko *et al.*, 1989).

2.3 Pathophysiology

The flow direction of the PDA is determined by the resistances of the pulmonary and systemic vascular beds (Moïse and Short, 1987). In most cases PDA is directed from left-to-right, or in other words from the aorta to the pulmonary artery. The shunt volume is directly related to the pressure gradient between the systemic pressure, the pulmonary pressure and the diameter of the duct. This continuous blood flow results in a grade VI/VI cardiac murmur, also called machinery murmur, increased pulmonary flow and overloading of the left ventricle and atrium. Due to the relatively high systemic pressure after birth, shunting occurs during systole as well as diastole.

Hyperkinetic arterial pulses are also characteristic of PDA. The blood runoff from the aorta into the pulmonary system causes a decrease in diastolic aortic pressure. The widened pulse pressure (systolic minus diastolic pressure) results in the palpably stronger (Waterhammer, Corrigan´s) pulse, detectable in dogs with substantial shunts. The vascular structures involved in the transport of the shunted blood enlarge in order to accommodate the extra volume flow. This causes dilatation of the proximal aorta, main pulmonary artery and over-circulation of the pulmonary vascular bed. As a consequence, left atrial dilatation and eccentric hypertrophy of the left ventricle develops. This mechanism, together with increased heart rate, permits compensation and maintenance of adequate systemic blood flow for a variable period of time. However, left ventricle and mitral annulus dilatation in turn cause mitral regurgitation and further volume overload. Excessive fluid retention, declining myocardial function arising from the chronic volume overload and arrhythmias contribute to the development of congestive heart failure.

In some cases, increased pulmonary blood flow leads to changes in the pulmonary vasculature, increased resistance and finally pulmonary hypertension, typically in dogs with several months of age. Normally these dogs have a history of prior left heart failure. Due to the occurrence of the left-to-right shunt at the level of the great vessels, the right atrium and ventricle never handle the shunted blood and do not undergo structural changes. However, this physiologic state can only be maintained as long as the pulmonary vascular resistance and pulmonary arterial pressure do not increase. If the pulmonary arterial pressure rises to equal aortic pressure, very little blood shunting occurs. However, if the pulmonary artery pressure exceeds aortic pressure, the direction of the blood flow reverses and shunting from right-to-left occurs. As a consequence, hypoxemia, secondary polycythaemia, cyanosis and seizures develop. The cyanosis, which is more pronounced in the caudal part of the body, is referred to as "differential cyanosis" and is the hallmark of a right-to-left shunting ductus arteriosus. This phenomenon can be explained by the anatomical location of the duct, which only supplies the descending part of the aorta with venous blood while sparing the cranial branches, therefore supplying oxygenated blood to the cranial part of the body.

The kidney, being supplied only with hypoxemic blood, reacts with an increased production or erythropoietin, leading to the secondary polycythaemia and hyper-viscosity as the PCV gradually rises up to 65% (Côté and Ettinger, 2001; Johnston S.A., Tobias K.M. (2018); Ettinger S.J., Feldman E.C. (2010).

2.4 Nicoladoni-Branham sign

As it is not pathological in its nature, but a mere physiologic response to hemodynamic changes in the circulation, the aetiology of Nicoladoni-Branham sign needs to be separated from the pathologic nature of the PDA (Wattanasirichaigoon, S., & Pomposelli, 1997).
Since its description in 1890, it has been considered the standard test of the hemodynamic significance of an arterio-venous (A-V) fistula in human vascular surgery (Summer 1995).
The study by Wattanasirichaigoon proposes that Branham's sign is an exaggerated Bezold-Jarisch (B-J) reflex. “Described in 1867, the B-J reflex causes bradycardia by stimulation of baroreceptors residing in the left ventricle. Certain receptors respond primarily to chemical stimuli (acetylcholine, 5-hydroxytryptamine), whereas others respond primarily to mechanical effects (volume loading, balloon distension) and some to both.” Both Branham's sign and the B-J reflex can be abolished by atropine administration (Wattanasirichaigoon, S., & Pomposelli, 1997).
When a hemodynamically significant A-V fistula or PDA is present in the circulation, cardiac output will rise in an attempt to return systemic blood flow to the baseline level (Summer 1995). In the case of PDA, the shunting of blood through the persistent Botallo duct resembles such an A-V fistula, in which blood is passed from a high-pressure to a low-pressure system. However, this is not the case if revers shunting occurs due to pulmonary hypertension leading to a right-to-left shunting PDA.

Occlusion of such a fistula or PDA, therefore, momentarily increases systemic blood pressure, during which the excess cardiac output is forced to pass throughout the higher-resistance peripheral vascular beds rather than the low-resistance fistula/PDA. The resulting bradycardic response that follows the occlusion of the PDA occurs within one or two heart beats. This immediate effect has been attributed to be the effect of blood pH on the action of choline esterase (BURCHELL, 1958). Furthermore, there is additional evidence that it is initiated by the baroreceptors and is also mediated through the vagus nerve (Summer 1995). It is therefore highly likely, that the occlusion of the PDA/A-V fistula leads to stimulation of left ventricular chemoreceptors and mechanoreceptors, initiating a bradycardic response (Wattanasirichaigoon, S., & Pomposelli, 1997).
A more recent study conducted at the University of Perugia in 2017 investigated the cardiovascular responses of PDA occlusion in 16 dogs with an Amplatz Canine Duct Occluder (ACDO). Heartrate, mean arterial and mean diastolic blood pressure were measured

for 20 minutes following PDA occlusion, coming to the conclusion that mean arterial blood pressure variation had a significant and moderate inverse correlation with diastolic and systolic flow velocity through the ductus. Transvascular patent ductus arteriosus occlusion in anaesthetised dogs caused a significant reduction in heart rate and an increase in diastolic and mean blood arterial pressure within 20 min of closure of the ductus (De Monte et al., 2017).

2.5 Clinical Findings

PDA is most commonly characterized by the much higher prevalence of female dogs affected, with a ratio of 3:1 compared to male dogs. Also, small breed dogs seem to be classically more prone to suffer from this disease. However, the most affected dog breeds appeared to have changed over the years reported by different studies. It is tempting to speculate that this is a result of geographical location of the study as well as the most popular dog breeds at the time. According to previous studies, small breeds like Chihuahua, Collie, Maltese, Poodle, Pomeranian, English Springer Spaniel, Keeshond, Bichon Frisé, and Shetland Sheepdog are most frequently affected, although others such as the Cavalier King Charles Spaniel may also be predisposed. Of the larger dog breeds the German Shephard appears to be the most commonly affected one throughout many studies, although Labrador Retriever and the Newfoundland are also prone to the disease (Patterson, 1971; Israel *et al.*, 2002; Saunders *et al.*, 2014; Eyster *et. al.*, 1976).

For the different presentations of PDA mentioned in Chapter 2.2, JW Buchanan categorized the 4 clinical types with the third having 2 further subgroups. The exact groupings can be seen in Table 1.

Type 1	Type 2	Type3		Type 4
		Type 3a (large PDA prior to congestive heart failure)	Type 3b (large PDA plus congestive heart failure)	
Asymptomatic left to right shunt	Asymptomatic left to right shunt	Usually reduced exercise capacity	All 3a features plus dyspnea due to pulmonary edema	Right-to-left or balanced shunt
High frequency continuous murmur only at the left heart base	Coarse continuous murmur at the left heart base and slightly audible at the left apex	Coarse continuous murmur and thrill over most of the left thorax	Usually poor body condition (cachexia)	Two weeks to 12 years old Hind leg weakness or collapse with exercise
Pre cordial thrill is faint or not present at the left heart base	Palpable continuous thrill at left heart base	Systolic murmur of mitral regurgitation often present at the left apex	Atrial fibrillation occasionally seen in ECG	Cyanosis usually limited to caudal part of body Pulse normal or weak
Heart rate and pulse quality are normal	Pulses normal or slightly bounding	Medium to large ductus aneurysm usually present	Pulmonary edema must be cleared as much as possible with cage rest, oxygen, digitalization and diuresis before surgery	Polycythemia (packed cell volume as high as 80%) Usually no murmur or precordial thrill after 1 month old
Radiographs and ECG are normal even at 1-2 years of age	Mild to moderate left heart enlargement before one year of age	Marked left heart enlargement before 6 months of age		Split and/or prominent second heart sound often detectable
Surgery is not urgent but is recommended for normal life span	Small to medium size ductus aneurysm may be present	Significant increase in pulmonary vascular markings		Right side apex beat stronger than left. Right axis deviation in electrocardiogram due to right ventricular hypertrophy
	Borderline increase in pulmonary vascular markings	Pulses bounding due to wide pulse pressure		Peripheral pulmonary artery size can appear normal or decreased and show slight tortuosity
	ECG Lead II R waves usually exceed 3 millivolts indicating left ventricular hypertrophy	ECG Lead II R waves may exceed 5 millivolts		Surgery is contraindicated because of severe pulmonary vascular disease
	Surgery is recommended but it can still wait a few weeks	Surgery recommended without delay		Treat polycythemia by periodic phlebotomy or chemotherapy

Table 1: Clinical types of PDA by JW Buchanan (Buchanan and Sci, 2001)

2.5.1 Left-to-right shunting PDA

A thorough physical examination is still a very efficient way to diagnose a left-to-right shunting PDA. In two studies including large populations, over 90% of dogs had an audible left basal continuous murmur with a grade IV/VI or even higher and 80% had a palpable precordial thrill (Israel *et al*., 2002; Saunders *et al*., 2014).

Other diagnostic methods like electrocardiography (ECG) or radiography can be used for tentative diagnosis as well. The ECG typically indicates an enlarged left ventricle (increased R-Wave voltages) or an enlarged left atrium (widened P-waves). Radiography of the thorax shows signs of enlarged pulmonary arteries on the lateral projection and the typical "aortic bulge" in the descending aorta on the ventro-dorsal view.

The golden standard for the confirmation of a PDA, however, is Colour Doppler echocardiography. It consistently demonstrates high-velocity continuous ductal flow directed toward the pulmonic valve on the interrogation of the pulmonary artery as well as left atrial, and, in more advanced cases, left ventricular enlargement. (Ettinger S.J., Feldman E.C. (2010): *Textbook of Veterinary Internal Medicine 7th edn*. St. Louis, Missuri: Saunders Elsevier, pp. 1256-1271).

A more recent option of visualisation is the already above mentioned transesophageal echocardiography (TEE), which can provide valuable 3D images for better morphological descriptions (Doocy *et al*., 2018). Such advances have also been made with multidetector-row computed tomography (MRCT) as well as cardiac MRI (cMRI), with both reaching satisfactory results (Henjes, Nolte and Wefstaedt, 2011; Lee *et al*., 2017).

2.5.2 Right-to-left shunting PDA

In a small percentage of dogs with PDA (5.5% in a multicenter clinical database, 15% in an experimental colony), pulmonary hypertension occurs and results in right-to-left shunting or ''reversal'' of blood flow through the PDA (Côté and Ettinger, 2001).

Reported signs include the above-mentioned exertional fatigue, hind limb weakness, shortness of breath, hyperpnea, differential cyanosis, and, more rarely, seizures. Clinical examination differs from left-to-right shunting PDA. Due to the size of the PDA, the flow of blood exhibits little turbulance and physical examination reveals either no murmur or only a soft, systolic murmur at the left base. Polycythaemia and hyperviscosity may occur during the first year of life, but rarely become severe before 18 to 24 months of age (Ettinger S.J., Feldman E.C., 2010).

The ECG, in contrast to the left-to-right PDA, shows signs of the right ventricular hypertrophy. Thoracic radiographs indicate right heart enlargement and the dilatation of the main pulmonary artery as a visible "ductus bump". Echocardiography reveals right ventricular concentric hypertrophy and a dilated main pulmonary artery (Ettinger S.J., Feldman E.C., 2010).

2.6 Clinical Management

Due to the fact that most dogs develop congestive heart failure by one year of age, surgical correction is highly advised in almost all young dogs diagnosed with left-to-right shunting PDA (Buchanan and Sci, 2001 pp. 7–16). Immature animals under one year of age appear to be the best candidates for surgery. Nevertheless, older patients should also undergo surgery as soon as possible. Even animals with severe secondary myocardial failure and functional mitral regurgitation would benefit from surgery. Animals suffering from congestive heart failure should be treated with diuretics and then undergo surgical ligation as soon as they are stable. Animals with pulmonary hypertension can also undergo patent ductus arteriosus surgery, as long as the pulmonary artery pressure does not exceed the systemic blood pressure (Seibert *et al*., 2010; Bureau S. *et al*., 2005; Eyster *et. al*., 1976). Geriatric patients suffering

from insignificant PDA with minimal or absent cardiomegaly may not be subjected to surgery and can be treated conservatively, if treatment is necessary (Saunders *et al.*, 2014).
Successful PDA surgery, meaning the permant luminal closure of the shunting duct, can be achieved by different techniques. The most common method is the "traditional" surgical ligation. Minimal invasive transcatheter closures have also been introduced and increasing in number since the first use of an embolization coil in 1994 (Miller MW *et al.*, 1994).

Also, the division of the ductus and over sewing the ends as well as the placement of haemostatic clips are 2 other options described for treatment of PDA. However, the clips are not favoured due to the reported risk for significant residual ductal flow and recanalization of the ductus arteriosus (Corti LB *et al.*, 2000; Eyster GE *et al.*, 1975).

Surgical ligation of PDA can be achieved by either the standard technique or the Jackson-Henderson technique. Due to the reported lower complication and reduced risk of residual flow (21% standard ligation vs. 53% Jackson-Henderson) the standard technique seems to be preferred by most of the surgeons (Johnston S.A., Tobias K.M. (2018).
According to one study, the different success rate can be caused by the higher amount of loose connective tissue entrapped within the medial aspect of the ligature impeding complete closure of the ductus (Stanley, Luis-Fuentes and Darke, 2003). However, this finding has not been consistently demonstrated (Israel *et al.*, 2002).
The minimal invasive techniques can use the already mentioned thrombogenic coils, the Amplatz vascular plug, the Amplatz Duct Occluder (AVP) or the Amplatz Canine Duct Occluder (ACDO), which is the only one specifically designed one to fit the shape of the canine PDA. All these methods and devices have their potential limitations and complications, including patient size with devices either not large enough to close the PDA or too big to advance into the femoral artery of small breed dogs, pulmonary arterial embolization and torsion or laceration of femoral artery or vein. In one study, however, the ACDO appears superior in the completeness of occlusion, complication rate and ease of use. (Stokhof AA *et al.*, 2000; Smith PJ *et al.*, 2007; Glaus TM *et al.*, 2002; Nguyenba TP *et al.*, 2007; Singh *et al.*, 2012,).

The surgical and minimally invasive interventional radiological treatments are both acceptable options of PDA in dogs. The results of various studies are quite comparable when it comes to procedure time, complications, mortality, and short-term outcome (Van Israël, Dukes-McEwan and French, 2003; Saunders *et al.*, 2014). However, only one study by K.

Goodrich in 2007 was truly dedicated to examine the differences between the two techniques, coming to the conclusion, that there was no significant difference in mortality between surgical ligation (5.6%) and trans-catheter occlusion (2.6%) (Goodrich et al., 2007). In addition, one should consider the fact that it was conducted before the latest and at the moment maybe most promising occlusion device, the ACDO, was available on the market (Singh *et al.*, 2012). This is also based on just a sole study, therefore the final conclusion cannot be reached at this point and will be subject to future studies.

Due to its vascular and haemodynamic properties, surgical correction of the right-to-left sided PDA is contraindicated. To this point, treatment options for this rare condition are limited and include most importantly regular management of the secondary polycythaemia. In one study, Etienne Cote and Stephen J. Ettinger examined the option of regular phlebotomy. The removal of a mean of 19% (maximum 28%) of circulating blood volume was spread out over two phlebotomies several hours apart. This resulted in a subjectively marked clinical improvement and longer survival time than expected. Other options to control the polycythaemia were not included in this study. As stated in the study, "drugs that suppress erythropoiesis such as hydroxyurea, cyclophosphamide and others may excessively suppress the bone marrow and require chronic monitoring for toxic effects", therefore safe usage cannot be guaranteed at this point. (Côté and Ettinger, 2001, pp. 39-42).

3 Materials and Methods

3.1 Data Collection

Twenty-one dogs diagnosed with PDA underwent surgical ligation at the Surgery Unit of the University of Veterinary Medicine Budapest and were involved in the prospective study in the period between October 2017 and May 2019. The inclusion criteria were complete baseline data such as age, breed, sex, bodyweight and presenting complaint, as well as recorded physical signs. Each patient underwent echocardiography prior to surgery and 6 weeks postoperatively. Recorded data included LA/Ao ratio (left atrium-to-aorta ratio), FS (fractional shortening), LVIDs (left ventricular internal dimension in systole) and LVIDd (left ventricular internal dimension in diastole). Intraoperative data such as surgical time (ST), heart rate (HR), possible complication and the presence or absence of Branham´s sign were also detected. Since no literature was to be found on how to define a positive Branham`s sign in dogs, we considered a drop of more than 12 bpm (beats per minute) as positive. All surgeries were performed by the same board-certified surgeon.

3.2 Anaesthesia Protocol

Perioperative antibiotic treatment was initiated right before anesthesia with Cefazoline (22 mg/kg iv., Cefazolin®, Sandoz). Each patient was premedicated with Fentanyl (0,005 mg/kg, Fentanyl® 0,25 mg/5 ml, Richter Gedeon), Morphine (0,3 mg/kg), Midazolam (0,25 mg/kg, Dormicum® 5 mg/1 ml inj., Egis) and Ketamine (0,5 mg/kg, Calypsol® 500 mg/10 ml inj., Richter Gedeon). General anaesthesia was induced with iv. Propofol 1% (5 mg/kg). The surgical plane of anaesthesia was maintained with Isoflurane in oxygen and intermittent positive pressure ventilation was applied. Intraoperative monitoring included electrocardiography, capnography, pulse oximetry and either rectal or oesophageal temperature monitoring. After clipping the hair from the operating site an intercostal block was performed infiltrating nerves caudal to the 3rd, 4th and 5th rib using Lidocaine (1,1 mg/kg, mg/kg, Lidokain Egis® 10 mg/ml inj., Egis) and Bupivacaine (1,1 mg/kg, Marcain® 5 mg/ml inj., Astra-Zeneca). Surgical Technique

In our study all 21 dogs underwent a standard ligation of PDA. The surgical approach was a left sided 4th intercostal lateral thoracotomy. The cranial left lung lobe was reflected caudally using a wet sponge. The PDA was identified right under the vagus nerve indicated by the most turbulent point of the heart base. A careful dissection of the duct was started extrapericardially, gently retracting the nerve dorsally, otherwise it was left untouched. Importance was given on a delicate deep dissection of the PDA with a right-angle forceps (Mixter dissector) first cranially and then caudally parallel to the ductus, in order to avoid traumatizing it. This was followed by blind dissection cranially and caudally around the duct (Figure 2). After completion, 2 pieces of non-absorbable 2-0 USP (in dogs under 4 kilograms 3-0 USP) monofilament polypropylene sutures (Vetsuture® LENE) were passed around the duct using the right-angled forceps. Finally, the ductus was closed by slowly tightening and tying both sutures, starting from the one closer to the aorta.

After closure, the surgeon palpated the duct, aorta and truncus pulmonalis to confirm the absence of any remaining fremitus. At the same time, we observed the anaesthetic monitor system to see if a positive Branham sign was visible. In the occurrence of extreme bradycardia, Atropine was at hand to counteract this phenomenon. If the result was to the surgeon's satisfaction, the swabs inside the thorax were taken out and the thoracotomy was closed in 4 layers restoring the negative pressure expanding the lung lobes without using thoracic drain.

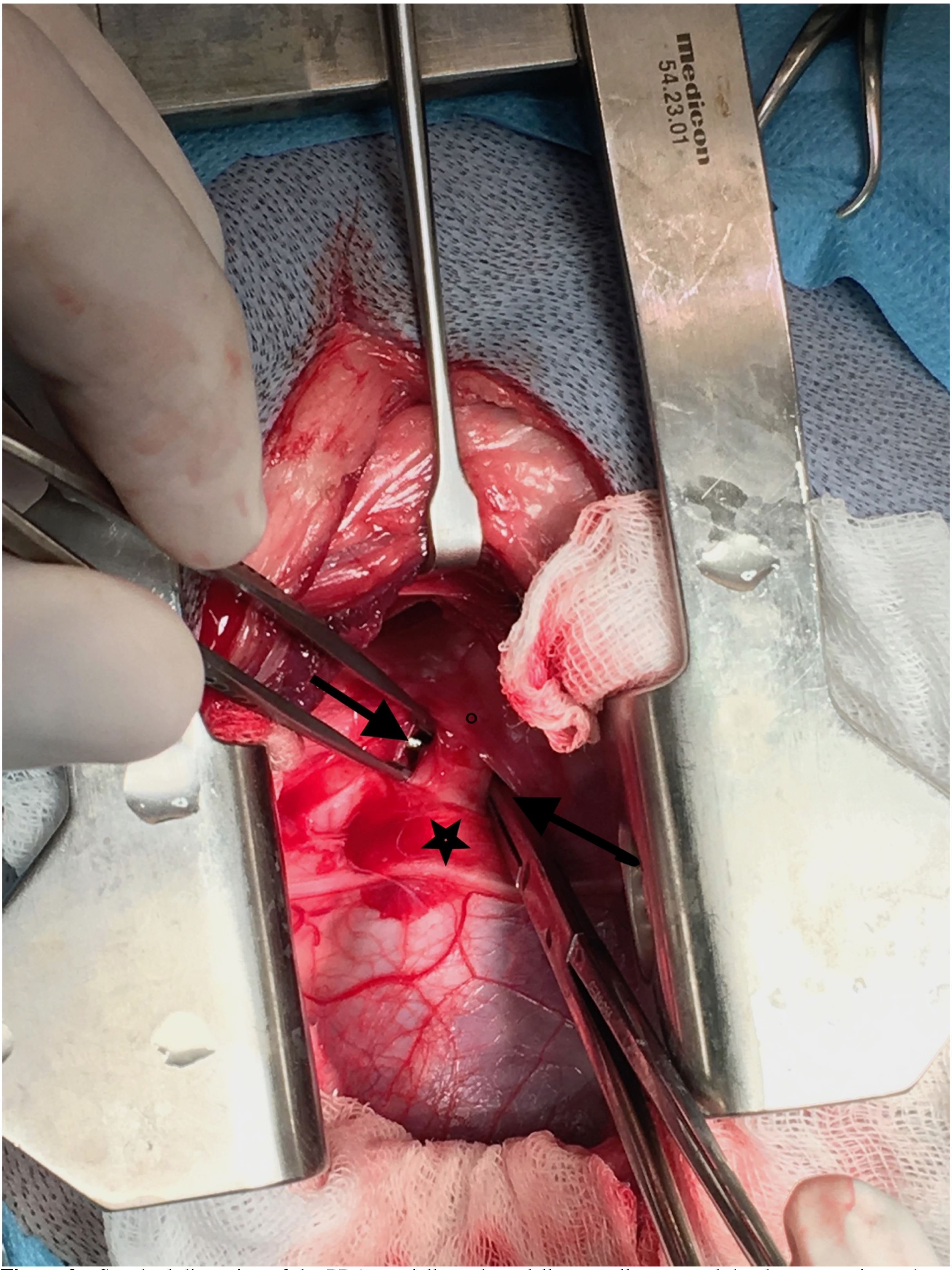

Figure 2: Standard dissection of the PDA cranially and caudally as well as around the ductus arteriosus (two arrows). Ventrally to the dissected area the pulmonary artery and below the phrenic nerve are visible (star). Dorsally the vagal nerve is still covered by pleura (circle).

3.3 Postoperative Care

All dogs received Meloxicam (0,2 mg/kg, Melovem® 5 mg/ml inj. A.U.V., Dopharma) subcutaneously as postoperative observation was done at the ICU, where they were checked for cardiac murmurs. If necessary, morphine (0,3 mg/kg, Morfina Cloridato Monico® 10 mg/1 ml inj., Monico) was administered as additional analgesic medication. The dogs stayed on approximately 1 to 2 days at the hospital before they were dismissed from the hospital. A follow up echocardiography was advised to the owners 6 weeks after the surgery.

4 Results

Results were divided into 3 subgroups. The population study included 21 (n=21) dogs, the echocardiographic results as well as the the investigation on the occurrence of the Branham reflex included the data of 12 dogs.

4.1 Clinical results

Baseline characteristics of the 21 dogs in our study are reported in Table 2 and 3. Five dogs were 24 months or older (n=5, 23.8%), the median age of the entire population was 4 months and ranged between 3 months and 39 months. Body weight (BW) ranged from 1.2 to 30.0 kg (median weight 4.0 kg). The most common breeds were Miniature Spitz (n=4, 19.0%), German Shepherd (n=3, 14.3%), Mix (n=2, 9.5%) Border Collie (n=2, 9.5%), Welsh Corgi (n=2, 9.5%). One dog was given of each Bichon Havanaise (n=1, 4.8%), Parson Russel Terrier (n=1, 4.8%), Miniature Dachshound (n=1, 4.8%), German Short Hair (n=1, 4.8%), Maltese (n=1, 4.8%), Chihuahua (n=1, 4.8%), Mudi (n=1, 4.8%) and Coton de Toulaire (n=1, 4.8%). Fourteen dogs were female (n=14, 66.6%) and 7 were male (n=7, 33.3%). Out of the 21 dogs, 15 showed no clinical signs (n=15, 71.4%), 2 showed slight tachypnea (n=2, 9.5%), 2 were panting during the examination (n=2, 9.5%) and 1 animal had faster respiration during sleeping (n=1, 4.8%). Of the 2 animals panting, 1 had concomitant exercise intolerance and the other one refused food and was generally lethargic. Bradycardia was detected in one dog (n=1, 4.8%) and another dog suffered from paroxysmal tachyarrhythmia (n=1, 4.8%) (Table 4). All twenty-one dogs had a cardiac murmur of grade IV or higher (n=21, 100%). Surgery time ranged from minimum of 30 to a maximum of 70 minutes, with a median time of 40 minutes (Table 5).

Age (month) by sex						
Sex	**n**	**mean**	**SD**	**median**	**min**	**max**
F	14	9.64	11.81	4.00	3.00	39.00
M	7	13.43	12.18	5.00	3.00	29.00
BW (kg) by sex						
Sex	**n**	**mean**	**SD**	**median**	**min**	**max**
F	13	5.98	6.56	3.20	1.20	22.50
M	7	11.87	10.14	7.60	1.30	30.00

Table 2: Age and bodyweight of the patients

Age (month) by breed						
Breed	**n**	**mean**	**SD**	**median**	**min**	**max**
Bichon Havanais	1	6.00		6.00	6.00	6.00
Border Collie	2	16.50	17.68	16.50	4.00	29.00
Chihuahua	1	34.00		34.00	34.00	34.00
Coton de Touloire	1	3.00		3.00	3.00	3.00
German Shepherd	3	7.67	5.51	5.00	4.00	14.00
German Shorthair	1	39.00		39.00	39.00	39.00
Maltese	1	26.00		26.00	26.00	26.00
Mini Dachshound	1	4.00		4.00	4.00	4.00
Miniature Spitz	4	3.25	0.50	3.00	3.00	4.00
Mix	2	14.50	13.44	14.50	5.00	24.00
Mudi	1	4.00		4.00	4.00	4.00
Parson Russel Terrier	1	9.00		9.00	9.00	9.00
Welsh Corgi	2	3.00	0.00	3.00	3.00	3.00

Table 3: Breed and age of patients taking part in the study

Cough	n
none	20
snort	1
Exercise intolerance	n
none	19
not eating, lethargic	1
yes	1
Cardiac Problems	n
Bradycardia + Arrhythmia	1
none	19
paroxysmal tachyarrhythmia	1
Dyspnea	n
faster respiration during sleeping	1
none	16
none, slight tachypnea	2
panting	2

Table 4: Recorded Clinical Signs

Surgery time (in minutes)					
n	**mean**	**SD**	**median**	**min**	**max**
12	40.00	11.51	38.50	25.00	70.00

Table 5: Surgery time in minutes

4.2 Echocardiographic Results

Complete echocardiological data was retrieved from 12 dogs, the rest was unfortunately lost to follow-up as many of the postsurgical echocardiographic check ups were performed outside our clinic (Table 6). The mean LA/Ao value prior to surgery varied from 1.55 ± 0.33 while the follow-up echocardiography showed a mean LA/Ao values of 1.23 ± 0.19 (Figure 4). Statistical analysis of the retrieved data revealed that one month increase of age increases the post op LA/Ao value by 0.0015, comparing to the pre op value (p=0.8238). In the echocardiographic check-ups, no residual shunting was detected at any of the 12 patients.

	Pre OP					
	n	**mean**	**SD**	**median**	**min**	**max**
FS (%)	14	36.30	8.52	37.05	15.00	48.00
LA/Ao	12	1.55	0.33	1.52	1.12	2.40
LVIDd (mm)	9	30.58	9.00	32.00	17.10	44.00
LVIDs (mm)	9	18.90	5.95	20.00	10.80	30.00
	Post OP					
	n	**mean**	**SD**	**median**	**min**	**max**
FS (%)	14	31.14	8.35	32.50	7.00	40.00
LA/Ao	14	1.23	0.19	1.20	0.91	1.56
LVIDd (mm)	11	34.65	16.75	27.40	18.00	67.80
LVIDs (mm)	11	25.16	15.78	20.10	12.00	62.90

Table 6: Echocardiographic values before and after the surgery (FS, fractional shortening; LA/Ao, left atrium-to-aorta ratio; LVIDd, left ventricular internal dimensions in diastole; LVIDs, left ventricular internal dimensions in systole)

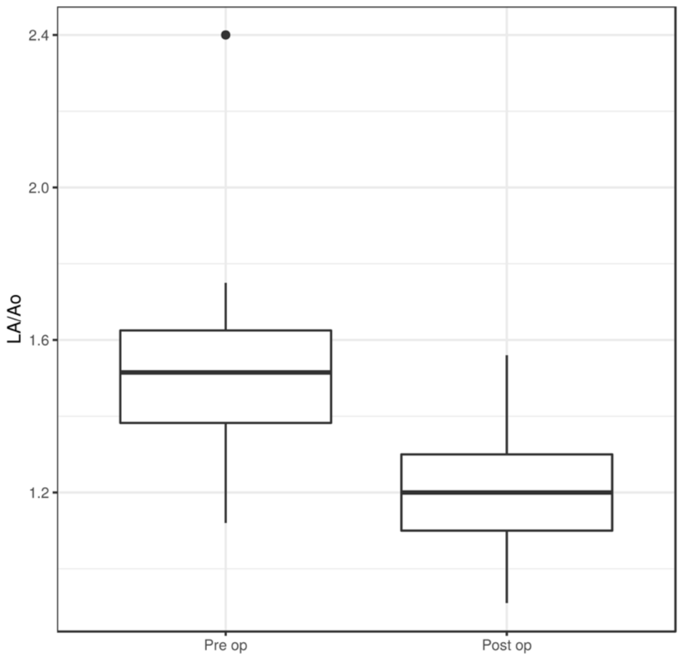

Figure 4: Left atrium-to-aorta ratio (LA/Ao) before and after surgery

4.3 Results retrieved during Anaesthesia

Complete anaesthesia records were reliable only in 12 dogs. As the change in heart rate was our point of interest, it was recorded every 5 minutes, resulting in a varying amount (n in Table 6) of values depending on the time of the surgery, which can be seen in Table 7.

Heart rates						
Code	**n**	**mean**	**SD**	**median**	**min**	**max**
265625	8	69.38	6.86	68.00	62.00	81.00
266376	4	120.00	14.58	117.50	105.00	140.00
267075	6	113.33	10.41	113.00	101.00	132.00
267136	8	77.00	16.97	73.00	57.00	113.00
269269	11	89.91	17.92	98.00	54.00	113.00
270842	9	95.44	15.66	91.00	76.00	119.00
271888	6	85.50	13.95	85.00	69.00	109.00
272657	6	136.17	29.88	124.00	110.00	176.00
274171	8	132.00	11.15	135.00	113.00	143.00
274289	7	131.43	21.76	127.00	108.00	161.00
275212	10	124.80	16.76	128.00	104.00	142.00
275246	6	128.17	9.77	126.50	116.00	144.00

Table 7: Overview of Heart rates

A positive Branham´s sign, which, by our definition, was an immediate drop of over 12 bpm, was seen in 5 cases (n=5, 38,5%) and could therefore not be seen in the remaining 8 (Figure 5). By logistic regression, there was no significant correlation between the age and Branham´s sign (OR: 1.02, 95% CI: 0.92-1.12, p=0.749), therefore rejecting our primary hypothesis. Considering the time frame from ligation until a maximum of 20 minutes after ligation there was a significant decrease in HR visible. After the occlusion, within 20 minutes, using the Δmax of HR is decreased by 37.13 bpm (95 % CI: 27.98 - 46.17, $p<0.0001$). Expressed by Δmax%, the average decrease was 28.50 ± 9.52 %. Nevertheless, as previously described, this was not considered a positive Branham´s sign in the spectrum of this study.

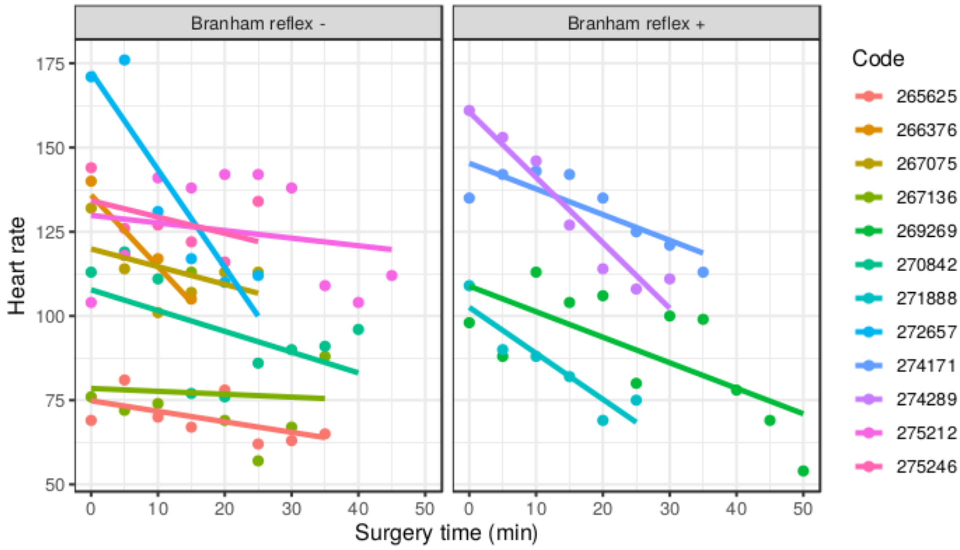

Figure 5: Change in heart rates during surgery. A drop of over 12 bpm was considered a positive (+) Branham´s sign.

Based on the contingency table in Figure 6, it seems the males have the sign with 9 times higher odds than females, but the result is statistically not significant (OR: 9.47, 95% CI: 0.54-682.27, p=0.1026).

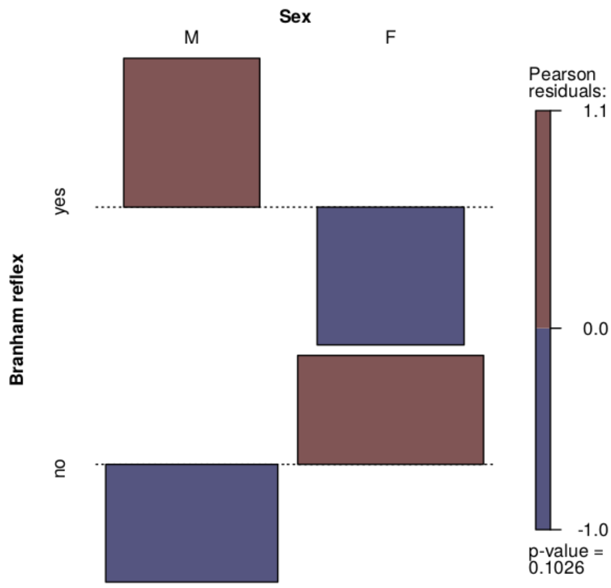

Figure 6: Association plot of Branham reflex and sex

5 Discussion

In our study, the majority of dogs where young females (n=14, 66,6%) under 6 months of age, with small breeds being overrepresented with fifteen dogs (n=15, 71.4%) compared to six large breed dogs (n=6, 28.6%) that all had a characteristic left base continuous murmur (n=21, 100%) without any characteristic clinical signs prior to presentation (n=15, 68,2%). This data is consistent with previous studies. The sex distribution, however, slightly differed from studies with larger case numbers, where a ratio of 3:1 in favour of females is normally the case (Bureau, Monnet and Orton, 2005; Campbell *et al*., 2006; Saunders *et al*., 2014). Nevertheless, a 2:1 ratio as in our case, has been reported before in a paper published in 2001. (Hunt *et al*., 2001) In another study coming from the University of Tokyo, sex ratio even dropped to 3:2 (Hamabe et al., 2015). At this point, one might assume that this was due to the similar case number, which was considerably lower compared to other studies (Saunders et al., 2014).

Five dogs (n=5, 23,8%) were over 24 months of age at their first presentation, similar to other studies with 21% to 25% (Saunders *et al*., 2014; Goodwin JK, Lombard CW 1992). Median body weight was 3.2 kg for females and 7.6 kg for males, which is on the lower end of previously reported results of 5.0 to 17.6 kg (Israel *et al*., 2002; Campbell *et al*., 2006) but similar to the results from AB Saunders in 2014. This study and the one by Saunders both included a high amount of small breed dogs with 71.4 %. (Saunders *et al*., 2014).

Under all the possible treatment options of PDA, standard ligation was chosen, which is preferred by our surgeon over the Jackson-Henderson technique. This is primarily due to the reported lower complication rate and reduced risk of residual flow as mentioned above as well as due to the experience of the surgeon with the standard technique (Stanley, Luis-Fuentes and Darke, 2003).

To truly analyse the full cardiovascular change and complete dynamics of the Nicoladoni-Branham sign following PDA occlusion, mean and diastolic arterial blood pressure measurement, next to heart rate measurement, is essential. We decided to leave out this parameter, which would otherwise be rather essential. This happened due to the fact, that although it was routinely measured intraoperatively, we believe that non-invasive mean and diastolic arterial blood pressure determined at the level of the left radial artery with non-invasive oscillometric technology is not always reliable. Invasive blood pressure measurement is unfortunately not regularly done at our clinic.

We did not manage to find evidence to support our primary hypothesis, which was to find a correlation between the age and the occurrence of a positive Branham´s sign. Rather the contrary happened, however, not being statistically significant ($p=0.749$). The amount of positive cases of Branham´s sign was with 38.5% surprisingly low for us in the first place, but we decided to analyse the developments not just immediately after ligation. Looking at the gradual drop in heart rate, there was a significant decrease of beats per minute with a mean maximal percentage variation of 28.50 ± 9.52% exactly 20 minutes after the ligation compared to the moment before ($p<0.0001$). This overlaps with the results of the previously published study by De Monte from 2017, showing a similar trend in a similarly sized population. Heart rates decreased even more after occlusion of the ductus with a mean maximal percentage variation of 41.0 ± 14.8% after 21.2 ± 13.7 min (De Monte *et al.*, 2017).

Echocardiographic results were attempted to be kept simple in this study. Since its first classification attempts by Buchanan in 2001, echocardiographic methods on diagnosing PDA as well as evaluating post-surgical cardiac function has developed dramatically the veterinary world (Henjes, Nolte and Wefstaedt, 2011; Hamabe *et al.*, 2015; Lee *et al.*, 2017; Doocy *et al.*, 2018). With this ever increasing possibilities, we decided to focus on one simple parameter that provides a short term feedback on successful PDA closure: the left atrium-to-aorta ratio (Hamabe *et al.*, 2015). The mean LA/Ao values prior to surgery varied from 1.55 ± 0.33 with follow-up LA/Ao values of 1.23 ± 0.19, which are similar to a previous report of LA/Ao values 1.71 ± 0.40 before and 1.28 ± 0.19 after ductal occlusion (Hamabe *et al.*, 2015). Assuming that young animals would heal faster, we hypothesized that their LA/Ao value would also decrease at a faster rate. The results showed that one month increase of age elevates the post-op LA/Ao value by 0.0015 ($p=0.8238$). Considering the low amount of cases, this was statistically not significant. Unfortunately there was no published literature to be found that further investigated this hypothesis, therefore it will remain to be determined by further studies whether or not there is a correlation between the pace of left atrial remodelling and age.

Although it has not been part of our original focus of interest, we encountered one interesting correlation between BW and LA/Ao ratio decrease postsurgical. One kg increase of body weight decreased post op LA/Ao value by 0.0088, comparing to the presurgical value (p=0.43777). Again, this result is statistically not significant and has to be subject to further studies.

One main limitation of this study lies in the relatively low amount of cases, which did not allow us to generate a more significant result for both of our hypothesis.

Another limitation in our eyes was the missing invasive blood pressure measurement during anaesthesia, which would be necessary to truly investigate the effects of PDA ligation on the cardiovascular system.

6 Abstract

Short-Term Outcome and Clinical Data Assessment of 21 Dogs undergoing Ductus Arteriosus Ligation (2017-2019)

Nikolaus Velich, Tibor Németh

Background: Published data of incidence of reflex bradycardia (Branham sign) following surgical closure of patent ductus arteriosus (PDA) is very limited.

Objective: Our goal was to find a correlation between clinical data of dogs undergoing PDA ligation hypothesising that (1) younger dogs are less likely to suffer from reflex bradycardia over the course of 10 minutes after complete ligation of PDA, and (2) younger dogs undergo more pronounced left atrial remodelling after surgery.

Animals: Twenty-one referred dogs.

Materials and methods: Prospective analysis of clinical data of 21 referred cases with PDA. Outcome was determined by contacting owners and referring veterinarians as well as cardiologists performing the post-surgical echocardiography. All dog owners were asked to do a control echocardiography four weeks post-surgery.

Results: Anaesthesiologic and echocardiographic data of changes in heart rate during the surgery could be assessed in thirteen cases, of which five animals showed an immediate drop in heart rate after the ligation. By logistic regression there was no found significant association between the age and Branham sign (p= 0.749). For the bodyweight the result is similar (p=0.340). Based on the sex ratio (6 male and 7 female) males had the sign with 9 times higher odds than females, but the result was statistically not significant (p=0.1026). Regarding the cardiac remodelling, the data could be retrieved from 12 patients. It shows a direct relation between the age of the animal and the increased Left Atrium/Aorta value (LA/Ao) postoperatively. In other words, one-month increase of age increases the post-surgical LA/Ao value by 0.0015 mm comparing to the presurgical value (p=0.8238). Another finding was that 1.0 kg increase of body weight decreases postsurgical LA/Ao value by 0.0088 mm comparing to the presurgical value (p=0 .43777).

Conclusion: The collected data rejected our first hypothesis, as there appeared to be no correlation between the age and occurrence of reflex bradycardia following the closure of a PDA. Our second hypothesis was confirmed, showing that younger animals in our study

appeared to have faster remodelling compared to older ones. Interestingly, large breed dogs seemed to benefit more as an increase in bodyweight was accompanied by a decrease in LA/Ao ratio after the ligation of the PDA.

Clinical importance: We could not see any association between the age and the appearance of a positive Branham sign. As for the cardiac remodelling, one might suspect that younger animals benefit from their overall increased healing capacity also in case of a distended left atrium.

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DECLARATION

I hereby declare that the thesis entitled "Short-Term Outcome and Clinical Data Assessment of 21 Dogs undergoing Patent Ductus Arteriosus Ligation (2017-2019)"
is identical in terms of content and formal requirements to the TDK research paper submitted in 2019.

Budapest, 22nd of November 2019 Student name and signature

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