



Quantification of equine aortic valve regurgitation by conventional echocardiography, tissue Doppler imaging and two-dimensional speckle tracking

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Dissertation submitted in the fulfilment of the requirements for the degree of Doctor in Veterinary Sciences (PhD)

2018

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The problem with the world is that the intelligent people are full of doubts, while the stupid ones are full of confidence.¹

- Charles Bukowski -

¹ Kruger J, Dunning D. Unskilled and unaware of it: how difficulties in recognizing one's own incompetence lead to inflated self-assessments. Journal of personality and social psychology 1999; 77 (6) 1121-1134

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List of Abbreviations

2D	two dimensional			
2DE	two-dimensional echocardiography			
2DST	two-dimensional speckle tracking			
3D	three dimensional			
4D	four dimensional			
A	late diastole (atrial contraction)			
ACE	angiotensin-converting enzyme			
al	calculated by the area-length method			
Ao	aorta			
AoDiastDecr	aortic diastolic decrease			
AoDiastDecr_rate	rate of aortic diastolic decrease			
AoV	aortic valve			
AR	aortic regurgitation			
bpm	beats per minute			
bullet	calculated by the bullet method			
BW	bodyweight			
CFD	colour flow Doppler			
СО	cardiac output			
ct	chordae tendineae			
СТ	computed tomography			
CW	continuous wave			
d / diast	end-diastolic			
DL	longitudinal displacement			
durA	duration of A-wave			
E	early diastole			
ECG	electrocardiography			
Ediast	early diastole			

EDP	end-diastolic pressure		
EF	ejection fraction		
EROA	effective regurgitant orifice area		
ET / LVET	left ventricular ejection time		
FAC	fractional area change		
FEI	Fédération Equestre International		
fps	frames per second		
FS	fractional shortening		
HR	heart rate		
IVC	isovolumic contraction		
IVRT	isovolumic relaxation time		
IVS	interventricular septum		
L	left		
LA	left atrium		
LAA	left atrial area		
LAD	left atrial diameter		
Ldiast	late diastole		
LLV	left part of the left ventricular wall		
LV	left ventricle		
LVA	left ventricular area		
LVFW	left ventricular free wall		
LVID	left ventricular internal diameter		
LVIV	left ventricular internal volume		
LVL	left ventricular length		
LVOT	left ventricular outflow tract		
LVV	left ventricular volume		
lx	long axis		
M-mode	motional mode		
MRI	magnetic resonance imaging		
PEP	pre-ejection period		

PISA	proximal isovelocity surface area
pm	papillary muscles
PV	pressure-volume
PW	pulsed wave
R	right
RLV	right part of the left ventricular wall
ROI	region of interest
RS	radial strain (TDI)
RWT	relative wall thickness
s / syst	end-systolic
S	systole
SC	circumferential strain
SD	standard deviation
SI	strain imaging
SL	longitudinal strain
SR	radial strain (2DST)
SrC	circumferential strain rate
SrL	longitudinal strain rate
SrR	radial strain rate
SV	stroke volume
SX	short axis
Т	time
TAVR	transcatheter aortic valve replacement
TDI	tissue Doppler imaging
TVI	tissue velocity imaging
Vel	velocity
Vmax	maximal velocity of the aortic blood flow
Vmean	mean velocity of the aortic blood flow
VO ₂ max	maximal oxygen uptake
VTI	velocity time integral

Chapter 1: Introduction

CHAPTER 1: INTRODUCTION

1. EQUINE CARDIOLOGY

1.1 The equine heart

Throughout evolution, horses depended on their stamina and speed to run for their survival and this made them evolve into extraordinary athletes.¹ Selective breeding further contributed to the development of a cardiovascular system highly adapted to exercise.¹ Skeletal muscle comprises more than 50% of body mass in horses, and oxygen supply to this large muscle mass is the limiting factor for peak performance.² To meet the high oxygen demand during exercise, a high cardiac output is needed. Horses are able to generate a high cardiac output (CO) by increasing heart rate (HR) from 27 beats per minute (bpm) at rest to 240 bpm at peak performance. Additionally, horses have proportionally large hearts, with a relative cardiac mass of up to 2% of their bodyweight. This allows them to produce large stroke volumes (SV).³⁻⁵ At rest, the SV reaches up to 800-900 mL (2-2.5 mL/kg). During submaximal exercise, the SV increases by 20-50%. At maximal exercise, the HR is very high which results in a shortened diastolic time duration. Despite this reduced time for diastolic filling, the SV is not reduced in horses at maximal exercise, resulting in a high CO. Furthermore, regular intensive training induces cardiovascular morphological and functional alterations. The heart will adapt to accommodate the higher demand for blood supply to the working muscles during exercise.³⁻⁵ In human medicine, it has been described that these adaptations depend on the type of work performed. The Morganroth hypothesis states that static strength training induces concentric hypertrophy, characterized by a normal end-diastolic left ventricular (LV) volume and an increased LV mass with increased wall thickness to compensate for the higher wall stress induced by the increased systolic pressure. Dynamic, longstanding endurance training on the other hand induces eccentric hypertrophy, which is characterized by an increased LV mass, increased LV end-diastolic volume and normal relative wall thickness. Recently this

hypothesis has been challenged. A study by Spence et al. (2011) confirmed that eccentric cardiac hypertrophy is evident in response to endurance training, but no substantive cardiac remodelling was found in resistance (static strength) trained athletes.⁶ Equine athletes do not perform static strength training, most of their work consists of dynamic endurance training. Aerobic endurance training requires a high blood supply to the working muscles. As a result, the ventricles will dilate to allow a larger SV. Dilatation of the ventricle results in increased wall stress and this will be compensated by hypertrophy of the wall, resulting in a higher cardiac mass.⁷⁻⁹ Eccentric hypertrophy in equine athletes has been extensively investigated for the left heart⁸⁻¹⁰, and recent studies have shown a similar effect for the right heart.¹¹ This phenomenon is commonly referred to as 'athletes' heart', and is also well known in human medicine. Top equine athletes are reported to produce a SV of up to 1700 mL per beat, resulting in a CO of up to 450 L/min and a maximal oxygen uptake of 220 mL/min/kg.^{1,5}

Aging also causes alterations of the cardiovascular system. In human medicine, it has been demonstrated that with increasing age, vascular stiffness of the large arteries increases due to elastin fragmentation and increased collagen production.¹² The increased stiffness results in a rise of systolic blood pressure with age, creating a higher afterload for the heart. The myocardial wall changes as well with increasing age. In the connective tissue of the heart, increased collagen deposition is found with increasing age. Functionally, the age-related cardiovascular alterations lead to changes in diastolic ventricular filling and increased filling pressure.¹² Normal diastolic filling of the ventricle occurs in two phases. During early diastole (E), the ventricle is filled due to relaxation of the ventricular myocardium which creates suction. During late diastole, atrial contraction (A) induces an additional active filling of the ventricle. In older individuals, early diastolic filling is reduced due to decreased LV diastolic compliance and increased myocardial wall thickness. This reduction in early diastolic filling is compensated by increased late diastolic filling. Thus, the pattern

of LV filling shifts from predominantly early diastolic in young individuals to late diastolic in older individuals.^{13,14}

Additionally, in human medicine it has been shown that maximal HR and oxygen extraction capacity decrease in aged individuals. This results in a lower maximal oxygen uptake (VO₂max).^{12,15} At rest, systolic function is unaltered with healthy aging. With exercise, the effects of aging become evident because functional cardiac reserve is reduced and this results in an overall decrease in exercise tolerance.^{12,13}

In dogs, similar alterations in cardiac structure and function with increasing age have been demonstrated.¹⁶⁻¹⁸ In clinically normal dogs, a gradual decrease in LV relaxation, increased myocardial stiffness and increased late diastolic filling are associated with aging.¹⁷ Early diastolic ventricular filling and E/A ratio decreased significantly with increasing age and late diastolic ventricular filling increased with age. Similar to human findings, systolic function at rest was unaltered in old dogs compared to young dogs.¹⁶

Few studies have evaluated the cardiac effects of aging in horses. A study in 18 Standardbred mares showed that older horses have a reduced aerobic capacity compared to young horses.¹⁹ The reduction in maximal oxygen uptake was attributed to alterations in peripheral and central mechanisms. At the peripheral level, oxygen extraction is reduced in old horses. With aging, horses experience loss of muscle mass, alterations in capillarisation of the muscle and shifts in muscle fibre type away from the aerobic metabolism. As a result of these changes, the muscles will extract less oxygen from the circulating blood. Central mechanisms affecting aerobic capacity involve determinants of CO. Similar to what has been reported in man, horses have a lower maximal HR with increasing age.¹⁹ Aging also influences several factors that determine the SV. It has been demonstrated that plasma volume decreases in old horses. This reduces their ability to thermoregulate, but also decreases preload. Additionally, increased vascular stiffness creates a higher afterload. The reduced preload and increased afterload

result in a smaller SV with increasing age.¹⁹ Cardiac output is determined by HR and SV. Since both parameters are reduced in older individuals, maximal CO and aerobic capacity will be lower. The impact of LV function on SV in aging horses is unknown, since studies evaluating alterations in systolic and diastolic function in the geriatric equine heart are lacking.

Additionally, there is a high incidence of degenerative alterations of the cardiac valves in older horses. The aortic valve (AoV) and the mitral valve are most frequently affected.^{20,21} Bowen et al. (2006) demonstrated age related alterations in both structural properties and dynamic function of the equine AoV.²² *In vitro* contractile force of the valve decreases with increasing age. At a structural level, an increased collagen content of the valve was found in older individuals. Whether the reduction in contractile force is a consequence of the higher collagen content of the valve or the increased collagen is a result of the reduction in contractile function.

1.2 CARDIAC DISEASE IN HORSES

For centuries, horses have been bred for performance. For obvious reasons, horses with performance-limiting cardiac diseases were not selected for breeding and thus congenital and hereditary cardiac pathologies in horses are rare. However, some defects are the result of abnormalities during the embryological development of the heart. Those congenital defects can be found in horses, although their prevalence is low, approximately 3-5% of all congenital organ defects. It is estimated to occur in approximately 1-5 out of 5000 births, although differences between breeds exist. Arabian horses appear to be more susceptible, whereas Thoroughbreds have a lower prevalence.²³ Ventricular or atrial septal defects, patent ductus arteriosus, tetralogy of Fallot and valvular dysplasia are the most commonly encountered congenital cardiac diseases in foals.^{23,24}

Acquired cardiac disease is more common in horses. Primary myocardial diseases have been reported sporadically. Sometimes a causative agent (infectious, toxic, nutritional, neoplastic, genetic) can be identified, but often these conditions are idiopathic.²⁵ In adult horses, valvular regurgitation is the most important cause of cardiac murmurs.²⁰ Valvular regurgitation in horses can be caused by physiological or pathological processes. There is a high prevalence of valvular regurgitation in well trained racehorses, with no impact on performance level. The tricuspid valve is most frequently affected (up to 89%), followed by the aortic valve (65%) and the mitral valve (52%).²⁶ This regurgitation is considered to be physiological and is probably a result of cardiac enlargement due to intensive training.²⁶ In some cases, however, regurgitation results from pathological changes. A high incidence of degenerative abnormalities of the cardiac valves with increasing age has been reported.²⁰ A study on 1557 equine hearts showed that almost 60% of horses older than 20 years had thickening of the valvular endocardium, which may be indicative for fibrosis.²⁰ These degenerative alterations of valvular morphology might result in insufficient closure of the valve. Endocarditis can also cause valvular deformation and regurgitation.^{27,28} When the regurgitant volume is small, hemodynamic consequences will be limited and performance will not be affected. In some cases, the disease is stable and will not affect performance on long-term. However, in other cases the valvular abnormalities and the severity of regurgitation might worsen and might become hemodynamically important or even performance limiting. Eventually there might even be progression to heart failure. Horses with valvular regurgitation should therefore be monitored carefully to determine progression of the disease.²⁹ Rarely, the disease progression is rapid. This is the case when the chordae tendineae of the atrioventricular valves rupture. This results in acute, severe regurgitation, so the heart has no time to adapt to the altered hemodynamics. In these cases, symptoms of congestive heart failure become evident rapidly.³⁰

2. AORTIC REGURGITATION

2.1 THE AORTIC VALVE

The AoV is located between the LV and aorta and consists of three semilunar valve leaflets: a left and right coronary cusp and a non-coronary cusp (Figure 1). The centre of the free edge of each cusp consists of a small firm nodule, called the corpus arantii. The junctions of the cusps are called commissures. The valves are attached to the aortic annulus and open into the sinus of Valsalva, the bulbous base of the aorta.^{24,31}



Figure 1: Anatomy of the equine cardiac valves. a,b,c represent the aortic valve. a is the left coronary cusp, b is the right coronary cusp, c is the non-coronary cusp. d,e,f represent the pulmonary valve. g,h represent the mitral valve. i,k,l represent the tricuspid valve. Adapted from Nickel et al. (1976)³²

Opening and closing of the AoV results from the difference in pressure in the aorta and in the LV (Figure 2). During systole, contraction of the LV causes pressure to rise above aortic pressure and the valve opens, allowing blood to flow into the aorta. During diastole, pressure in the ventricle drops and the pressure difference with aortic pressure causes the valve to close. A competent AoV will prevent blood flowing back from the aorta into the LV during diastole.



Figure 2: Pressure and volume curves of the left heart throughout the cardiac cycle. The upper panel shows the changes in pressure in the aorta, the left ventricle and the left atrium. The lower panel displays the alterations in left ventricular volume during the cardiac cycle. The cardiac cycle is divided in different phases. Phase 1 is diastole, phase 2 is isovolumic contraction, phase 3 is systole, phase 4 is isovolumic relaxation.³³ Failure of the AoV to competently close the aortic orifice during diastole will lead to a reversed blood flow from the aorta into the LV. This aortic regurgitation (AR) causes hemodynamic alterations in the cardiovascular system.³⁴ The aetiology of AR is not always easy to identify. Well-trained horses appear to have a higher prevalence of AR, so cardiac adaptations to training might predispose to the development of AR. However, this regurgitation is usually mild and not progressive and does not limit performance.³⁵ Reported AR prevalences depend on the population studied and the technique used to diagnose AR. In a hospital population, a prevalence of 2.1% was found³⁶ and in a mixed population of working horses a prevalence of 2.2% was reported.³⁷ Small riding breeds are at increased risk of having AR.³⁸ With increasing age, the prevalence of AR increases reaching more than 15% in older horses.³⁹ This is a result of degenerative alterations of the valve with increasing age.²⁹ The exact mechanism of this process is not fully understood. With increasing age, an increased collagen content and reduction in in *vitro* contractile force of the AoV have been demonstrated.^{20,22} A post-mortem study on histopathological alterations in equine hearts revealed that nodular lesions of the AoV were more common than general thickening.⁴⁰ These nodules consisted of subendothelial proliferations of fibrous connective tissue. The authors suggest that an initial lesion of the valve might initiate a vicious circle of AoV deterioration. The initial lesion, such as trauma, infection, parasites, ..., might cause valvular distortion which in turn leads to aberrant flow that may cause additional damage to the valve. Additional age-related changes, such as alterations in connective tissue due to cellular degeneration and replacement fibrosis, will exacerbate the valvular lesions over time (Figure 3).⁴⁰



THE AORTIC VALVE LESION CYCLE

Figure 3: Factors involved in the aetiology of aortic valve lesions.⁴⁰

Bishop et al. (1966) performed a histological study of the AoV of 15 horses with diastolic murmurs.⁴¹ They found two types of lesions: bands and nodules. The bands consisted of fibrous thickening and loss of elastic tissue, with intact valvular endothelium. The nodular lesions appeared to be nonbacterial thrombotic endocarditis. After loss of endothelium, blood proteins and platelets precipitate on the surface. Although both types of lesions have different morphological characteristics, they both occur on the occlusal area of the valve cusp. Their development is probably caused by a combination of generalized stress and local trauma acting on tissue which is degenerated by alterations due to the normal aging process.⁴¹

2.2 HEMODYNAMIC CONSEQUENCES OF AORTIC REGURGITATION

In the normal heart, the LV SV flows into the aorta and the systemic circulation. With an incompetent AoV, a certain amount of blood flows back into the ventricle with every heartbeat. This results in LV volume and pressure overload.⁴² The heart will adapt to accommodate this new hemodynamic state with ventricular dilatation and an increased end-diastolic volume. The increased pre-load will trigger the Frank-Starling mechanism and ventricular inotropy is increased, producing a larger SV (Figure 4). However, the effective forward SV is unaltered, because part of this larger SV reverses to the ventricle during diastole. The increased wall stress resulting from ventricular dilatation is compensated by hypertrophy of the LV wall (eccentric hypertrophy).^{42,43}

These adaptations cause hemodynamically important changes. The larger SV creates a higher systemic blood pressure during systole. During diastole, a rapid decline in systemic blood pressure occurs, due to the large diastolic run-off through the AoV.⁴² The large difference between systolic and diastolic pressure, also known as pulse pressure, can be palpated at the facial artery in horses with AR as a bounding pulse.²⁷ End-diastolic systemic blood pressure will be lower in horses with AR compared to healthy horses, whereas end diastolic LV pressure will be higher. These pressures are the driving force for opening of the AoV at the onset of systole and thus the valve will open earlier in horses with AR.



Figure 4: Pressure-volume (PV) loop of the cardiac cycle. The black line depicts a normal PV loop of a healthy subject. The red line/area shows the PV loop of a patient with chronic aortic regurgitation (AR). In horses with AR, the end-diastolic left ventricular (LV) volume and pressure are increased. During contraction, a larger stroke volume is ejected, resulting in close to normal end-systolic LV volume and pressure. A, aortic valve closure; A-B, isovolumic relaxation; B, mitral valve opening; B-C, ventricular filling; C, mitral valve closure; C-D, isovolumic contraction; D, aortic valve opening; D-A, ventricular ejection

Using adaptive mechanisms, the heart can compensate for the regurgitant volume for a long time. However, the dilated ventricle becomes susceptible to the development of exercise-induced ventricular premature depolarisations.⁴⁴ Several mechanisms have been proposed to explain the association between AR and ventricular dysrhythmias. First, chronic impingement of the regurgitant jet might result in fibrosis of the myocardial wall, promoting ventricular dysrhythmias through altered conduction or triggered activity.²⁰ Second, AR results in an augmented cardiac preload and afterload, resulting in increased cardiac work and oxygen demand. However, diastolic blood pressure is reduced in these horses and thus coronary perfusion pressure is lower. During exercise, diastole shortens which further reduces the coronary perfusion. It has been suggested that ventricular hypoxia due to insufficient coronary perfusion may contribute to the increased risk of ventricular dysrhythmias during exercise in horses with AR.⁴⁴ Third, through a complex process of hemodynamic burden and alterations in myocardial function, AR induces neuroendocrine changes, including alterations in circulating catecholamines. It has been suggested that this may make the myocardium more susceptible to the development of ventricular arrhythmias.²⁵ Therefore, horses with AR are at increased risk for ventricular tachycardia and even sudden cardiac death.

Notwithstanding the big impact of ventricular fibrillation and sudden cardiac death, the risk for such an event to happen is relatively small. More often, horses with chronic AR develop severe LV dilatation. Longstanding abnormal loading and increasing severity of regurgitation might result in failure of the LV to compensate for the regurgitation. Failure of the LV to adequately evacuate the blood will hamper emptying of the left atrium and consequently result in pulmonary congestion. Due to mitral annular dilatation, mitral regurgitation can develop, which also leads to left atrial dilatation and eventually pulmonary hypertension. Loading conditions for the right ventricle will be altered and right heart failure develops. This indicates end-stage heart disease. Myocardial contractility decreases, the horse becomes hemodynamically compromised and clinical signs of heart failure become evident.

2.3 CLINICAL SIGNS

The severity of clinical signs caused by AR depends on chronicity of regurgitation. Progression is usually slow and many horses never develop obvious clinical signs due to the regurgitation. Slow progressive degeneration of the valvular cusps allows the heart to compensate for the regurgitant volume and adapt to the altered hemodynamics. However, in some horses, degeneration of the valve evolves faster. When the heart is no longer able to compensate for the regurgitation, clinical signs of heart failure will become evident. This usually happens after longstanding AR. The clinical signs of left-sided heart failure are related to the incompetence of the LV to develop sufficient contractility to pump blood into the systemic circulation, resulting in pulmonary congestion.^{25,45} In mild cases, tachypnoea and exercise intolerance will be the first signs of pulmonary oedema and insufficient oxygen supply. Auscultation of the lungs may reveal moist bronchovesicular sounds and thoracic radiographs show an interstitial pattern and pulmonary venous congestion. In more severe cases, horses show signs of dyspnoea, coughing and nasal discharge. Weight loss, weak arterial pulses, weakness, ataxia and syncope may indicate a severe reduction in cardiac output. Pulmonary hypertension creates an increased afterload for the right ventricle and may eventually lead to right-heart failure. Inability of the right ventricle to pump blood in the pulmonary circulation leads to congestion in the systemic circulation. Clinical signs include distension of jugular and other peripheral veins. Oedema of the ventral abdomen and extremities may become evident. Abdominal ultrasound may reveal congestion of the liver and ascites.²⁵ When the onset of severe AR occurs more acutely, the heart has no time to adapt to the hemodynamic disturbance and severe symptoms (dyspnoea, pulmonary oedema, exercise intolerance), will become evident rapidly.²⁷

As AR is a risk factor for the development of ventricular arrhythmias, the consequences of these arrhythmias may be the first and only clinical sign. Ventricular arrhythmias are often unnoticed, but may result in collapse or even sudden death. Therefore, it is important to include exercise electrocardiography when examining horses with AR to detect the presence of ventricular premature depolarisations and assess the risk for riding the horse.

2.4 DIAGNOSIS

The diagnosis of AR and assessment of severity and hemodynamic consequences is made based on a thorough clinical examination, echocardiography and electrocardiography.²⁹

Cardiac auscultation in horses with AR usually reveals an often decrescendo diastolic murmur with the point of maximal intensity over the AoV.^{46,47} The murmur is typically holo- or even pandiastolic and often sounds musical due to vibrations of cardiac structures. The grade of the murmur is not always related to the severity of the regurgitation. Especially with musical murmurs, intensity of the murmur is often not well correlated with severity.²⁵ Palpation of the arterial pulse is a good clinical indicator of AR severity, with hyperkinetic pulses in severe cases.⁴⁶ If available, invasive or non-invasive measurement of peripheral blood pressure can provide additional information regarding hemodynamic consequences of the regurgitation.

Systolic blood pressure above 125 mmHg, diastolic blood pressure below 50 mmHg and pulse pressure higher than 60 mmHg are indicative of significant hemodynamic alterations.^{29,48}

Echocardiography is necessary to confirm valvular regurgitation and to evaluate the hemodynamic impact on the heart. Different ultrasound techniques are available and the exam should focus on visualization of the regurgitant jet, assessment of valvular pathology and hemodynamic impact on the heart.^{29,49}

For evaluation of structural conformation of the heart, two-dimensional (2D) echocardiographic images are recorded to measure cardiac and large vessel dimensions and to visualize abnormalities of the cardiac valves and myocardial walls. Horses with AR develop eccentric hypertrophy and this can be assessed from the standard 2D images by subjective evaluation of LV dilatation and by measuring ventricular dimensions. Additionally, M-mode images allow measurement of functional parameters and timing of different events.^{50,51} Colour flow Doppler can visualize the regurgitant jet. The size and area occupied by the jet are indicative of AR severity.⁵² However, evaluation of the size of the jet is very subjective and a jet that sprays widely into the ventricle may be classified as severe regurgitation although the effective regurgitant volume may be relatively small.

In human medicine, several echocardiographic methods have been developed to estimate the size of the regurgitant volume.⁵³ In human patients, cardiac ultrasound can be performed from a parasternal, apical, suprasternal or transoesophageal view. Estimation of the regurgitant volume is never based on a single parameter, but results from combining qualitative and (semi-) quantitative methods. Table 1 contains reference values for the most important measures to quantify the size of the regurgitant volume. 2D and M-mode images display an overall view of the heart, the cardiac dimensions, valve anatomy and valve function. They provide indirect signs of the chronicity and severity of the regurgitation and allow differentiation between severe regurgitation or a minor leak. The regurgitant jet can be displayed for visual assessment with colour flow Doppler imaging. The resulting image and size of the jet depend on machine settings and care should be taken to always use the same settings. Methods have been developed for planimetry of the regurgitant jet area, but the results are influenced by direction of the jet, loading conditions and blood pressure. Central jets are often overestimated and eccentric jets are often underestimated.⁵³

Table 1: Recommendations for quantification of the regurgitant jet in humanmedicine, adapted from Lancellotti et al. (2013)⁵³

AR severity	Mild	Moderate	Severe
Qualitative			
Aortic valve morphology	Normal/ abnormal	Normal/ abnormal	Abnormal/flail/large coaptation defect
Colour flow AR jet width (Nyquist limit of 50-60 cm/s)	Small	Intermediate	Large in central jet, variable in eccentric jets
CW signal of AR jet	Incomplete/faint	Dense	Dense
Semi-quantitative			
VC width (mm)	< 3	Intermediate	<u>></u> 6
Pressure half-time (ms)	> 500	Intermediate	< 200
Quantitative (PISA)			
EROA (mm²)	< 10	10-19; 20-29*	<u>></u> 30
R Vol (mL)	< 30	30-44; 45-59*	<u>></u> 60

AR, aortic regurgitation; CW, continuous wave Doppler; EROA, effective regurgitant orifice area; LV, left ventricle; R Vol, regurgitant volume; VC, vena contracta.

* mild-to-moderate (EROA of 10-19 mm² or an R Vol of 20-44 mL); moderate-to-severe (EROA of 20-29 mm² or an R Vol of 45-59 mL).

A semi-quantitative method that has been used in human medicine is measurement of the vena contracta (Figure 5). This is the narrowest point of the regurgitant jet downstream of the regurgitant orifice. It provides an estimate of the effective regurgitant orifice area (EROA). A small vena contracta width of less than 3 mm is considered to be mild AR, a large vena contracta width of more than 6 mm is considered as severe AR in human patients.⁵³ This method is inaccurate when multiple jets or jets with non-circular orifice are present.



Figure 5: Measurement of vena contracta (VC) from parasternal long axis view of the aortic valve.

The proximal isovelocity surface area (PISA) or flow convergence method is a quantitative method to estimate the regurgitant volume. The flow convergence zone is visualized from an apical or parasternal view (Figure 6). The radius of the PISA is measured in diastole from the centre to the first aliasing signal. Based on the radius, the aliasing velocity and the time velocity integral, the regurgitant volume and the EROA can be calculated. In human patients, an EROA of less than 10 mm² is graded as mild AR. When EROA exceeds 30 mm², regurgitation is considered to be severe. In human medicine, this method is considered to be one of the best echocardiographic methods to quantify the regurgitant volume.⁵³





Figure 6: Flow convergence method for quantification of the regurgitant flow. The left panel displays a schematic representation of the flow convergence method. The right panel pictures regurgitant flow from the aortic valve with aliasing velocity adjusted to facilitate measurement of the hemisphere radius. VC: vena contracta; Va: aliasing velocity; PISA: proximal isovelocity surface area; r: radius; Reg Jet: regurgitant jet; EROA: effective regurgitant orifice area; TVI(AR): time-velocity integral of the aortic regurgitation.⁴²

With the Doppler volumetric method the regurgitant volume is calculated as the difference in SV between a competent cardiac valve and the insufficient aortic valve. Using pulsed wave (PW) Doppler, the mitral inflow is measured to calculate the systemic SV (if mitral regurgitation is present, the pulmonary flow can be used). The difference between the calculated SV from mitral flow or pulmonary flow and the SV measured at the aortic valve represents the regurgitant volume. For human patients, a regurgitant volume of less than 30 mL represents a mild regurgitation. When more than 60 mL flows back with every heartbeat it is considered to be severe AR. However, this method is time consuming.⁵³

Continuous wave Doppler can be used to sample the regurgitant flow from the aortic valve. This requires good alignment of the ultrasound beam with the blood flow to obtain correct measurements. From this flow curve, the rate of deceleration of the diastolic regurgitant jet can be evaluated. The derived pressure half-time reflects the degree of regurgitation and the ventricular end-diastolic pressures. A long pressure half-time (> 500 ms for human patients) indicates mild AR. When pressure half-time is less than 200 ms this indicates severe regurgitation.⁵³

In horses, echocardiography is a non-invasive technique that can easily be applied. However, equine cardiologists are limited to parasternal images due to the conformation of the horse. Apical, suprasternal and transoesophageal images cannot routinely be recorded in the adult awake horse or provide a limited imaging window. Therefore, it is often not possible to achieve good alignment with the blood flow to apply the methods used in human medicine. Assessing the regurgitant volume in horses is therefore difficult.

In addition to the echocardiographic examination, electrocardiography should be performed to detect concurrent dysrhythmias. Horses with AR often develop ventricular dysrhythmias and this should be monitored with exercising electrocardiography because the combination of AR with ventricular dilatation and ventricular ectopy predisposes to exercise related collapse or even sudden cardiac death.^{29,48} Although the risk for sudden cardiac death is increased in horses, the occurrence of such an event is rare. However, when a horse dies from sudden cardiac death, this might have a big impact on rider safety and public opinion. Therefore, it is important to monitor these horses closely.

2.5 PROGNOSIS

Horses with AR should be examined regularly to monitor progression of the disease. Prognosis is more reserved when AR is detected in young horses and depends on the aetiology of the regurgitation. Early onset of degenerative alterations of the valve leads to longstanding abnormal loading of the heart and increases the risk of heart failure. Older horses with AR that continue to be ridden should also be monitored closely.²⁹

In most horses, AR is slowly progressive and does not limit exercise capacity or life expectancy.³⁸ However, some horses progress more rapidly to severe AR and this

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can have an important clinical impact. Acute onset of severe regurgitation holds a poor prognosis as the heart has no time to adapt and clinical signs of heart failure become evident almost immediately.²⁷

When LV dilatation is more slowly progressive, altered hemodynamics including altered loading conditions for the heart and reduced coronary perfusion increase the risk for ventricular premature depolarisations and sudden cardiac death.²⁰ Exercise induced ventricular dysrhythmias are considered as a negative prognostic indicator and these horses are less safe to ride.²⁹

With increasing age, some horses develop heart failure due to longstanding abnormal loading of the heart and extreme dilatation of the ventricle. Assessment of prognosis is important in prepurchase examination and for rider safety. Several clinical parameters have been described in literature to estimate prognosis. Bounding arterial pulse and a pulse pressure of more than 60 mmHg indicate an important hemodynamic impact. Severe valvular lesions, moderate to severe LV volume overload, hyperdynamic or decreased LV systolic function, concurrent mitral valve regurgitation secondary to AR and presence of ventricular arrhythmias indicate poor prognosis.²⁹ However, it is difficult to determine the prognosis accurately based on the current guidelines and diagnostic techniques. In human medicine, several techniques are available to determine the severity of AR, but some of these techniques are not applicable in horses due to their size and conformation.⁵³

2.6 TREATMENT

Horses with compensated AR and no clinical signs of heart failure do not require treatment. These horses should be monitored regularly to follow progression of the disease and to detect early signs of onset of decompensation and myocardial failure.²⁹ Echocardiography and electrocardiography should be performed twice yearly, or at least annually to assess hemodynamic alterations. A bounding arterial

pulse, onset of exercise intolerance and irregular heart rhythm are indications that AR severity may have worsened. A large regurgitant jet or multiple jets, excessive dilatation of the LV, a pulse-pressure of more than 60 mmHg, decreased contractility of the myocardium and frequent ventricular arrhythmias indicate that the horse should be retired from work.²⁹

Both in human medicine and in small animals, it has been shown that medical treatment delays the onset of congestive heart failure in patients with heart disease of various aetiologies.^{54,55} In contrast, horses without clinical signs of heart failure are usually left untreated as the beneficial effects of medical treatment have not been demonstrated. Additionally, medical treatment renders the horse unsuitable for competition due to doping regulations.

Even when signs of heart failure emerge, medical treatment is not as common in horses as it is in man or small animals. Most cardiac drugs are not approved for use in horses and therapeutic protocols are often extrapolated from data from other species.⁵⁶ Medical treatment should focus on improvement of cardiac output and stimulation of diuresis. Diuretics help lowering the preload, but can also lead to metabolic disturbances following long-term use. Angiotensin-converting enzyme (ACE) inhibitors help reducing the cardiac afterload in humans and small animals. Studies examining the effectiveness of enalapril in horses showed contradicting results. Benazepril, ramipril and quinapril are probably more effective in horses.^{27,56} Digoxin is a positive inotropic agent that can be used to enhance myocardial contractility.⁵⁶ This drug should be used with caution to avoid toxic side-effects. There is a wide variability in individual horses response to digoxin and horses treated with digoxin should be monitored closely. When signs of toxicity emerge, treatment should be discontinued immediately. Other positive inotropic agents, such as pimobendan and milrinone are available but are often too expensive to be used in horses. Long term prognosis for equine heart failure is poor. As the defect causing heart failure cannot be corrected, medication should be continued for life.56,57

2.7 AORTIC REGURGITATION IN OTHER SPECIES

In human medicine, AR is a common valvular disease and is usually the result of morphological abnormalities of the aortic leaflets or dilatation of the aortic root, making correct apposition of the leaflets impossible.⁵³ Patients often remain asymptomatic for long time, although some develop symptoms of LV dysfunction. Several techniques and modalities are available for diagnosis and assessment of AR, including echocardiography, magnetic resonance imaging (MRI), computerized tomography (CT)-scan and angiography. Management of these patients focusses on the determination of severity of regurgitation and its impact on LV function. In patients with severe AR, surgical repair or replacement of the AoV is indicated. However, determining the optimal timing for surgery is difficult and it has been demonstrated that best clinical outcome is obtained if surgery is performed before the onset of LV dysfunction.⁵⁸

In small animals, acquired diseases of the AoV are very rare. A small jet of AR can sometimes be diagnosed by colour flow Doppler in older dogs with chronic degenerative valvular disease. But such lesions are rarely associated with an audible murmur or clinical signs.⁵⁹ Aortic regurgitation in dogs occurs most often as a result of bacterial endocarditis or congenital heart defects.

3. ECHOGRAPHIC EVALUATION OF THE EQUINE HEART

3.1 Echocardiography

Echocardiography is the most important non-invasive diagnostic tool in equine cardiology. In human and small animal medicine, radiography, fluoroscopy, CT-scan and MRI are frequently used to evaluate cardiac function.⁵⁸ These techniques cannot be applied in horses due to the size of the animals. In horses, echocardiography is used to visualize the heart and to identify the aetiology and consequences of cardiac abnormalities.
A standardized echocardiographic examination comprises short- and long-axis images from left and right parasternal views. The ultrasound probe is placed in the third to fifth intercostal space. For optimal image quality, the skin should be clipped free of hair, cleaned with surgical spirit and ultrasonic coupling gel should be applied. From the right side, the entire heart can be visualized and relative sizes of the cardiac chambers and vessels can be assessed.²⁵ For adult horses of approximately 500kg, the image depth should be set at 26-30 cm to fit the entire heart on the ultrasound image. The examination is usually performed with a 2.0-2.5 MHz transducer, with 1.7/3.4 MHz (octave harmonics) as the most commonly used frequency. For small horses and foals a higher frequency probe can be used to generate more detailed images at lower depth.⁶⁰ Apical images, as recorded in human medicine, can be obtained in foals but not in adult horses due to their conformation.

Different modalities are used in a standardized echocardiographic examination: 2D echocardiography, motional-mode (M-mode) and Doppler based imaging.⁶⁰ 2D echocardiography allows recording of grayscale images, showing cardiac anatomy moving in real-time. These images provide an overall evaluation of cardiac structure and function. M-mode visualizes a one-dimensional representation of the movement of cardiac structures. The movement of the myocardial walls and valves is displayed over time. This allows accurate timing of cardiac events due to the high sampling rate and simultaneous recording of an electrocardiogram.²⁵ Doppler echocardiography is based on the Doppler effect, a physical phenomenon that describes the shift in sound wave frequency when reflected by a moving object.⁶¹ The shift in frequency is called Doppler shift and depends on the direction and velocity of the moving object. From the Doppler equation, the velocity (*v*) can be calculated from the Doppler shift (*fD*), the velocity of sound (*c*), the transmitted frequency (*fT*) and the insonation angle (α) between the ultrasound beam and the velocity vector of the moving object.

$$v = -\frac{fD.c}{2fT.\cos(\alpha)}$$

The velocity will have a positive or negative sign depending on the direction of the movement. In cardiology, the Doppler principle is used to measure the velocity of blood and cardiac structures. The maximal velocity that can be measured depends on the Nyquist limit, which is defined as half of the pulse repetition frequency that is used to sample the moving structure. If the velocity is higher than the Nyquist limit, aliasing will occur and no accurate measurement of velocity can be performed. Pulse repetition frequency is influenced by the depth of the sample volume. When the sample volume is placed close to the transducer, pulse repetition frequency will be higher and thus higher velocities can be measured.⁶² In echocardiography, continuous wave (CW) and pulsed wave (PW) Doppler are used to measure blood flow velocity. CW Doppler is most accurate for measuring high velocities, but does not allow depth discrimination. PW Doppler measures velocity at a specific place (e.g. the cardiac valve), but is limited by aliasing effect when the velocity is too high. Both techniques require good alignment of the ultrasound beam with the blood flow, which is not always easy to achieve. From the resulting curves, peak flow velocity, velocity time integral and flow acceleration can be measured.^{25,62}

Colour flow Doppler is an ultrasound technique that allows colour-coded visualization of the blood flow.⁶³ By convention, blood moving away from the transducer is coded in blue and blood moving toward the transducer is displayed in red. Regurgitant flow from the cardiac valves is high-velocity turbulent flow and is displayed in green (Figure 7). This technique allows visual assessment of the regurgitant jet. The area of the jet, timing and duration of the regurgitation can be evaluated.



Figure 7: Colour flow Doppler image of regurgitant flow from the aortic valve. Left parasternal long axis view of the left ventricular outflow tract showing regurgitant flow (in green) from the aorta into the left ventricle. Ao, aorta; AoV, aortic valve; LV, left ventricle.

A standardized echocardiographic examination yields a general overview of cardiac structure and function. Cardiac dimensions can be measured from 2D and M-mode images. Additionally, functional parameters can be calculated. Fractional shortening (FS) is a commonly used parameter for evaluation of LV systolic function.⁶³ LV internal diameter (LVID) is measured from short-axis M-mode images at chordal level at end-diastole (d) and at end-systole (s). Fractional shortening is calculated as FS = (LVIDd – LVIDs)/LVIDd. However, this parameter is load-dependent and since valvular disease may alter cardiac loading significantly, FS may not be useful in such cases.⁶⁴

Grayscale images also allow to assess functional abnormalities of the myocardial walls and cardiac valves. A visual scoring system for abnormal wall motion has been proposed, but remains rather subjective.⁶⁵ Subtle alterations of the myocardial function cannot always be detected by a standardized echocardiographic

examination using 2D, M-mode and Doppler techniques. Cardiac contraction is a three-dimensional event, with wall motion in longitudinal, radial and circumferential direction (Figure 8).⁶⁶



Figure 8: Schematic representation of the three major directions of left ventricular contraction.

Due to conservation of mass, these movements are not independent of each other. Longitudinal and circumferential shortening during systole are compensated by radial thickening. Alterations in cardiac function might affect the different components of contraction in different ways, therefore evaluation of all three directions might be useful. Furthermore, the pattern of myocardial contraction is not homogeneous over the ventricular wall. Differences in contraction between the epicardial and endocardial side of the myocardium have been documented.^{67,68} This might be of importance when evaluating myocardial function. Additional ultrasound techniques, such as tissue Doppler imaging (TDI) and speckle tracking (2DST), are increasingly being used in human medicine and have shown to be able to detect subtle alterations in myocardial function.^{69,70}

3.2 TISSUE DOPPLER IMAGING

While colour flow Doppler measures velocity of red blood cells, which is colourcoded and superimposed on 2D grayscale images, TDI measures the velocities of myocardial walls. To discriminate blood flow from myocardial wall motion, a filter is applied. Red blood cells typically move at high speeds (>50 cm/s) and yield a low intensity signal. For colour flow Doppler a high pass filter is applied and increased gain is used to enhance the low intensity signal. Myocardial walls move at lower speed (<35 cm/s) and the reflected signal has a higher intensity. Therefore a low pass filter is applied to visualize movement of myocardial walls by TDI (Figure 9).^{71,72}



Figure 9: The Doppler signal of myocardial wall motion has low frequencies and high amplitudes, whereas the Doppler signal of blood flow has high frequencies with a wider distribution and lower amplitudes. The upper panel demonstrates the application of a low-pass filter that will reject high frequency signals of the moving red blood cells (indicated by grey colour). The lower panel shows the effect of gain adjustment, which will eliminate low amplitude signals of the blood flow (indicated by grey colour). TDI can be used in two different modes. In PW TDI a sample volume is placed in the myocardium at a fixed distance from the transducer. Throughout the cardiac cycle, the myocardium moves through the sample volume. The resulting velocity curve thus reflects different parts of the myocardium. With 2D colour TDI, colour-coded velocity information of the myocardial walls is superimposed on the grayscale images. The velocity curves are generated after image acquisition (off-line) by placing a sample area on the myocardial wall. The sample area is set to track the movement of the wall during the cardiac cycle so that the same part of myocardium is analysed throughout the cardiac cycle. This technique thus allows evaluation of the movement of a specific segment of the myocardium. PW TDI and 2D colour TDI are different techniques that use different algorithms to calculate velocity. Therefore, results cannot be compared directly.^{73,74}

With TDI, velocity is calculated relative to a fixed point, the ultrasound transducer. Thus, not only velocity of the myocardial wall is measured, but total movement of the heart in the thorax, e.g. due to breathing, is also included in the measurement. In apical images, the transducer is close to the apex that is more or less fixed in the thorax and the error will be small. However, in short axis images, total heart movement might lead to misinterpretation of myocardial velocities. In human medicine, this drawback can be reduced by taking images at breath hold, but this is not possible in equine patients. Another disadvantage of measuring velocity is that dysfunctional myocardial segments are dragged passively by neighbouring functional segments (tethering).⁷⁵ Despite being dysfunctional and not contractile, these segments will show motion at a certain velocity, equal to or lower than the neighbouring segments.

Strain and strain rate are measures of myocardial deformation that overcome some of the limitations of velocity measurements.⁷⁶ Strain is the deformation of the myocardial wall. It is the amount of deformation relative to the initial wall thickness or length and is expressed as a percentage. The strain rate is the change in strain per time unit, which is expressed as 1/s. Both measures can be calculated based on

velocity measurements from different regions within the myocardial wall. Strain and strain rate can then be derived from the myocardial velocity gradient. This way some of the limitations of velocity measurements can be overcome as myocardial deformation is less influenced by total heart motion or tethering. Strain and strain rate can also be colour-coded on top of grayscale images, yielding a good visual representation.⁷⁷

Colour tissue Doppler imaging offers the advantage that several myocardial segments can be assessed from a single image. From a short axis image of the LV, both the LV free wall and the interventricular septum can be measured simultaneously, which facilitates the evaluation of ventricular contraction synchronicity. Additionally, the differences between epicardial and endocardial function can be evaluated by placing the sample area accordingly.

However, the technique also has some limitations. Both for PW and colour TDI, correct measurement of velocity requires a good alignment of the ultrasound beam with the movement of the myocardial wall. Since the resulting velocity depends on the angle between the ultrasound beam and the velocity vector of the wall, an insonation angle less than 15-20 degrees is advised.⁷⁸ During image acquisition, this should be taken into account. In horses it is not possible to take apical images. Therefore, longitudinal function of the ventricles cannot be evaluated by TDI in horses.

Another disadvantage of the colour TDI technique is that images cannot be processed in real time. Images are recorded and stored for off-line analysis. Processing of the images is time consuming and requires operator expertise.

Sampling rate should be sufficiently high to assess myocardial motion throughout the cardiac cycle. To guarantee sufficient temporal resolution, a high frame rate (> 180 frames per second) is required.⁷⁹ The sector width of the image should be reduced to limit the time needed to sweep from one side to another. Decreasing the depth of the image also increases frame rate because time from sending to

receiving the pulses is reduced. Reducing the number of beams per frame would also increase the frame rate, but reduces the lateral resolution of the image, especially in deeper parts of the images. Images should be optimized by balancing temporal and spatial resolution.

As TDI is a Doppler technique, measurements are limited by the Nyquist limit. If sampling rate is too low compared to the velocity that should be measured, aliasing occurs. In TDI images this phenomenon is visible as a fast shift of velocities from negative to positive (or opposite). The sampling rate or pulse repetition frequency should be at least twice the maximal Doppler shift. Aliasing can be eliminated by increasing the velocity scale during image acquisition.⁶⁰

Image artefacts have an extensive impact on TDI measurements.⁷⁸ Non-random artefacts, such as drop-out and reverberations, should be avoided during data-acquisition and by repositioning the sample area during off-line processing. Random noise can be counteracted by smoothing the curves. Temporal averaging can be achieved by averaging consecutive cardiac cycles. Spatial averaging can be done by increasing the size of the sample area.

Strain curves are also subject to integrational drift, due to an accumulation of errors. This is corrected by the software drift compensation of the strain curve.⁶⁶

Use of TDI in veterinary medicine

In human medicine, TDI has been studied and used in a wide range of cardiac problems.⁸⁰ In small animals, the use of TDI has been investigated extensively as well. The technique has been proven to be reliable and was able to detect an impaired LV function in various cardiac diseases such as dilated cardiomyopathy and mitral valve disease.^{68,74,81-83}

Decloedt et al. (2013) investigated the feasibility and reliability of colour TDI for quantification of radial myocardial function in healthy horses.⁷⁹ Short axis images of

the LV from 10 healthy trotters were evaluated by TDI to determine intra- and inter-observer measurement variability.



Figure 10: Tissue Doppler curve from a right parasternal short axis image of the left ventricle. The green curve shows the velocity of the interventricular septum throughout the cardiac cycle. The yellow curve displays the velocity of the left ventricular free wall. IVC: isovolumetric contraction; S: systole; E: early diastole; A: late diastole.

Peak values and timings were measured from velocity and strain rate curves at systole (S), during early diastole (E) and late diastole (A) (Figure 10). Isovolumic phases yielded smaller peaks, were often biphasic or were absent. Strain was only measured at peak systole. Peak values showed a moderate to high variability, whereas timings of cardiac events showed a low variability. Strain and strain rate could not be measured reliably in the LV free wall, probably because this segment was too far from the transducer. It was concluded that TDI can be used reliably to evaluate radial function of the LV.⁷⁹ Sepulveda et al. (2005) used PW TDI and colour TDI to determine repeatability of both techniques to measure LV function in 5

segments of the ventricular walls. Twenty young Thoroughbreds were involved in this study and it was concluded that PW Doppler produces more reproducible data.⁸⁴ Another study in 3 Standardbreds and 3 Thoroughbreds used the same techniques to evaluate LV radial function. The authors concluded that it was feasible and reliable, although PW TDI was more reliable than colour TDI.⁸⁵

TDI could also detect myocardial dysfunction in horses with various cardiac diseases. In horses with atypical myopathy, TDI measurements revealed a prolonged duration of contraction, increased isovolumic relaxation time and a decreased ratio of early to late diastolic LV radial velocities. These findings indicate impaired diastolic LV function.⁸⁶ TDI was also used to evaluate LV function in horses that were accidentally exposed to the ionophore drug lasalocid, which causes myocardial damage. TDI was able to demonstrate a decreased radial systolic velocity and strain in these horses.⁸⁷ In a horse with nutritional masseter degeneration, pulsed wave TDI was used to quantify myocardial dysfunction.⁸⁸ In the acute phase, TDI demonstrated an impaired LV diastolic function. During recovery, LV function returned to normal. TDI was more sensitive than 2D echocardiography to detect alterations in LV function.⁸⁸ Gehlen et al. (2013) investigated the cardiac effects of therapeutic dosages of clenbuterol. Compared to conventional 2D echocardiography, TDI proved to be a sensitive technique to detect myocardial remodelling after administration of clenbuterol.⁸⁹ TDI was also used in exercise stress echocardiography.⁹⁰ Both colour-coded TDI and PW TDI were evaluated and proved to be feasible at rest and after exercise. After exercise, only a short time frame is available to record images. Colour-coded TDI may be more favourable than PW TDI in these circumstances since images can be analysed off-line. In horses with cardiac valvular disease, TDI has not been used extensively.

3.3 TWO-DIMENSIONAL SPECKLE TRACKING

Two-dimensional speckle tracking (2DST) is an ultrasound technique that is based on tracking of spatial motion of speckle patterns in grayscale images. It provides an objective and quantitative method for evaluation of global and regional myocardial function. Speckle patterns in a 2D ultrasound image are created by reflections and scattering of ultrasound beams. These patterns are usually relatively stable throughout the cardiac cycle. The algorithm defines clusters of speckles (kernels) and tracks these kernels from frame to frame during cardiac contraction and relaxation. Spatial movement of the kernels allows calculation of strain, strain rate, velocity and displacement.^{70,78}

Similar to TDI, 2DST allows measurement of myocardial velocities and deformation parameters (Figure 11). However, 2DST uses a completely different algorithm to calculate deformation, and so it overcomes some of the limitations of TDI.⁹¹



Figure 11: Evaluation of myocardial function by 2D speckle tracking. The upper panel shows the four chamber view of the left ventricle. The coloured curves on the right display the longitudinal velocity of the different myocardial segments. The lower panel shows the short axis image of the left ventricle. The coloured curves on the right depict the radial strain rate of the different myocardial segments. S, systole; E, early diastole; A, atrial contraction.

Movement of speckles is not measured relative to a fixed point, but relative to other speckles. Therefore, results are less influenced by total heart motion and insonation angle. Additionally, speckles are tracked in two dimensions so results can be obtained for myocardial motion in the radial, circumferential and longitudinal direction.^{70,91} In human medicine, longitudinal function has been reported to be one of the best markers of subtle myocardial dysfunction.^{67,92} Longitudinal shortening of the ventricle is responsible for the largest part of the volume reduction during systole and might therefore be more sensitive to alterations in myocardial function. Additionally, radial and circumferential deformation is not homogeneous over the thickness of the myocardial wall, the endocardial part shows a larger deformation than the epicardial part. Consequently, the results for radial and circumferential function are influenced by the placement of the region of interest (ROI). Longitudinal deformation is homogeneous over the thickness of the myocardial wall, making the results less dependent on ROI placement. In horses, longitudinal myocardial function cannot be evaluated by TDI because their conformation does not allow apical images to be recorded. Bidimensional tracking of the speckles with 2DST makes it possible to evaluate longitudinal function from parasternal images in horses. Another disadvantage of TDI is the need for high frame rates. 2DST can be performed at lower frame rates compared to TDI, although if frame rate is too low, tracking quality will be insufficient. Low frame rates lead to undersampling and may lead to misinterpretation of peak values. Short isovolumic peaks may disappear and fast diastolic peaks may be underestimated. A frame rate of 50-70 frames per second is advised.^{70,78} With TDI velocity measurements, tethering leads to overestimation of myocardial function in dysfunctional segments. In 2DST, strain is calculated directly from the frame to frame speckle movement, so the tethering effect is not an issue.⁹¹ Dysfunctional myocardial segments will not deform actively, but they may be deformed passively by neighbouring segments.^{66,91}

However, 2DST has some limitations of its own. First, image quality is crucial to enable the software to adequately track the speckles. Poor image quality will result

in loss of speckles and poor tracking of the myocardial walls. Therefore, during image acquisition it is important to record images of good quality and to avoid artefacts. Second, correct tracing of a ROI containing the myocardium is paramount. Most software applications require manual delineation of endocardial borders. The software then assumes a uniform thickness of the myocardium, which may not be correct.^{78,91} The ROI should be sufficiently large to increase the number of speckles that can be tracked. However, the ROI should not include the epicardium or other surrounding structures since this will result in poor tracking guality. Third, the movement of the heart causes out of plane motion of speckles. The speckle pattern might therefore change too much from one frame to the next and this may lead to misinterpretation of the speckle pattern in the next frame. The software will select another kernel that resembles the original kernel. As a consequence, cumulated drift will develop throughout the cardiac cycle, which needs to be corrected. When the ventricle is very large or dilated, it might be difficult to image the entire myocardium and often the apical segments are not suitable for analysis.^{78,91} A final major limitation of 2DST is that the software algorithms are unknown and may vary from vendor to vendor. Therefore, results from different scanners and software applications might not be comparable.^{78,91}

Use of 2DST in veterinary medicine

In human medicine, 2DST has been studied in a variety of cardiac diseases.⁹³ Compared to conventional 2D and M-mode echocardiography, an increased sensitivity for detection of subtle myocardial dysfunction has been demonstrated. In dogs, the feasibility of the technique was established and results showed good correlation with TDI measurements.^{94,95} Suzuki et al. (2013) investigated the use of 2DST in dogs with mitral valve insufficiency.⁹⁶ This study demonstrated that longitudinal deformation was not different in normal dogs compared to dogs with mitral valve insufficiency. Radial and circumferential function showed alterations depending on the severity of mitral valve insufficiency. Therefore, measuring

multidirectional myocardial deformation might be important to detect early onset of myocardial dysfunction.⁹⁶

The same author investigated the effect of age on myocardial function and demonstrated significant differences in diastolic function between young and old dogs.¹⁶

In horses, the technique of 2DST has been validated. Systolic longitudinal LV myocardial function can be measured reliably using 2DST, with a low to moderate inter- and intra-observer, within- and between-day variability. Diastolic parameters showed a higher variability and this is probably due to the relatively low frame rate of 2DST making it difficult for the software to track the fast motion of the myocardial walls during diastole. This leads to underestimation of early diastolic peak values.⁹⁷ Schwarzwald et al. (2009) investigated the feasibility of 2DST for measuring radial and circumferential LV function in horses.⁹⁸ They found that systolic radial motion could be measured reliably but circumferential parameters showed more variation. Diastolic measurements had a higher variability, probably as a result of inaccurate tracking of the myocardial wall. However, only 6 horses were involved and analysis was only performed in short-axis images of the LV at chordal level.⁹⁸ Decloedt et al. (2013) did a similar study, in 10 horses, using images both at the level of the papillary muscles and at chordal level.⁹⁹ They concluded that measurement of global and regional radial and circumferential LV wall motion is feasible. Again, early diastolic measures showed a higher variability than systolic parameters. At chordal level, variability was slightly higher than at papillary muscle level. This might be explained by a thicker LV wall at the level of the papillary muscles, resulting in a more accurate tracking of the larger ROI.⁹⁹

2DST has been used in a number of clinical applications in horses. After accidental lasalocid intoxication, 2DST was able to detect a decrease in LV systolic function in horses with severe myocardial damage.⁸⁷ In horses with atypical myopathy, a lower LV global longitudinal strain and increased mechanical dispersion between myocardial segments was found by 2DST.⁸⁶ After prolonged exercise in endurance

athletes, 2DST was able to demonstrate a decreased early diastolic velocity and strain rate, indicating an impaired LV diastolic function. This might be similar to cardiac fatigue as seen in human medicine after endurance exercise.¹⁰⁰ A study in 5 Warmblood horses performing treadmill exercise demonstrated that 2DST can be used for quantitative analysis of stress echocardiograms.¹⁰¹ Significant alterations in strain and strain rate could be detected by 2DST after standardized treadmill exercise.¹⁰¹ For detection of stress-induced alterations in myocardial function 2DST based measures appeared to be more sensitive and more reliable than 2D echocardiographic and TDI measures. Gehlen et al. used 2DST to evaluate the effect of clenbuterol on myocardial remodelling. After clenbuterol treatment, increases in systolic and diastolic velocities were found, indicating physiologic hypertrophy of the LV.⁸⁹

4. SUMMARY

Aortic regurgitation is quite common in highly trained athletic horses and in older horses. Most horses never develop clinical signs, but some horses progress to severe regurgitation. The regurgitant volume causes hemodynamic alterations and the heart will adapt to accommodate these changes. The ventricle will develop eccentric hypertrophy to maintain a normal forward SV. With increasing severity of regurgitation, limits of cardiac adaptation may be reached and the heart is no longer able to compensate for the regurgitation. Eventually this may even result in heart failure or sudden cardiac death. A good follow-up of these horses is therefore paramount. However, AR severity is difficult to assess in horses. In human medicine, different diagnostic modalities are available, but most are not applicable to horses, due to their size and conformation. Echocardiography is a non-invasive method that can easily be applied in the unsedated, standing horse and is therefore the most important diagnostic tool in equine cardiology. 2D and M-mode echocardiography allow real-time evaluation of cardiac structures. The hemodynamic impact of the regurgitation on the heart can be evaluated. Colour

flow Doppler can be used to visualize the intracardiac blood flow. The size, area and timing of the regurgitant jet can be assessed visually. However, this is a rather subjective evaluation and depends on machine settings.

Tissue Doppler imaging is an ultrasound technique that measures myocardial velocities based on the Doppler principle. The technique has been validated in horses. In human medicine, small animals and horses, TDI was able to detect alterations in myocardial wall motion in several clinical applications. The technique has not been used in horses with aortic regurgitation. Given the hemodynamic alterations caused by AR, TDI might be able to detect an early onset of myocardial dysfunction in these patients.

With 2DST, kernels of speckles are tracked and myocardial velocity, strain and strain rate can be calculated by the software. This technique has also been validated in horses and applied in different clinical diseases. Similar to TDI, 2DST was also able to demonstrate significant alterations in myocardial wall motion under influence of toxic or therapeutic agents, after exercise and in horses with myopathy. Studies using 2DST in horses with AR are scarce, although an altered myocardial motion may be expected in these horses.

Assessment of AR severity in horses remains difficult and is based on measurement of ventricular dimensions and subjective evaluation of LV dilatation and the evaluation of the regurgitant jet from colour flow Doppler images. This requires operator experience and makes it difficult to compare results between operators. More objective parameters that can accurately measure AR severity and determine the onset of myocardial failure in an early stage would simplify the follow-up of these horses.

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Chapter 2: Scientific aims

CHAPTER 2: SCIENTIFIC AIMS

Aortic regurgitation causes important hemodynamic alterations. Slowly progressive, chronic AR allows the equine heart to adapt and compensate for the regurgitant volume. As long as the heart is able to compensate, no clinical symptoms of heart failure will become evident. However, due to altered loading and wear and tear of the myocardial wall, the risk for ventricular premature depolarisations and sudden cardiac death is increased. If severity of the regurgitation increases beyond the compensatory capacity of the heart, decompensation occurs and clinical signs of heart failure may develop. Horses diagnosed with aortic regurgitation need to be monitored regularly to assess evolution of the disease and to determine safety to ride these horses. With the current echocardiographic techniques, it remains challenging to objectively and accurately determine the severity of regurgitation.

This research was intended to gain more insight in cardiac alterations caused by aortic regurgitation. Tissue Doppler imaging and 2D speckle tracking are echocardiographic techniques that can track and quantify the myocardial wall motion. The hypothesis was that both techniques are able to detect alterations in myocardial wall motion in horses with different grades of aortic regurgitation.

Increasing age also causes alterations in the cardiovascular system. Since aortic regurgitation is more common in older horses, it is difficult to separate the alterations caused by aortic regurgitation from those induced by aging. An additional aim of this thesis was to determine the alterations in left ventricular dimensions and myocardial function caused by increasing age. Finally, aortic regurgitation has a higher prevalence in well-trained horses. Therefore, an additional study aimed at assessing the effects of high-level endurance training on the equine left heart.

The specific aims of this thesis were:

- To investigate the differences in cardiac dimensions and function between control horses and horses with different grades of aortic regurgitation, using standard echocardiography, including B-mode, M-mode and pulsed wave Doppler. (Chapter 3)
- To determine the ability of tissue Doppler imaging to detect alterations in left ventricular radial function in horses with different grades of aortic regurgitation. (Chapter 4)
- To determine the ability of 2D speckle tracking to detect alterations in left ventricular radial, circumferential and longitudinal myocardial function in horses with different grades of aortic regurgitation. (Chapter 5)
- To investigate alterations in dimensions and function of the equine left heart caused by increasing age (Chapter 6) and by high-level endurance training (Chapter 7), using different echocardiographic modalities.

Chapter 3: Assessing aortic regurgitation severity from 2D, M-mode and pulsed wave Doppler echocardiographic measurements in horses

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Assessing aortic regurgitation severity from 2D, M-mode and pulsed wave Doppler echocardiographic measurements in horses.

The Veterinary Journal (2016) 210:34-38

Part of this work was presented at the 47th European Veterinary Conference (Voorjaarsdagen), Amsterdam, The Netherlands, April 2014

CHAPTER 3: ASSESSING AORTIC REGURGITATION SEVERITY FROM 2D, M-MODE AND PULSED WAVE DOPPLER ECHOCARDIOGRAPHIC MEASUREMENTS IN HORSES.

Abstract

BACKGROUND: Aortic regurgitation (AR) in horses can lead to left ventricular (LV) eccentric hypertrophy, ventricular arrhythmias and heart failure. Objective quantification of the severity of regurgitation is difficult.

OBJECTIVE: The aim of this study was to evaluate dimensional measurements, systolic time intervals and blood flow velocities, acquired by standard 2D, M-mode and pulsed wave Doppler echocardiography, for quantification of AR.

MATERIAL AND METHODS: Echocardiography was performed in 32 healthy horses and 35 horses with AR that were subdivided in three groups (mild, moderate or severe AR). From the recorded images LV, left atrial and aortic dimensions, systolic time intervals and aortic blood flow velocities were measured. Diastolic run-off in the aorta (Ao_{DiastDecr}) was calculated as the difference in aortic diameter between early diastole and late diastole. The rate of Ao_{DiastDecr} was calculated as Ao_{DiastDecr} multiplied by heart rate. Stroke volume (SV) was calculated from pulsed wave Doppler measurements, by the bullet method (SV_{bullet}) and by the area-length method. Pre-ejection period (PEP) and ejection time (LVET) were determined from the M-mode images.

RESULTS: Horses with AR showed enlargement of the LV, left atrium and aorta compared to the control group. The SV, the Ao_{DiastDecr} and the rate of Ao_{DiastDecr} were significantly larger compared to controls. PEP decreased significantly in horses with AR, whereas LVET did not change.

CONCLUSION: PEP and the newly defined variable Ao_{DiastDecr} proved to be easy to measure parameters that provided a good indication of AR severity. All three methods to calculate SV indicated an increased SV in horses with AR, but SV_{bullet} performed best in detecting an increase in AR severity.

INTRODUCTION

Aortic regurgitation (AR) is common in horses, especially in the aged population.^{1,2} Prevalences of up to 65% have been reported in racehorses.³ Horses with AR usually have a normal performance level and life expectancy, but in some horses progression of the condition is more rapid.^{4,5} As the regurgitant flow increases, left ventricular (LV) end diastolic pressure (EDP) increases and may lead to compensatory eccentric hypertrophy.⁶ The increased preload and LV contractile function (Frank-Starling mechanism) result in a larger stroke volume (SV) and aortic root dilatation.⁷ If AR severity further increases and the limits of LV remodeling are reached, the heart will fail to maintain its normal function and clinical signs, including exercise intolerance, dyspnea and ventricular arrhythmias, may become apparent.⁸ The increased likelihood of ventricular arrhythmias in horses with moderate or severe AR make these horses unsafe to ride due to the increased risk of collapse and sudden cardiac death.² As a consequence, horses diagnosed with AR need to be monitored closely and a good follow-up of the progression is paramount.²

AR severity may be difficult to quantify in horses and includes estimation of the regurgitant volume, evaluation of long-term hemodynamic impact on the heart and evaluation of the valvular structure. The regurgitant jet can be visualized with color flow Doppler (CFD) which allows evaluation of the area, direction and duration of the jet. This method is semiquantitative at best and depends on the settings of the ultrasound equipment, the direction of the jet and the ability to visualize it.^{3,9-11} Echocardiographic evaluation of AR severity therefore remains largely subjective and requires experience.³ The subjective nature of AR evaluation in clinical practice makes the judgment operator dependent and limits comparison of the results of different operators. The aim of this study was to describe echocardiographic changes associated with different degrees of AR.

MATERIALS AND METHODS

Study population

The study was approved by the ethical committee of the Faculty of Veterinary Medicine and Bioscience Engineering (approval number EC2012_57; date of approval 26 February 2013). The study population consisted of 35 horses with AR (16 mares, 13 geldings, six stallions) aged 18 ± 4 years with a bodyweight (BW) of 503 ± 84 kg, presented at the Faculty of Veterinary Medicine, Merelbeke, Belgium for cardiac examination. The group comprised 24 Warmbloods, 6 Arabians, 2 Anglo-Arabians, 2 Thoroughbreds and 1 Lusitano.

The control group consisted of 32 healthy horses (14 mares, 15 geldings, three stallions) aged 8 \pm 4 years with BW of 563 \pm 49 kg. This group included 30 Warmbloods, 1 Thoroughbred and 1 Trotter.

Echocardiographic examination

All horses underwent a physical examination, cardiac auscultation and transthoracic ultrasound, including two-dimensional (2DE), M-mode echocardiography, CFD of all valves and pulsed wave Doppler (PW) of the aortic blood flow, using a GE Vivid 7 Dimension ultrasound with 3S phased array transducer (GE Healthcare). An electrocardiogram was recorded simultaneously and images were stored for off-line analysis using dedicated software (EchoPAC Software Version 112, GE Healthcare). Only horses free of cardiovascular and respiratory disease were included in the control group. If horses with AR showed regurgitation from other cardiac valves, they were only included in this study if this regurgitation was no more than trivial or mild.
All measured chamber dimensions were normalized to a BW of 500 kg, using the formulae of allometric scaling:¹²⁻¹⁴

- for diameters: diameter(500) = measured diameter/ $BW^{1/3} \times 500^{1/3}$
- for areas: area(500) = measured area/BW^{2/3} × 500^{2/3}
- for volumes: volume(500) = measured volume/BW × 500

Left atrial (LA) size was evaluated from the right parasternal long axis (lx) four chamber image. The maximal LA diameter at end systole (LAD_{Ix,syst}) was measured parallel to the mitral annulus, one or two frames (depending on image quality) before mitral valve opening. The LA area was measured by tracing the inner edge of the LA in the same image (LAA_{Ix,syst}) and again at end diastole (LAA_{Ix,diast}), one or two frames after mitral valve closure. LA size was also assessed from the right parasternal short axis image of left atrium and aorta. The short axis (sx) diameter of the LA (LAD_{sx,syst}) was measured at end systole, at aortic valve (AoV) closure with a maximal LA size, in a line extending from the commissure between the noncoronary and left coronary aortic valve cusps.^{15,16}

From the right parasternal left ventricular outflow tract (LVOT), the aortic diameter at the level of the sinotubular junction was measured in systole (Ao_{lx,syst}) as the maximal diameter in one of the first three frames after AoV opening. In the same image, the LVOT diameter (LVOT_{diam}) was measured at the base of the valve.¹⁷ Measurement of the aortic diameter was repeated in early diastole (Ao_{lx,Ediast}), after AoV closure, and again in late diastole (Ao_{lx,Ldiast}), before AoV opening (Figure 1).



Figure 1: Left ventricular outflow tract with measurement of the aortic diastolic diameter. The diameter is measured in early diastole (upper panel) and in late diastole (lower panel). Ao_{lx,Ediast}, early diastolic aortic diameter; Ao_{lx,Ldiast}, late diastolic aortic diameter.

From these variables, the diastolic decrease in aortic diameter per beat was calculated:

Since the amount of Ao_{DiastDecr} depends on the duration of diastole, the rate of Ao_{DiastDecr} was also calculated:

From the short axis image of left atrium and aorta, the short axis diameter of the aorta ($Ao_{sx,syst}$) was measured at end systole at AoV closure, along the commissure between the non-coronary and the right coronary aortic valve cusps.^{15,16}

The LV internal diameter and the LV free wall thickness were measured from the M-mode image of the LV at chordal level in diastole (LVID_{diast}, LVFW_{diast}), at onset QRS, and in systole (LVID_{syst}, LVFW_{syst}) at the point of maximal excursion of the ventricular septum. The fractional shortening (FS) was calculated according to the formula:^{7,8,18}

$$FS = ([LVID_{diast} - LVID_{syst}]/LVID_{diast}) \times 100$$

From the four chamber view, the long axis LV area was measured by tracing the inner edge of the ventricle both in systole (LVA_{lx,syst}), as the smallest LV area, and in end diastole (LVA_{lx,diast}), just after mitral valve closure (Figure 2).



Figure 2: Measurement of the long axis left ventricular area (LVA_{lx}) from the right parasternal four chamber view.

The ventricular length was measured from this long axis image in systole (LVL_{syst}) and in diastole (LVL_{diast}) as the distance between the middle of the straight line connecting the opposite sections of the mitral ring and the ventricular apex (Figure 3).¹⁹



Figure 3: Measurement of the diastolic left ventricular length (LV length) from the right parasternal four chamber view.

The short axis LV area was measured from the short axis image at chordal level in systole ($LVA_{sx,syst}$) as the smallest area at end systole, and in diastole ($LVA_{sx,diast}$) as the area at end diastole (Figure 4).



Figure 4: Measurement of the short axis left ventricular area (LVA_{sx}) from the right parasternal short axis image of the left ventricle.

The fractional area change was calculated both for the long axis (FAC_{1x}) and for the short axis (FAC_{5x}) by the formula:¹⁸

Based on the measured LV area and LV length, two different methods were used to calculate LV volume.^{20,21} The area-length method uses the long axis area:

 $LVV_{al}(500) = (0.85 \times LVA_{lx}(500)^2) / LVL(500)$

The bullet method uses the short axis area:

$$LVV_{bullet}(500) = 5/6 \times LVA_{sx}(500) \times LVL(500)$$

For each method, the difference between systolic and diastolic volume was used to calculate the SV (SV_{al} (500); SV_{bullet} (500)) and the ratio of SV over LVV to calculate the ejection fraction (EF_{al} (500); EF_{bullet} (500)).^{18,19}

From a left parasternal view, the aortic flow was recorded with PW Doppler without angle correction to determine velocity time integral (VTI), maximal (Vmax) and mean (Vmean) velocity of the aortic blood flow. SV was calculated as:

$$SV_{Doppler} = (VTI \times \pi \times LVOT_{diam}^2)/4$$

From the LVOT M-mode image of the AoV, the pre-ejection period (PEP) was measured from onset QRS to AoV opening. LV ejection time (LVET) was measured from opening to closure of the AoV. The ratio of PEP/LVET was calculated.

All measured variables were the mean of three not necessarily consecutive cardiac cycles. Cardiac cycles after arrhythmias were not included.

Assessment of severity

Horses with AR were categorized as mild, moderate or severe AR based on the scoring of three different criteria. All horses were scored by two operators (GvL and SV), who were unaware of any previous classifications for each horse. The scores of both operators were averaged for final classification of the horses.

LV size was assessed subjectively from the four chamber view and then scored 1 (no enlargement) to 5 (severe enlargement), as follows: (1) normal ventricle, no signs of enlargement, normal apex shape; (2) normally shaped ventricle, mild enlargement, normal apex shape; (3) large ventricle with normal shape, apex slightly rounder than normal; (4) large ventricle, apex with globoid aspect, and (5) severe enlargement with a very large, rounded ventricle.

The regurgitant jet, as visualized by CFD, was also graded subjectively, based on the size and area of the jet. A small jet that occupied <1/3 of LVA_{lx,diast} was scored as mild (1 point), a larger jet that reached <2/3 of LVA_{lx,diast} was graded as moderate (2 points) and a very large jet that filled > 2/3 of LVA_{lx,diast} was called severe (3 points). Based on LVID_{diast} (500) additional points were assigned: 1 point if the diameter was 12-13 cm, 2 points if the diameter was 13-14 cm and 3 points if the diameter was 14 cm or more.

Based on these criteria, a total score was calculated for each horse. Horses with a total score of 8 points or more were classified as severe AR, horses with 5, 6, or 7 points were classified as moderate AR and horses with 4 points or less were classified as mild AR.

Statistical analysis

Statistical analysis was performed using commercially available software (SPSS Statistics, version 22). Data are reported as mean ± standard deviation (SD). Means from the four groups were compared using a one-way ANOVA with Bonferroni multiple comparisons test for post-hoc pairwise comparisons. Normal distribution

of all variables was checked by evaluating the residuals using visual inspection, the Kolmogorov-Smirnov test and Shapiro-Wilk test. Homogeneity of variance across the groups was checked by Levene's test for equality of variances. Variables that were not normally distributed parameters or did not show homogeneous variances were compared by a non-parametric Kruskal-Wallis test with post-hoc pairwise comparison using the Dunn test. Means from control horses and all horses with AR were compared by an independent-samples *t* test. The level of statistical significance was set at 0.05.

Results

Table 1 shows age, BW, heart rate (HR) and height for the different groups. Fourteen horses were classified as having severe AR, 11 horses had moderate AR and 10 horses showed only mild AR. There was a significant difference in age (P < 0.001) between the horses with AR (17 ± 4 years) and the control group (7 ± 3 years). Horses with AR weighed significantly less than control horses (P=0.001) and had a significantly lower height at withers (P=0.001). HR was not significantly different between groups.

	Control	Mild	Moderate	Severe	AR
Number of horses	32	10	11	14	35
Age (years)	7.6 ± 3.6^{a}	18.3 ± 4.3 ^b	16.2 ± 5.3⁵	18.1 ± 5.0 ^b	17.5 ± 4.9*
Bodyweight (kg)	563 ± 49^{a}	482 ± 75⁵	508 ± 63^{a}	513 ± 105ª	503 ± 84*
Heart rate (beats/min)	39.4 ± 6.3	38.4 ± 5.5	40.5 ± 6.1	41.0 ± 7.9	40.2 ± 6.6
Height at withers (cm)	168 ± 4ª	161 ± 10 ^{a,b}	161 ± 7⁵	164 ± 11 ^{a,b}	162 ± 9*

Table 1: Descriptive statistics for the study groups (mean \pm standard deviation). Different superscripts indicate significant differences (*P*<0.05), * indicates significant difference from control group (*P*<0.05)

In Table 2, the results for the LV size are listed. As LVID_{diast} (500) was included as a criterion for determining AR severity, it was to be expected that this parameter was significantly different between the different groups. Our results show LV enlargement in horses with AR compared to control horses. LVID_{syst} (500) was significantly different between both groups (*P*<0.001). The systolic and diastolic LV area were significantly larger in horses with AR, both on long axis (LVA_{lx,diast} (500), *P*<0.001; LVA_{lx,syst} (500), *P*<0.001) and on short axis images (LVA_{sx,diast} (500), *P*<0.001; LVA_{sx,syst} (500), *P*<0.001; LVL_{syst} (500), *P*<0.001). Consequently, the systolic and diastolic LV volume were larger in horses with AR (LVV_{al,syst} (500), *P*<0.001; LVV_{al,diast} (500), *P*<0.001; LVV_{bullet,syst} (500) *P*=0.001; LVV_{bullet,diast} (500), *P*<0.001). FS, LVFW_{diast}(500) and LVFW_{syst}(500) were not significantly different between control horses and horses with AR.

Table 2: Results for LV dimension	s.
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Different superscripts indicate significant differences (P<0.05), * indicates significant difference from control group (P<0.05).

Variable	Control group	Mild AR	Moderate AR	Severe AR	AR
2D Echocardiography					
LVA _{lx,diast} (cm ²)	145.3±19.7ª	153.6±13.4 ^{a,b}	166.1±17.7 ^{b,c}	188.9 ± 29.3℃	171.6±26.4*
LVA _{lx,diast} (500) (cm ²)	134.6±18.3ª	159.0±18.3 ^₅	164.9±15.7⁵	187.7±24.7℃	172.3±23.7*
LVA _{lx,syst} (cm ²)	67.9±12.4ª	70.0±9.2ª	71.3±7.9ª	95.8±23.5⁵	80.7±20.2*
LVA _{ix,syst} (500) (cm ²)	63.0±11.7ª	72.5±11.3ª	70.9±8.1ª	94.8±18.5⁵	80.9±17.8*
LVA _{sx,diast} (cm ²)	94.4±12.1ª	86.9±7.9 ^a	100.8±9.2 ^{a,b}	127.7±30.6 ^₅	107.6±26.6*
LVA _{sx,diast} (500) (cm ²)	87.3±11.1ª	89.8±8.9ª	100.5±12.1ª	126.0±25.5 ^₅	107.6±23.7*
LVA _{sx,syst} (cm ²)	33.2±7.6ª	28.9±2.9ª	33.4±5.7ª	48.2±17.5⁵	38.0±14.2
LVA _{sx,syst} (500) (cm ²)	30.8±7.3ª	30.0±4.0 ^a	33.2±5.8ª	47.6±16.3 ^₅	38.0±13.4*
FAC _{1x} (500) (%)	53.25±6.16 ^{a,b}	54.21±6.51 ^{a,b}	56.91±4.47ª	49.70±5.68 ^₅	53.25±6.26
FAC _{sx} (500) (%)	64.80±6.78	66.67±2.30	67.00±3.86	62.82±4.75	65.23±4.29
LVL _{diast} (cm)	16.6±1.4ª	17.3±1.0ª	17.4±0.9ª	17.9±1.4⁵	17.6±1.1*
LVL _{diast} (500) (cm)	16.0±1.4ª	17.6±1.2⁵	17.4±0.8⁵	17.9±1.3⁵	17.6±1.1*
LVL _{syst} (cm)	11.7±1.3ª	12.5±0.7 ^{a,b}	12.1±1.1 ^{a,b}	12.8±1.2⁵	12.5±1.1*
LVL _{syst} (500) (cm)	11.3±1.2ª	12.7±0.8⁵	12.1±1.3 ^{a,b}	12.8±1.0 ^b	12.5±1.1*
LVV _{al,diast} (500) (mL)	968±189ª	1226±206 ^{a,b}	1333 ± 201⁵	1689±387⁵	1445±351*
LVV _{al,syst} (500) (mL)	306±95 ^a	356±88ª	357±63ª	608±208 ^b	457±188*
SV _{al} (500) (mL)	672±164ª	870±193⁵	976±186⁵	1081±250 ^b	987±227*
LVV _{bullet,diast} (500) (mL)	1161±194ª	1320±186 ^{a,b}	1455±175 ^{b,c}	1883 ± 429⁰	1588±389*
LVV _{bullet,syst} (500) (mL)	291±81ª	318±57ª	333±68ª	512±197⁵	400±160*
SV _{bullet} (500) (mL)	870±152ª	1002±135 ^{a,b}	1122±129⁵	1372 ± 255⁰	1188±245*
EF _{al} (500) (%)	69.36±9.72	70.61±7.23	72.94±4.74	64.39±6.90	68.86±7.29
EF _{bullet} (500) (%)	75.06±5.35	75.97±1.62	77.21±3.01	73.39±3.88	75.33±3.46

Variable	Control group	Mild AR	Moderate AR	Severe AR	AR
M-Mode					
LVID _{diast} (cm)	11.2±0.7ª	11.2±0.6ª	11.9±0.7 ^{a,b}	13.4±1.4⁵	12.3±1.4*
LVID _{diast} (500) (cm)	10.8±0.7ª	11.4±0.8 ^{a,b}	11.8±0.9⁵	13.3±1.2⁰	12.3±1.3*
LVID _{syst} (cm)	6.4±0.8ª	6.5±0.8ª	6.9±0.7ª	8.1±1.2⁵	7.3±1.1*
LVID _{syst} (500) (cm)	6.2±0.8ª	6.7±0.9ª	6.8±0.7ª	8.1±1.1⁵	7.3±1.1*
LVFW _{diast} (cm)	2.45±0.33	2.45±0.20	2.45±0.45	2.50±0.32	2.47±0.33
LVFW _{diast} (500) (cm)	2.36±0.34	2.49±0.19	2.44±0.40	2.50±0.34	2.48±0.32
LVFW _{syst} (cm)	4.45±0.51	4.14±0.43	4.36±0.46	4.04±0.56	4.17±0.50*
LVFW _{syst} (500) (cm)	4.28±0.50	4.19±0.25	4.34±0.37	4.03±0.54	4.17±0.43
FS (%)	43.1±6.1	41.5±6.3	42.3±3.9	39.5±7.6	40.9±6.2

Table 2 continued

LVA, left ventricular area; FAC, fractional area change; LVL, left ventricular length; LVV, left ventricular volume; SV, stroke volume; EF, ejection fraction LVID, left ventricular internal diameter; LVFW, left ventricular free wall; FS, fractional shortening; lx, measured from the long axis view; sx, measured from the short axis view; diast, measured in diastole; syst, measured in systole; al, calculated by area-length method; bullet, calculated by bullet method

The results for the different echocardiographic measurements are listed in table 3. In horses with AR LA dimensions (LAD_{Ix,syst} (500), *P*<0.001; LAD_{sx,syst} (500), *P*<0.001; LAA_{Ix,diast} (500), *P*=0.001; LAA_{Ix,syst} (500), *P*<0.001) were significantly larger than in the control group.

The AO_{lx,syst} (500) (P=0.001), AO_{lx,Ediast} (500) (P<0.001) and AO_{sx,syst} (500) (P=0.001) were significantly larger in horses with AR, compared to the control group. Also AO_{DiastDecr} (500) (P<0.001) and AO_{DiastDecr_rate} (500) (P<0.001) were significantly larger in horses with AR (Table 3).

PEP was significantly shorter (P<0.001) and PEP/LVET significantly smaller (P<0.001) in horses with AR compared to the control group.

Aortic Vmax (P<0.001), Vmean (P=0.001) and VTI (P=0.010) were significantly larger in horses with AR. SV_{Doppler} (500) (P<0.001), SV_{al} (500) (P<0.001) and SV_{bullet} (500) (P<0.001) were also significantly larger in horses with AR.

Variable	Control group	Mild AR	Moderate AR	Severe AR	AR
2D Echocardiography	g. • • p				
LAA _{lx,diast} (cm ²)	53.2±7.3 ^{a,b}	49.3±7.3ª	54.7±8.4 ^{a,b}	59.5±11.2⁵	55.1±10.1
LAA _{lx,diast} (500) (cm ²)	49.3±6.8ª	50.5±4.3ª	54.1±5.9 ^{a,b}	58.7±7.2⁵	54.9±6.8*
LAA _{ix,syst} (cm ²)	77.4±7.9	75.7±11.4	85.5±13.2	85.1±11.4	82.5±12.4*
LAA _{lx,syst} (500) (cm ²)	71.7±7.2ª	77.6±6.8 ^{a,b}	84.4±7.1 ^b	84.3±7.3 ^b	82.4±7.5*
LAD _{Ix,syst} (cm)	11.0±0.6ª	11.3±0.7 ^{a,b}	11.9±0.8 ^b	11.8±0.9⁵	11.7±0.8*
LAD _{Ix,syst} (500) (cm)	10.6±0.6ª	11.5±0.6⁵	11.8±0.7⁵	11.8±0.7⁵	11.7±0.7*
Ao _{sx,syst} (cm)	7.3±0.6ª	7.5±0.6 ^{a,b}	7.2±0.5ª	7.9±0.8⁵	7.6±0.7
Ao _{sx,syst} (500) (cm)	7.1±0.5ª	7.6±0.4 ^{a,b}	7.1±0.6ª	7.9±0.7⁵	7.6±0.7*
LAD _{sx,syst} (cm)	8.6±0.5	8.7±0.7	8.7±0.8	9.1±0.7	8.9±0.7
LAD _{sx,syst} (500) (cm)	8.3±0.4ª	8.8±0.4 ^b	8.6±0.6 ^{a,b}	9.1±0.5⁵	8.9±0.5*
LAD _{sx,syst} (500)/Ao _{sx,syst} (500)	1.17±0.06	1.16±0.06	1.22±0.12	1.15±0.09	1.18±0.09
Ao _{lx,Ediast} (cm)	6.5±0.7ª	$6.7 \pm 0.6^{a,b}$	$6.7 \pm 0.5^{a,b}$	7.2±0.7⁵	6.9±0.6*
Ao _{lx,Ediast} (500) (cm)	6.3±0.6ª	6.8±0.6 ^{a,b}	$6.7 \pm 0.5^{a,b}$	7.2±0.6 ^b	6.9±0.6*
Ao _{lx,Ldiast} (cm)	6.2±0.6	6.2±0.6	6.1±0.6	6.4±0.7	6.23±0.62
Ao _{lx,Ldiast} (500) (cm)	6.0±0.6	6.3±0.4	6.0±0.5	6.3±0.6	6.22±0.53
Ao _{DiastDecr} (500) (cm)	0.3±0.1ª	$0.5 \pm 0.3^{a,b}$	0.6±0.3 ^b	0.8±0.4 ^b	0.6±0.3*
Ao _{DiastDecr_rate} (500) (cm/min)	9.7±4.7ª	20.4±8.8 ^{a,b}	25.7±9.3⁵	35.2±9.5⁵	27.9±10.9*
Ao _{ix,syst} (cm)	6.6±0.5	6.8±0.5	6.9±0.5	7.2±0.7	7.0±0.6
Ao _{lx,syst} (500) (cm)	6.6±0.5ª	6.9±0.5 ^{a,b}	6.9±0.4 ^{a,b}	7.2±0.6 ^b	7.0±0.5*
LVOT _{diam} (cm)	6.2±0.5	6.4±0.5	6.7±0.6	6.5±0.6	6.5±0.6*
LVOT _{diam} (500) (cm)	6.0±0.5ª	6.5±0.5 ^b	6.6±0.6 ^b	6.5±0.4 ^b	6.5±0.5*
M-Mode					
PEP (ms)	86±13ª	69±25 ^b	60±11⁵	53±15⁵	59±17*
LVET (ms)	434±50	436±36	438±43	461±44	447±42
PEP/LVET	0.20±0.03ª	0.16±0.06 ^₅	0.14±0.03 ^{b,c}	0.11±0.03℃	0.13±0.04*

Table 3: Results for all measured and calculated variables, except LV dimensions. Different superscripts indicate significant differences (P<0.05), * indicates significant difference from control group (P<0.05).

Variable	Control group	Mild AR	Moderate AR	Severe AR	AR
Pulsed Wave Doppler					
Vmax (m/s)	0.72±0.09ª	0.79±0.09ª	0.77±0.12ª	0.91±0.08 ^b	0.83±0.12*
Vmean (m/s)	0.51±0.07ª	0.55±0.08 ^{a,b}	0.56±0.10 ^{a,b}	0.65±0.08 ^b	0.59±0.10*
VTI (cm)	24.38±4.89ª	26.13±5.77 ^{a,b}	27.20±4.06 ^{a,b}	29.74±6.09 ^b	27.93±5.45*
SV _{Doppler} (mL)	749±177ª	878±217 ^{a,b}	988±236 ^b	1071±346⁵	992±282*
SV _{Doppler} (500) (mL)	674±181ª	913±131 ^{a,b}	992±310 ^b	1020±287⁵	982±261*

Table 3 continued

LAA, left atrial area; LAD, left atrial diameter; Ao, aortic diameter; LVOT_{diam}, left ventricular outflow tract diameter; PEP, pre-ejection period; LVET, left ventricular ejection time; Vmax, maximal velocity of the aortic blood flow; Vmean, mean velocity of the aortic blood flow; VTI, velocity time integral of the aortic blood flow; lx, measured from the long axis view; sx, measured from the short axis view; diast, measured in diastole; syst, measured in systole; Ediast, measured in early diastole; Ldiast, measured in late diastole; Ao_{DiastDecr}, aortic diastolic decrease; Ao_{DiastDecr_rate}, rate of aortic diastolic decrease; SV_{Doppler}, stroke volume calculated from Doppler measured variables

DISCUSSION

Horses with AR showed an enlarged LA, LV, Ao and SV, a higher Vmax, a larger diastolic run-off, a shorter PEP and a smaller PEP/LVET.

AR severity is usually graded based on LV size and subjective assessment of the regurgitant jet, which requires experience.³ In this study, horses with AR were categorized based on three criteria that mimic as much as possible the subjective evaluation as done by most clinicians. None of these criteria have been validated for the quantification of AR in horses as there is no gold standard, but by combining these independently evaluated criteria a good approximation of the severity of regurgitation can be expected. Objective measurements that quantify AR reliably would simplify the classification of horses with AR.

Cardiac measurements have been reported to depend on HR and loading conditions.¹¹ HR was not significantly different between groups. However, cardiac loading conditions change with AR. The regurgitant flow causes LV volume-overload and thus preload increases.¹¹ In humans, afterload is increased in patients with AR, due to the larger SV stretching the aorta, making it less compliant to pressure.²² The pressure-volume relation in horses with AR has not been studied, but a similar mechanism in horses can be assumed.

Several authors have reported normal reference values for LVID_{diast} for different breeds, but most were not scaled to adjust for BW.^{17,23,24} We applied allometric scaling because different breeds were included in our study. Our results show an increase in LV cavity dimensions in horses with AR due to volume-overload. LV wall dimensions did not alter with AR, indicating LV eccentric hypertrophy. Remarkably, horses with AR also have a significantly longer LV than normal horses, while no increase in length appeared with increasing AR severity. SV increases in horses with AR. The results for SV_{al} (500) and SV_{Doppler} (500) were comparable, while SV_{bullet} (500) was slightly larger. SV_{Doppler} has been shown to correlate well with thermodilution recordings, although it was systematically lower.²⁵ The Bullet method has been reported to provide the best approximation of the SV.²⁰

Surprisingly, FS, FAC and EF, indicators of systolic heart function that indicate onset of myocardial failure,^{11,22} were not significantly different between groups. Increased preload causes an increase of these indicators but an increased afterload counteracts this effect.^{8,11} Horses with severe AR showed tendency to a decreased FS, FAC and EF, but the difference was not significant.

Horses with AR showed aortic dilatation, as previously reported, probably as a result of the larger SV stretching the aortic root.^{7,8,26} Ao_{DiastDecr} (500) measures the amount of diastolic run-off in the aorta. In horses with AR this was significantly larger because blood flows both forward into the aorta and backward to the LV. Ao_{DiastDecr_rate} (500) was an even better indicator of AR severity, as it relates to heart rate and takes into account the duration of the aortic run-off during diastole.

LV volume overload and increased EDP hamper emptying of the LA at the end of diastole, leading to LA dilatation and sometimes even to premature mitral valve closure.⁸ The ratio LAD_{sx,syst}/Ao_{sx,syst} is commonly used for assessment of LA size^{15,16} but was not significantly different between groups because the aortic diameter also increased. Therefore, the use of LAD_{sx,syst}/Ao_{sx,syst} for evaluation of LA dilatation is probably less useful in horses with AR.

PEP and LVET are measures of systolic function, but they also depend on HR and loading conditions.¹¹ In horses with AR, PEP was significantly shorter, most likely due to the decreased aortic EDP and increased LV EDP.^{11,27} LVET was not significantly different between groups. A high preload causes an increase in LVET¹¹, but the effect is probably counteracted by an increased afterload.²² The ratio PEP/LVET was a good indicator of AR severity.

The main limitation of this study was the lack of a reference standard to classify AR severity in horses. The classification of horses in different groups was performed by two operators based on three unvalidated, but frequently used criteria. This could

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have resulted in misclassification bias, but it can be assumed that it was nonetheless a good approximation. Another limitation of this study was the difference in age between the control group and horses with AR.

CONCLUSION

In this study, the new parameters Ao_{DiastDecr} and Ao_{DiastDecr_rate} were easy to measure and proved to be reliable and objective indicators of AR severity. Also, PEP and PEP/LVET were reliable for quantification of AR severity. SV_{bullet} was most sensitive for detecting an increase in SV in horses with increasing AR severity. FS, FAC and EF were not significantly different between AR and healthy horses.

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Chapter 4: Detection of subclinical left ventricular dysfunction by tissue Doppler imaging in horses with aortic regurgitation.

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Detection of subclinical left ventricular dysfunction by tissue Doppler imaging in horses with aortic regurgitation.

Equine Veterinary Journal (2018) 50(5):587-593

Part of this work was presented at the 50th European Veterinary Conference (Voorjaarsdagen), Amsterdam, The Netherlands, April 2017

CHAPTER 4: DETECTION OF SUBCLINICAL LEFT VENTRICULAR DYSFUNCTION BY TISSUE DOPPLER IMAGING IN HORSES WITH AORTIC REGURGITATION.

Abstract

BACKGROUND: Aortic regurgitation (AR) can have an important clinical impact and even lead to left ventricular (LV) failure. Tissue Doppler imaging (TDI) is an echocardiographic technique that has been used in horses to detect LV dysfunction.

OBJECTIVES: The aim of this study was to examine if TDI can detect changes in radial myocardial wall motion in horses with AR compared to control horses.

STUDY DESIGN: Case-control study.

METHODS: Echocardiography was performed in 30 healthy Warmblood horses and 34 Warmblood horses with AR, subdivided in groups with mild, moderate or severe AR. TDI measurements were performed on six segments of the short axis images of the LV myocardial wall. Myocardial wall motion was evaluated by measuring velocity and deformation during isovolumetric contraction, systole, early and late diastole. Timing of different events was also measured.

RESULTS: In most segments, a significantly higher systolic myocardial velocity was found in horses with AR. Horses with AR also showed a higher late diastolic velocity, although the difference was not significant in all segments. Measurement of timing intervals by TDI showed less significant differences.

MAIN LIMITATIONS: There was a significant difference in age between the control group and horses with AR, which may confound the results. The assessment of AR severity was based on subjective criteria as there is no gold standard.

CONCLUSIONS: TDI showed significant differences in radial systolic and late diastolic myocardial velocity in horses with AR. This could indicate an altered LV function in these horses, but further research is needed to investigate the prognostic value of these measurements.

INTRODUCTION

Aortic regurgitation (AR) in horses can have an important clinical impact, depending on the severity of regurgitation. In most horses the condition remains stable for several years, but in some horses it progresses rapidly to severe regurgitation.¹ Severe regurgitation will have hemodynamic consequences, including left ventricular (LV) enlargement. Clinical signs, such as dyspnea, exercise intolerance, ventricular arrhythmias and sudden cardiac death may become evident.² Horses with AR should be checked regularly to monitor the evolution of AR severity.¹ However, quantification of AR severity is difficult and is usually based on measurement of LV internal diameter and subjective assessment of the regurgitant jet and LV enlargement.^{3,4}

Tissue Doppler Imaging (TDI) is an ultrasound technique that allows to quantify myocardial wall motion. Evaluation of myocardial velocity and deformation can be used to assess LV function.⁵⁻⁹ Compared to 2DE and M-mode images, TDI measurements are more sensitive for detection of subtle myocardial dysfunction.^{10,11} Tissue velocity imaging (TVI) depicts the velocity of the myocardial wall, but is also affected by total heart motion and tethering by neighboring segments. Strain imaging (SI) measures the deformation of the myocardial wall and is less affected by total heart motion and tethering.¹⁰ Both in human medicine¹² and in small animals⁷ the technique is used for early detection of LV dysfunction in different conditions. In horses, the feasibility of the technique was demonstrated.⁸ The aim of this study was to examine if an altered LV radial function could be demonstrated by color TDI in horses with AR, compared to healthy control horses.

MATERIALS AND METHODS

Study population

The study was approved by the ethical committee of the Faculty of Veterinary Medicine and Bioscience Engineering of Ghent University (approval number EC2012_57). Owner informed consent was obtained for all horses. Thirty healthy Warmblood horses (14 mares, 13 geldings, 3 stallions) aged 8±4 years with a bodyweight (BW) of 565±48 kg were included in the control group.

The study population consisted of 34 Warmblood horses with AR (15 mares, 16 geldings, 3 stallions) aged 16±6 years with a BW of 544±80 kg, presented at the the Faculty of Veterinary Medicine (Ghent University, Belgium) for cardiac examination.

Echocardiography

All horses were submitted to a physical examination, cardiac auscultation and transthoracic cardiac ultrasound, using a GE Vivid 7 Dimension ultrasound with 3S phased array transducer (GE Healthcare, Diegem, Belgium), with simultaneous recording of an electrocardiogram. Images were stored for off-line analysis using dedicated software (EchoPAC Software Version 11.2, GE Healthcare, Diegem, Belgium). None of the horses were sedated during examination. Only horses free of cardiovascular and respiratory disease, assessed by thoracic auscultation and absence of clinical signs, were included in the control group. In the group of horses with AR, patients with more than mild concomitant other valvular disease were excluded. None of the horses had clinical signs of heart failure.

Standard 2DE, M-mode and color flow Doppler (CFD) images were recorded. From a right parasternal view (R) short axis TDI images of the LV were recorded at the level of the chordae tendineae (ct) and at the level of the papillary muscles (pm) for evaluation of the interventricular septum (IVS) and the LV free wall (LVFW). From a left parasternal view (L) short axis TDI images of the LV were recorded at the level of ct for assessment of the left (LLV) and the right (RLV) region of the LV wall.⁸ All tissue Doppler images were recorded at a frequency of 1.7/3.4 MHz with imaging depth set at 26-30 cm. The grayscale image was reduced to 30° and TDI sector was set as narrow as possible. The velocity scale was set at +32 to -32 cm/s. This resulted in a frame rate of 183 frames per second.

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Off-line analysis

From the M-mode images, LV internal diameter at systole (LVIDs) and at diastole (LVIDd) were measured. Fractional shortening (FS) was calculated by the software. To calculate the LV volume, the LV area was measured in systole and diastole from the parasternal short axis view at chordal level. From the four chamber view, the length of the ventricle was measured as the distance between the middle of the straight line connecting the opposite sections of the mitral ring and the ventricular apex. Systolic (LVIVs) and diastolic (LVIVd) volumes were calculated using the Bullet method (LVIV = (5 * LV area * LV length) / 6).

In the TDI images, a sample area (12mm x 6mm) was placed in the middle of the wall segments of interest and anchored to track the myocardial motion at specific time intervals (onset QRS, at T wave, early diastole, at P wave and atrial contraction). Visual control assured adequate tracking of the middle of the myocardium.

Three consecutive cycles were averaged into the last cycle by the cine compound function of the software. Horizontal sweep was set to display and measure this averaged cycle. The resulting curves were filtered by a 30 ms temporal smoothing filter. From the velocity (Vel) curves, the maximal value at isovolumetric contraction (IVC), at systole (s), in early diastole (E) and at atrial contraction (A) were measured (Figure 1). Several timing intervals were also measured. Preejection period (PEP) was measured from onset QRS to the start of systolic excursion. Time (T) to maximal systole and to maximal E wave were measured with regard to onset QRS. Time to maximal A wave was measured from onset P wave. Duration of the A wave (durA) was measured from onset to end of the A wave (Figure 1).



Figure 1: Tissue velocity curve from the left ventricular free wall from a short axis image of the left ventricle at the level of the chordae tendineae from a right parasternal view. Timing intervals are shown in white, peak values are shown in yellow. Vel_{IVC}, maximal velocity during isovolumic contraction; Vel_s, maximal velocity during systole; Vel_E, maximal velocity during early diastole; Vel_A, maximal velocity during atrial contraction; T Vel_s, timing of Vel_s; T Vel_E, timing of Vel_a; PEP, pre-ejection period; ET, ejection time; durA, duration of A-wave

Strain was calculated over the length of the sample area and linear drift compensation was applied. From the strain curve, the maximal radial strain (RS) and time to RS were measured (Figure 2). LVFW was only evaluated by TVI, since previous research had demonstrated poor quality of strain curves in this segment.⁸



Figure 2: Strain curve of the interventricular septum from a short axis image of the left ventricle at the level of the chordae tendineae from a right parasternal view. Timing intervals are shown in white, peak values are shown in yellow. RS, maximal radial strain; T RS, timing of maximal radial strain

Assessment of severity

Horses with AR were divided into 3 groups (mild, moderate or severe AR), based on the subjective scoring of three different criteria as previously described.⁴ LV dilatation and the size of the regurgitant jet were scored subjectively by two operators (GvL & SV), who were unaware of any previous classifications of the patient. LV internal diameter at end diastole was measured and scaled to a bodyweight of 500kg using the formula of allometric scaling (LVID(500) = measured LVID/bodyweight^{1/3} x 500^{1/3}).^{4,13,14} Based on the scoring of these criteria a total score was calculated for each horse.

Horses with a total score of 8 points or more were classified as severe AR, horses with 5, 6 or 7 points were classified as moderate AR and horses with 4 points or less were classified as mild AR.

Statistical analysis

Statistical analysis was performed using commercially available software (SPSS Statistics, version 23, Chicago, IL). Variables with normal distribution are reported as mean±standard deviation (SD), non-normally distributed variables are reported as median (range). Normal distribution of all variables was checked by evaluation of the residuals by visual inspection, the Kolmogorov-Smirnov test and Shapiro-Wilk test. Homogeneity of variance across groups was checked by Levene's test for equality of variances. Variables that showed normal distribution and homogeneous variances were evaluated using one-way ANOVA with Bonferroni correction to compare means of the four groups (control, mild, moderate and severe AR). All other variables were compared by a non-parametric Kruskal-Wallis test with posthoc pairwise comparison using the Dunn test. Means from control horses and horses with AR were compared by an independent-samples *t*-test. Level of significance was 0.05.

Results

Table 1 shows age, BW, heart rate (HR) and height at withers for the different groups. Horses with AR (16±6 years) were significantly (*P*<0.001) older than the control group (8±4 years). There was no significant difference in weight and height at withers between horses with AR and the control group. Eleven horses showed mild AR, 15 horses had moderate AR and 8 horses were classified as having severe AR.

Table 1: Population descriptives.

Different superscripts indicate significant (P<0.05) differences between groups (^{a,b,c}) or between control and the whole AR group (*). Variables with normal distribution are reported as mean±standard deviation. Variables with non-normal distribution are reported as median (range).

	Control	Mild AR	Moderate AR	Severe AR	All AR
Number of	30	11	15	8	34
Age (years)	8±4ª	15±7 ^b (<i>P</i> =0.001)	16±7 ^b (<i>P</i> <0.001)	18±7 ^b (<i>P</i> <0.001)	16±6* (<i>P</i> <0.001)
Body weight (kg)	565±48	535±100	544±61	555±90	544±80
Height at withers (cm)	169±4	167±9	166±7	169 ± 8	167±8
HR (beats/min)	39±7	37±6	40±6	43±9	40±7
LVIDd(500) (cm)	11.1(9.9-13.5)ª	11.2(10.2-13.5) ^{a,b}	12.3(10.7-13.2) ^b (<i>P</i> =0.004)	13.3(12.4-15.9)° (<i>P</i> <0.001)	12.3(10.6-15.9)* (<i>P</i> <0.001)
LVIDs(500) (cm)	6.4(4.6-8.1) ^a	6.6(5.5-8.1) ^{a,b}	7.2(6.0-8.4) ^{b,c} (<i>P</i> =0.039)	8.3(6.1-10.8)° (<i>P</i> <0.008)	7.3(5.5-10.8)* (<i>P</i> <0.001)
LVIVd(500) (ml)	1141(640-1449)ª	1271(1025-1587) ^{a,b}	1438(1018-1774) ^b (<i>P</i> =0.005)	1818(1471-2914)⁰ (<i>P</i> <0.001)	1439(1018-2914)* (<i>P</i> <0.001)
LVIVs(500) (ml)	283(99-433)ª	298(245-418)ª	356(223-467)ª	481(357-1098) ^b (<i>P</i> <0.001)	349(223-1098)* (<i>P</i> =0.003)
FS (%)	43±6	41±5	42±4	39±8	41±6
EF (%)	75±5	76±3	76±3	72±5	75±3

HR, heartrate; LVIDd(500), left ventricular internal diastolic diameter (scaled for bodyweight); LVIDs(500), left ventricular internal systolic diameter (scaled for bodyweight); LVIVd(500), left ventricular diastolic volume (scaled for bodyweight); LVIVs(500), left ventricular systolic volume (scaled for bodyweight); FS, fractional shortening; EF, ejection fraction In Table 2 the results from the right parasternal images are listed. Peak Vel_s of the IVS segments was higher in horses with moderate or severe AR compared to the control group, or when comparing all horses with AR to the control group.

A significantly higher RS was found in IVS_{ct} in horses with mild AR compared to control horses or when comparing all horses with AR to the control group.

Peak Vel_E was lower in the LVFW segments in horses with AR. When the results of all horses with AR were combined, a significantly higher Vel_A was found in several different segments. The PEP was significantly shorter in horses with moderate and severe AR compared to the control group, but only in the IVS segment at papillary muscle level.

Table 2: Results from tissue Doppler imaging (TDI) of the right parasternal images (R) for the left ventricular free wall (LVFW) and the interventricular septum (IVS) at chordal level (ct) and at papillary muscle level (pm). Different superscripts indicate significant (P<0.05) differences between groups (^{a,b}) or between control and the whole AR group (*). Variables with normal distribution are reported as mean±standard deviation. Variables with non normal distribution are reported as median (range).

	Control	Mild AR	Moderate AR	Severe AR	All AR
TVI measurements of					
R-TVI-LV _{ct} -Vel _{IVC} (cm/s) -Vel _s (cm/s)	5.34±2.26 9.19±1.58	5.22±3.00 9.11±2.53	6.23±3.65 9.60±1.63	4.97±3.38 8.74±0.68	5.58±3.31 9.23±1.82
-T Vel _s (ms)	222 (157-576)	231 (183-268)	250 (181-280)	247 (216-271)	244 (181-506)* (<i>P</i> =0.014)
-Vel _E (cm/s)	24.97±3.74ª	19.16±5.55 ^b (<i>P</i> =0.006)	20.22±5.72 ^b (<i>P</i> =0.017)	18.75±5.08 ^b (<i>P</i> =0.010)	19.51±5.38* (<i>P</i> <0.001)
-T Vel _E (ms) -Vel _A (cm/s)	663±38 8.21±3.28	687±32 9.05±3.65	645±33 10.06±3.09	663±48 9.50±3.33	663±40 9.59±3.26
-T Vel _A (ms) -dur _A (ms)	184±29 138±35	190±41 156±21	189±28 141±22	184±30 142±22	188±32 146±22
-PEP (ms) -ET (ms)	98±18 524±35	107±24 531±27	93±16 499±31	106±18 535±45	101±20 518±37
R-TVI-LV _{pm} -Vel _{IVC} (cm/s) -Vel _s (cm/s)	4.06 (1.76-17.54) 7.17±1.17 ^{a,b}	4.70 (1.62-9.61) 6.54±1.26ª	5.02 (3.47-11.57) 8.00±1.00 ^b (<i>P</i> =0.009)	3.73 (0.45-11.02) 7.53±0.76 ^{a,b}	5.01 (0.45-11.57) 7.42±1.20
-T Vel _s (ms) -Vel _E (cm/s)	229(160-376) 16.85±3.75ª	238(160-286) 11.85±2.56 ^b (<i>P</i> =0.001)	248(169-271) 14.73±3.95 ^{a,b}	252(240-281) 13.80±2.59 ^{a,b}	245(160-471) 13.58±3.41* (<i>P</i> =0.001)
$\text{-}T \; \text{Vel}_{\text{E}} \left(\text{ms} \right)$	701±40 ^{a,b}	735±35ª	677±47 ^b (<i>P</i> =0.009)	702±59 ^{a,b}	702±52
-Vel _A (cm/s)	7.31±3.33ª	8.28±3.30 ^{a,b}	10.84±2.74 ^b (<i>P</i> =0.007)	9.22±3.14 ^{a,b}	9.59±3.15* (<i>P</i> =0.007)
-T Vel _A (ms) -durA (ms)	198(127-343) 139±33	179(151-260) 142±22	187(161-242) 128±16	201(150-220) 133±17	194(150-260) 133±19
-PEP (ms) -ET (ms)	105±19 520±38	117±25 526±24	108±23 507±33	119±30 539±46	113±25 521±36

TVI measurements of the	Control	Mild AR	Moderate AR	Severe AR	All AR
IVS					
R-TVI-IVS _{ct} -Vel _{IVC} (cm/s)	3.70(0.76-8.72) ^a	4.52(2.56-7.04) ^{a,b}	5.88(3.63-14.40) ^b (P=0.009)	5.03(3.09-9.78) ^b (P=0.033)	5.03(2.56-14.4)* (<i>P</i> =0.006)
-Vel _s (cm/s)	4.22±2.05ª	5.66±2.35 ^{a,b}	8.11±2.91 ^b (<i>P</i> <0.001)	8.53±3.14 ^b (<i>P</i> <0.001)	7.40±2.78* (<i>P</i> <0.001)
-T Vel₅ (ms) -Vel _E (cm/s)	122±20 12.44±3.35	127±35 12.40±2.63	118±25 12.24±3.15	119±15 12.38±3.29	121±27 12.33±2.93
-T Vel _E (ms) -Vel _A (cm/s)	713±42 1.81±1.29ª	728±30 4.80±1.95 ^b (<i>P</i> =0.001)	687±49 4.03±2.72 ^b (<i>P</i> =0.009)	729±44 4.21±2.78 ^b (<i>P</i> =0.027)	711±46 4.32±2.46* (<i>P</i> <0.001)
-T Vel _A (ms)	220±49	181±46	198±48	207±42	195±46* (<i>P</i> =0.044)
-dur _A (ms)	214±51ª	179±31 ^{a,b}	169±47 ^b (<i>P</i> =0.020)	176±31 ^{a,b}	174±38* (<i>P</i> =0.001)
-PEP (ms)	89(60-182)	78(50-130)	69(42-95)	62(52-98)	71(43-123)
R-TVI-IVS _{pm} -Vel _{IVC} (cm/s)	4.39 (1.12-11.50)	4.15 (2.87-6.41)	5.37 (0.45-15.35)	6.07 (4.18-11.50)	431±32 5.65 (0.45-15.35)* (<i>P</i> =0.049)
-Vel _s (cm/s)	4.75 (1.20-9.76) ^a	6.59 (3.79-8.63) ^b (<i>P</i> =0.020)	9.24 (5.28-13.58) ^b (<i>P</i> <0.001)	9.05 (5.75-11.87) ^b (<i>P</i> <0.001)	7.45 (3.79-13.58)* (<i>P</i> <0.001)
-T Vel₅ (ms) -Vel _E (cm/s)	129 (75-434) 14.03±3.38	117 (86-157) 14.04±2.81	129 (101-154) 14.76±3.54	121 (105-140) 11.87±2.15	120 (86-157) 13.85±3.17
-T Vel _E (ms) -Vel _A (cm/s)	732±42 3.64±2.10	728±61 4.76±1.98	709±42 4.48±3.25	754±40 3.92±1.87	726±50 4.43±2.57
-T Vel _A (ms) -durA (ms)	215±31 189±40°	195±50 185±54 ^{a,b}	203±41 146±31 ^b (<i>P</i> =0.010)	231±48 182±44 ^{a,b}	207±46 167±45* (<i>P</i> =0.043)
-PEP (ms)	86±20ª	74±27 ^{a,b}	66±25 ^b (<i>P</i> =0.032)	59±23 ^b (<i>P</i> =0.023)	67±25* (<i>P</i> =0.001)
-ET (ms)	431±31	449±40	419±31	428±24	431±34
SI measurements of the IVS					
R-SI-IVSct-RS (%)	40.17±11.23ª	52.11±13.41 ^b (P=0.045)	50.61±11.12 ^{a,b}	47.39±15.09 ^{a,b}	50.32±12.68* (P=0.002)
-T RS (ms)	539±64	589±69	567±82	546±68	569±74
R-SI-IVSpm-RS (%)	46.76±10.34	46.55±5.04	51.00±10.80	52.51±11.19	49.88±9.42
-T RS (ms)	486±41	535±71	498±67	501±87	511±73

Table 2 continued

AR, aortic regurgitation; TVI, tissue velocity imaging; SI, strain imaging; Vel, velocity; s, systole; T, time; E, early diastolic filling; A, atrial contraction; durA, duration of A; PEP, preejection period; ET, ejection time; IVC, isovolumic contraction; RS, maximal radial strain

Table 3 shows the results from the left parasternal images. For the LLV, similar results as for the right parasternal images were found, including higher Vel_s, RS and Vel_A. RLV showed few significant differences.

Table 3: Results from tissue Doppler imaging (TDI) of the left parasternal images (L) for the left (LLV) and right (RLV) part of the left ventricular wall at level of the chordae tendineae (ct). Different superscripts indicate significant (P<0.05) differences between groups (^{a,b}) or between control and the whole AR group (*). Variables with normal distribution are reported as mean±standard deviation. Variables with non normal distribution are reported as median (range).

	Control	Mild AR	Moderate AR	Severe AR	All AR
TVI measurements of the LLV					
L-TVI-LLV _{et} -Vel _{ive} (cm/s)	3.35 (0.22 - 8.50)	4.15 (1.12 - 10.86)	3.73 (2.15 - 8.61)	2.89 (0.81 - 12.91)	3.49 (0.81 - 12.91)
-Vel, (cm/s)	3.55±2.10°	4.37±1.79°	6.75±2.05°	5.55±1.54°.°	5.71±2.09*
	(P< 0.001)	(P=0.033)			(P<0.001)
-TVel, (ms)	261±110	275±97	249±76	268±130	262±94
-Vel _e (cm/s)	9.70±4.02°	9.18±2.39 ^{8,8}	13.17±4.49 ^b (<i>P</i> =0.048)	9.77±3.08 ^{8,0}	11.11±4.00
-T Vel _e (ms)	673±31	693±48	681±45	673±44	683±45
-Vel _A (cm/s)	2.92±2.25"	3.85±2.03 ^{a,b}	5.32±1.83 ^b (P=0.017)	4.81±3.32 ^{8,b}	4.73±2.30* (P=0.005)
-T Vel _A (ms)	185±31	179±46	187±26	203±28	188±34
-dur _A (ms)	141(21-197)°	152(118-187) ^{a,b}	166(146-199) ^b (₽=0.004)	147(127-206) ^{a,b}	154(118-206)* (P=0.003)
-PEP (ms)	95 (47-177) ^{s,b}	117 (81-143) ^e	91 (43-111) ^{e,b}	80 (64-103) ^b (P=0.025)	92 (43-143)
-ET (ms)	513±32	517±27	498±35	517±47	508±36
SI measurements of the LLV					
L-SI-LLV _{et} -RS (%)	82.49±32.89°	101.81±31.83 ^{8,0}	115.01±24.33°	111.21±32.41°.0	109.52±28.77*
			(P=0.018)		(P=0.001)
-T RS (ms)	523±53	535±50	519±55	556±67	534±57
TVI measurements of the RLV					
L-TVI-RLV _{et} -Vel _{ive} (cm/s)	7.14±2.97	7.05±2.28	6.78±3.18	7.28±1.60	6.98±2.53
-Vel, (cm/s)	6.69±1.34	7.17±2.23	6.54±1.08	7.94±2.40	7.08±1.90
-T Vel, (ms)	197 (96-265)	180 (124-241)	178 (103-262)	215 (134-228)	191 (103-262)
-Vel _e (cm/s)	18.66±5.05	16.26±4.13	16.90±4.27	16.93±7.65	16.69±5.07
-T Vel _e (ms)	675±42	685±33	675±46	665±57	676±45
-Vel _A (cm/s)	2.51±1.43	2.86±1.29	2.84±1.75	3.11±0.92	2.91±1.40
-T Vel _a (ms)	127 (81-377)	102 (79-378)	176 (112-323)	339 (114-405)	158 (79-405)
-dur _A (ms)	100±49	103±32	120±43	133±40	118±39
-PEP (ms)	103±39	96±53	90±32	103±41	96±41
-ET (ms)	504 (462-567)	515 (424-545)	475 (421-614)	514 (418-548)	504 (418-614)
SI measurements of the RLV					
L-SI-RLV _{et} -RS (%)	48.80 (17.12-132.53) ^{8,6}	80.59 (46.63-89.42) ^a	71.56 (35.80-81.79) ^{4,6}	56.63 (41.87-88.38) ^b (P=0.024)	72.47 (35.80-89.42)* (P=0.020)
-T RS (ms)	555±82	589±67	564±77	543±81	566±75

AR, aortic regurgitation; TVI, tissue velocity imaging; SI, strain imaging; Vel, velocity; s, systole; T, time; E, early diastolic filling; A, atrial contraction; durA, duration of A; PEP, pre-ejection period; ET, ejection time; IVC, isovolumic contraction; RS, maximal radial strain
DISCUSSION

This study aimed at measuring changes in LV radial function by TDI in horses with chronic AR. In the IVS significant differences in wall velocity and strain could be detected in maximal systole and late diastole, but not in early diastole. In the LVFW only velocity was measured and significant differences in systole, early and late diastole were found. LLV also showed several variables to be significantly different.

Aortic regurgitation resulted in a higher systolic maximal velocity in the LVFW at pm level and in the IVS at pm and ct level. Chronic AR causes LV diastolic enlargement and in the compensated stage of the disease, end systolic dimensions increase proportionally.⁴ The myocardial walls thus make a larger displacement during ejection, resulting in a higher stroke volume and velocity of the walls in horses with moderate or severe AR. Fractional shortening (FS) is the ratio of the difference between diastolic and systolic left ventricular diameter over the diastolic left ventricular diameter. In horses with AR both the numerator and the denominator are increased, so FS is unaltered. FS is a poor indicator of left ventricular function in horses with chronic AR. Only in the decompensated stage, the end-systolic dimensions will increase more relative to the end-diastolic dimensions and FS will decrease.⁴

In LVFW, E wave velocity was significantly lower in horses with AR. This may be in part due to the significantly higher age of the horses with AR, resulting in a stiffer ventricle and slower relaxation as described in humans and dogs.^{7,15,16} In several segments A wave was significantly larger in horses with AR. This is probably also an age effect to compensate for the reduced early ventricular filling, similar to what has been reported in humans and dogs.^{7,15,16} Figure 3 clearly demonstrates this. However, the effect was not straightforward in all segments and in some groups of horses with AR, E and A were not significantly different from the control group (despite a significant difference in age). This could be due to the low numbers of horses per group or to the effect of AR on early and late diastolic filling. The data in this study do not allow to differentiate between the effects of age and AR. In

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human medicine, meta-regression analysis resulted in a calculated measure (Z-score) for E velocity, standardized for age and region. This provides an easy to interpret measure of diastolic function, independent of age.¹⁷ Such data are unfortunately not available in equine cardiology.

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Figure 3: Tissue velocity images of the left ventricular free wall from the right parasternal short axis image of the left ventricle from a normal horse (upper panel) and from a horse with severe aortic regurgitation (lower panel). S, systole; E, early diastole; A, atrial contraction

Left ventricular enlargement due to AR, may lead to LV dysfunction. In human medicine it has been shown that longitudinal function decreases early in the development of AR, and radial compensation to preserve ejection fraction has been suggested. With progression towards more severe disease, radial function also decreases.¹⁸ In horses longitudinal function has been assessed by speckle tracking¹⁹, but cannot be determined by TDI as apical views cannot be obtained. In the current study, radial strain increases in the mild and moderate group. In horses with severe AR, RS was lower than in horses with mild or moderate AR, although the difference was not always significant. This may indicate the onset of myocardial failure in the group of horses with severe AR. However, a strain gradient from epito endocardial wall has been demonstrated in horses, dogs and humans.^{7,18,20} A small deviation in the placement of the sample area thus affects the results and might also contribute to the outcome for radial strain. A smaller sample area, tracked closely to the endocardial or epicardial wall might result in a more conclusive outcome.²⁰

Koenig et al. investigated the use of pulsed wave TDI to measure LV function in healthy horses and horses with different cardiac diseases.²¹ Their results from horses with AR were inconclusive. However, pulsed wave TDI measures peak velocities at a fixed point whereas color TDI measures mean velocities from a sample area that tracks the movement of the myocardial wall.²² These are different techniques that might produce different results and should not be used interchangeably.^{9,23}

In human medicine it has been shown that deformation indices provide a good estimation of LV function and reflect an early sign of myocardial damage. This helps identifying the best moment for aortic valve replacement before the patient develops irreversible heart failure.²⁴ In horses determination of subtle changes in LV function might contribute additional information for risk assessment for riding a horse with AR.¹ More research is needed to identify the prognostic value of the results of this study.

This study was intended to screen for variables that might be of interest for detecting myocardial dysfunction in horses with AR. Therefore a large number of variables was included. This might lead to false positives due to multiple comparisons, so results should be interpreted with caution. The difference in age between horses with AR and control horses reflects the nature of the disease but is also a limitation of the study. Several indices of myocardial function are age dependent^{7,15,16}, making it difficult to separate the effects of AR from the age-related alterations. Further research on the effects of age on cardiac function in horses is needed.

Another limitation is the division of horses in groups of different AR severity, which was partially based upon subjective assessment. However, there is no gold standard available for quantification of AR. The criteria used to classify horses (evaluating the size of the regurgitant jet, visual inspection of LV enlargement and measurement of the LV diameter) are commonly used in clinical practice by most operators⁴, but might not accurately reflect true AR severity.

The last limitation is related to the TDI technique. Measuring tissue velocity is subject to tethering and is influenced by total heart motion.⁸ In apical images wall velocity is measured relative to a more or less steady point, the apex of the heart. In short-axis images, there is no steady reference point and thus the measurements will be influenced by total heart motion. Strain and strain rate imaging are less influenced by tethering and total heart motion, but strain rate has been shown to have a high variability in horses.⁸

CONCLUSION

TDI of LV midwall myocardium was able to detect alterations in radial function in horses with AR, especially in moderate AR. Not all segments showed the same significant differences, so further research is needed to determine the prognostic value of these findings and to examine the influence of aging on myocardial function in horses.

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Chapter 5: Assessment of left ventricular function in horses with aortic regurgitation by 2D speckle tracking.

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Assessment of left ventricular function in horses with aortic regurgitation by 2D speckle tracking.

BMC – Veterinary Research (2018). Manuscript submitted.

Part of this work was accepted for poster presentation at the International Conference on Equine Exercise Physiology, Lorne, Australia, November 12-16, 2018

CHAPTER 5: ASSESSMENT OF LEFT VENTRICULAR FUNCTION IN HORSES WITH AORTIC REGURGITATION BY 2D SPECKLE TRACKING.

Abstract

BACKGROUND: Aortic regurgitation (AR) may lead to left ventricular (LV) dilatation, cardiac arrhythmias and heart failure. Echocardiography with 2D speckle tracking (2DST) has been used in horses to evaluate LV function and might be useful to detect early myocardial dysfunction in horses with chronic AR.

OBJECTIVES: The aim of this study was to examine if 2DST is able to detect changes in myocardial wall motion in horses with AR compared to control horses.

ANIMALS: The control group included 29 healthy Warmblood horses. The study population included 57 Warmblood horses with AR, subdivided in groups with mild (23 horses), moderate (21 horses) or severe AR (13 horses).

METHODS: Case-control study. Speckle tracking was performed on six segments of the short axis images and four segments of the long axis images of the LV myocardial wall. Wall motion was evaluated by measuring strain, strain rate and displacement for all segments separately and the average was calculated over all segments.

RESULTS: Radial and circumferential strain were significantly higher in horses with AR. Longitudinal strain did not show significant differences, but longitudinal displacement was larger in horses with AR, especially in the interventricular septum. Diastolic longitudinal strain rate was lower in early diastole and higher in late diastole.

CONCLUSIONS: 2DST is able to detect an altered myocardial motion in horses with AR. Further research is needed to demonstrate whether 2DST can be used to make a more accurate diagnosis and prognosis in clinical cases.

INTRODUCTION

Aortic regurgitation (AR) is quite common in horses, especially in older animals.¹ In most horses, the regurgitation is only mild and remains stable for several years, but some horses progress to more severe regurgitation and may develop left ventricular (LV) dilatation, cardiac arrhythmias and heart failure.² Follow up of these horses is paramount to detect early signs of decompensation.³ Evaluation of myocardial function can be used to monitor the progression of the disease. Traditionally, fractional shortening (FS) or ejection fraction (EF) are used to estimate LV function, but in the compensated stage of the disease, FS and EF are unaltered.^{4,5} Tissue Doppler imaging (TDI) is an ultrasound technique that has been validated in horses⁶ and is able to detect alterations in radial myocardial function in horses with different grades of AR.⁷ However, since apical images cannot be obtained in horses, longitudinal myocardial function cannot be evaluated by TDI. In human medicine, longitudinal function has been shown to be the best marker of subtle myocardial dysfunction.^{8,9}

Two-dimensional speckle tracking (2DST) is an ultrasound technique that tracks speckles in the myocardial wall and allows evaluation of radial, circumferential and longitudinal function in horses. The technique has been shown to be feasible in horses, although diastolic measurements have a higher variability that systolic measurements.¹⁰⁻¹² 2DST has also been used to monitor myocardial function under various circumstances.¹³⁻¹⁶

The aim of this study was to investigate whether 2DST is able to detect changes in radial, circumferential or longitudinal LV function in horses with different stages of AR severity, compared to healthy control horses.

MATERIALS AND METHODS

Study population

This is a case-control study. Twenty-nine healthy Warmblood horses (13 mares, 13 geldings, 3 stallions) aged 8±4 years with a bodyweight (BW) of 566±48 kg represented the control group. The study population consisted of 57 Warmblood horses with AR (22 mares, 26 geldings, 9 stallions) aged 15±6 years with a BW of 548±68 kg, presented at the Faculty of Veterinary Medicine (Merelbeke, Belgium) for cardiac examination. Thorough physical examination and thoracic auscultation was performed in all horses to exclude other cardiovascular and respiratory diseases. The study was approved by the ethical committee of the Faculty of Veterinary Medicine and Bioscience Engineering (approval number EC2012_57). Owner informed consent was obtained for all horses.

Echocardiography

Echocardiography was performed in all horses without sedation, using a GE Vivid 7 Dimension ultrasound with 3S phased array transducer (GE Healthcare, Diegem, Belgium) at a frequency of 1.6/3.2 MHz with simultaneous recording of an electrocardiogram. Images were stored for off-line analysis. Standard 2DE, M-mode and colour flow Doppler images were recorded. Horses with AR were not included if they had more than mild regurgitation of another cardiac valve. For 2DST examination from a right parasternal view a slightly modified four chamber image was recorded so that the mitral annulus was visible throughout the cardiac cycle. As a result, in some horses, the apex did not remain in the image. Left ventricular short axis images at chordal level were also recorded from a right parasternal view. In all images sector width was reduced to 55° to achieve a frame rate of at least 40 frames per second.

Off-line analysis

Off-line analysis was performed using dedicated software (EchoPAC Software Version 11.2, GE Healthcare, Diegem, Belgium). For each image, 3 cardiac cycles were analysed. To evaluate the longitudinal function of the LV, the four chamber images were analysed and the region of interest (ROI) was set by tracing the LV endocardial border from the septal insertion of the mitral valve until the insertion point on the lateral myocardial wall. The width of the ROI was set to cover the myocardial wall but not the epicardium (Figure 1) and was automatically divided by the software into 6 segments: basal (basSept), mid (midSept) and apical (apSept) part of the interventricular septum, and basal (basLat), mid (midLat) and apical (apLat) part of the LV free wall (LVFW). Both apical segments were not analysed.



Figure 1: Right parasternal long axis four chamber image of the left ventricle. The image is optimized to keep the mitral annulus visible throughout the cardiac cycle. The region of interest is automatically divided into six segments (basSept, midSept, apSept for the interventricular septum; basLat, midLat, apLat for the left ventricular free wall).

To evaluate LV radial and circumferential function, short axis images were analysed. The ROI was set by tracing the endocardial border and adapting the width of the ROI. The software divided the ROI automatically in 6 segments: AntSept and Sept represent the interventricular septum, Inf and Post represent the cranial LVFW and Lat and Ant define the caudal LVFW (Figure 2).



Figure 2: Right parasternal short axis image of the left ventricle, taken at end-systole. The region of interest is automatically divided into six segments (Sept and AntSept for the interventricular septum; Ant and Lat for the caudal left ventricular free wall; Inf and Post for the cranial left ventricular free wall).

Tracking quality of all images was calculated by the software and verified visually by the operator. When necessary, ROI was adapted manually until tracking quality was good.^{10,11} For the longitudinal motion, strain (SL), strain rate (SrL) and displacement (DL) curves were displayed. From the short axis images, curves for radial strain (SR)

and strain rate (SrR) and for circumferential strain (SC) and strain rate (SrC) were derived. From the strain and displacement curves maximal segmental strain or displacement was measured and average strain over all segments was calculated. From the strain rate curves, segmental systolic (S), early diastolic (E) and late diastolic (A) peak values were measured and average strain rate over all segments was calculated.^{10,11}

Assessment of severity

All horses with AR were scored as having mild, moderate or severe AR. This scoring was done based on three criteria that have been described previously.⁴ Dilatation of the LV and size of the regurgitant jet were scored subjectively by two operators (GvL & SV), blinded to previous classifications of the patient. The end diastolic LV internal diameter (LVIDd) was scaled to a BW of 500kg, using the formula of allometric scaling: LVIDd(500) = LVIDd/BW^{1/3} x 500^{1/3}.^{4,17} The scores for these three criteria were added and the total score determined the grade of AR severity for each horse.^{4,7} Horses with a total score of 8 points or more were classified as severe AR (13 horses), horses with 5, 6 or 7 points were classified as moderate AR (21 horses) and horses with 4 points or less were classified as mild AR (23 horses).

Statistical analysis

Statistical analysis was done using commercially available software (SPSS Statistics version 25, Chicago, IL, USA). Normal distribution of all variables was verified by evaluation of the residuals by visual inspection, the Kolmogorov-Smirnov test and Shapiro-Wilk test. Levene's test for equality of variances was performed to check homogeneity of variance across groups. Variables with normal distribution and homogeneous variances were evaluated by one-way ANOVA with Bonferroni correction to compare means of the four groups (control, mild, moderate and severe AR). Means from control horses and all horses with AR were compared by an independent-samples *t*-test. A non-parametric Kruskal-Wallis test with post-hoc

pairwise comparison using the Dunn test was applied to compare all non-normal distributed variables. Level of significance was 0.05. Variables with normal distribution are reported as mean ± standard deviation (SD), non-normally distributed variables are reported as median (range).

Results

Descriptive values of all groups are listed in Table 1. There was a significant difference in age between control horses and horses with AR. Bodyweight, height at withers, heart rate, FS and EF were not significantly different between groups.

Table 1: Population descriptives.

Different superscripts indicate significant (P<0.05) differences between groups (^{a,b}) or between control and the whole AR group (*). Variables with normal distribution are reported as mean ± standard deviation. Variables with non-normal distribution are reported as median (range).

	Control	Mild	Moderate	Severe	All AR
Number of horses	29	23	21	13	57
Age (years)	8±4ª	14±6 ^b	15±6 ^b	16±6 ^b	15±6*
		<i>P</i> =0.001	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001
Body weight (kg)	566±48	548±61	542±72	559±78	548±68
Height at withers (cm)	168±4	166±6	167±7	169±8	167±7
Heart rate (beats/min)	40±7	40±6	39±6	42±10	40±7
LVIDd(500) (cm)	11.1 (9.9-13.5) ^a	11.2 (9.5-13.2) ^a	12.4 (10.7-13.5) ^b	13.7 (12.4-18.9) ^b	12.2 (9.5-18.9)*
			P<0.022	<i>P</i> <0.001	<i>P</i> <0.001
LVIDs(500) (cm)	6.4 (4.6-8.1) ^a	6.4 (4.4-8.1) ^a	7.3 (6.0-8.5) ^b	8.5 (6.1-10.8) ^b	7.1 (4.4-10.8)*
			<i>P</i> <0.027	<i>P</i> <0.001	<i>P</i> =0.002
FS (%)	43±6	44±7	41±5	39±11	41±7
EF (%)	72 (49-83)	71 (50-86)	69 (61-76)	70 (39-80)	70 (39-86)

LVIDd(500), left ventricular internal diastolic diameter scaled for bodyweight; LVIDs(500), left ventricular internal systolic diameter scaled for bodyweight; FS, fractional shortening; EF, ejection fraction; AR, aortic regurgitation. Table 2 shows the results of systolic peak values and average values of the longitudinal, circumferential and radial measurements. SL did not show significant differences, but horses with severe AR had a larger standard deviation, indicating that some horses showed values below reference range. In the IVS, longitudinal displacement was higher in horses with moderate-to-severe AR. A significantly higher SR was found in horses with moderate AR or when comparing all horses with AR to the control group. SrRs of the LVFW was higher in horses with moderate AR and when comparing all horses with AR to the control group.

Table 2: Segmental and averaged systolic peak values of 2DST measurements. Different superscripts indicate significant (P<0.05) differences between groups (a,b,c) or between control and the whole AR group (*). Variables with normal distribution are reported as mean ± standard deviation. Variables with non-normal distribution are reported as median (range).

		Control	Mild	Moderate	Severe	All AR
SLG	basSept	20.7±3.7	20.5±3.5	22.9±4.3	22.6±4.8	21.9±3.8
(%)	midSept	21.7±2.6	21.2±3.4	23.3±3.0	22.9±4.7	22.4±3.7
	midLat	19.7±4.2	19.7±4.5	20.9±5.1	17.2±5.7	19.6±4.7
	basLat	19.3±4.1	20.4±5.1	19.6±6.0	17.7±8.7	19.6±6.3
	average	20.3±2.5	20.5±2.7	21.7±2.8	19.9±5.2	20.8±3.5
SrLs	basSept	0.82±0.14ª	0.85±0.17 ^{a,b}	0.97±0.24 ^b ₽=0.036	0.92±0.16 ^{a,b}	0.91±0.20*
(s-1)	midSept	0.86±0.14	0.87±0.14	0.94±0.14	0.88±0.11	0.90±0.14
	midLat	0.91±0.15	0.86±0.13	0.97±0.17	0.89±0.13	0.91±0.15
	basLat	1.05±0.19	1.08±0.16	1.07±0.18	1.18±0.37	1.10±0.23
	average	0.91±0.12	0.91±0.11	0.99±0.12	0.96±0.12	0.95±0.12
DLs	basSept	30.7±4.9ª P<0.001	33.4±6.0 ^{a,b} P=0.001	36.9±5.1 ^{b,c} P=0.002	41.7±8.2°	36.6±6.9* P<0.001
(mm)	midSept	17.6±4.6ª	20.0±3.7 ^{a,b}	22.9±4.2 ^{b,c}	26.6±4.7°	22.6±4.8*
	midLat	20.6±4.0 ^{a,b}	19.5±7.2ª	22.4±5.0 ^{a,b}	24.7±5.8 ^b	21.8±6.4
	basLat	33.9±6.2	34.7±9.3	35.7±7.7	37.6±9.9	35.7±8.8
	average	25.7±4.0ª <i>P</i> <0.001	26.9±5.3 ^{a,b} <i>P</i> =0.008	29.5±4.1 ^{b, c} <i>P</i> =0.039	32.4±6.1°	29.1±5.4* <i>P</i> =0.004
SC	Ant	18.3±4.6 ^{a,b}	15.6±5.0ª	20.4±5.4 ^b P=0.009	18.6±3.3 ^{a,b}	18.0±5.3
(%)	AntSept	24.9±6.1	22.7±6.1	22.0±5.4	19.6±6.8	21.7±6.0* <i>P</i> =0.023
	Inf	9.8 (1.3-22.1) ^a	13.3 (3.8-25.2) ^{a,b}	14.4 (3.4-34.2) ^b P=0.048	14.7 (7.2-25.5) ^{a,b}	14.0 (3.4-34.2)* P=0.002
	Lat	16.7±5.7	17.0±4.3	20.3±6.2	17.7±4.6	18.4±5.3
	Post	12.1±5.2ª <i>P</i> <0.001	18.4±5.8 ^{b,c} <i>P</i> =0.005	21.2±7.5 ^b	14.3±8.3 ^{a,c} <i>P</i> =0.024	18.6±7.4* <i>P</i> <0.001
	Sept	18.1±5.3	17.4±5.5	17.9±4.7	14.7±6.9	17.0±5.6
	average	16.8±3.0ª <i>P</i> =0.040	17.4±3.1 ^{a,b}	19.3±3.2 ^b	16.3±3.5ª <i>P</i> =0.047	17.9±3.4
SrCs	Ant	1.02±0.21	0.97±0.16	1.09±0.23	1.08±0.23	1.04±0.21
(s-1)	AntSept	1.18±0.30	1.12±0.24	1.18±0.28	1.00±0.32	1.11±0.28
	Inf	0.77 (0.43-1.07)ª	0.85 (0.48-1.55) ^{a,b}	0.87 (0.60-1.51) ^{a,b}	0.93 (0.79-1.07)⁵ <i>P</i> =0.017	0.88 (0.48-1.55)* <i>P</i> =0.020
	Lat	1.07±0.18	0.99±0.23	1.18±0.28	1.10±0.24	1.09±0.26
	Post	1.06±0.20	1.15±0.24	1.25±0.27	1.16±0.25	1.19±0.25* <i>P</i> =0.023

		Control	Mild	Moderate	Severe	All AR
	Sept	0.92±0.22	0.91±0.21	0.94±0.21	0.77±0.23	0.89±0.22
	average	1.00±0.14	1.00±0.14	1.09±0.16	0.99±0.19	1.03±0.16
SR	Ant	53.3±18.9ª	56.5±19.7 ^{a,b}	73.8±23.4 ^b <i>P</i> =0.007	64.2±26.4 ^{a,b}	64.7±23.4* <i>P</i> =0.027
(%)	AntSept	55.5±17.6ª	58.9±20.1 ^{a,b}	73.4±21.8 ^b <i>P</i> =0.036	76.2±32.8 ^b <i>P</i> =0.038	68.2±24.9* <i>P</i> =0.017
	Inf	53.6±20.4ª	57.1±21.4 ^{a,b}	75.8±28.4 ^b <i>P</i> =0.008	62.9±23.3 ^{a,b}	65.3±25.6* <i>P</i> =0.036
	Lat	54.6±21.1ª <i>P</i> =0.018	56.0±21.7ª <i>P</i> =0.048	76.4±29.2 ^b	59.4±29.9 ^{a,b}	64.3±27.8
	Post	52.7±19.4ª <i>P</i> =0.004	56.8±21.7ª <i>P</i> =0.042	76.0±26.2 ^b	62.4±27.0 ^{a,b}	65.2±25.7* <i>P</i> =0.023
	Sept	57.3±18.2ª	58.1±23.7 ^{a,b}	77.4±26.0 ^b <i>P</i> =0.023	71.8±27.6 ^{a,b}	68.3±26.5* <i>P</i> =0.049
	average	54.5±18.0ª <i>P</i> =0.006	57.2±20.1ª <i>P</i> =0.037	75.5±24.3 ^b	65.5±26.2 ^{a,b}	65.8±24.1* <i>P</i> =0.029
SrR _S	Ant	1.84 (1.17-2.98)ª	1.99 (1.36-3.37) ^{a,b}	2.35 (1.01-3.46) ^b <i>P</i> =0.007	1.94 (1.28-4.01) ^{a,b}	2.11 (1.01-4.01)* <i>P</i> =0.041
(s ⁻¹)	AntSept	1.85±0.51	1.98±0.51	2.26±0.64	2.16±0.83	2.13±0.64* <i>P</i> =0.044
	Inf	1.86 (1.04-3.23) ^a	2.02 (1.37-3.23) ^{a,b}	2.41 (1.58-4.95) [♭] <i>P</i> =0.011	2.09 (1.27-3.50) ^{a,b}	2.18 (1.27-4.95)* <i>P</i> =0.021
	Lat	2.22 (1.26-9.09) ^a	2.06 (1.36-3.47) ^{a,b}	2.50 (1.55-4.86) [♭] <i>P</i> =0.049	1.96 (0.96-4.25) ^{a,c} <i>P</i> =0.041	2.20 (0.96-4.86)
	Post	2.05 (1.15-6.86) ^a	2.05 (1.40-3.29) ^{a,b}	2.38 (1.56-3.19) ^ь <i>P</i> =0.021	2.05 (1.08-4.06) ^{a,b}	2.17 (1.08-4.06)
	Sept	1.95±0.51	2.00±0.55	2.34±0.57	2.21±0.70	2.17±0.60
	average	1.96 (1.19-3.65)	2.02 (1.31-3.29)	2.37 (1.48-3.70)	2.06 (1.17-3.92)	2.16 (1.17-3.92)

SL_G, longitudinal strain; SrL_S, systolic longitudinal strain rate; DL_S, longitudinal displacement; SC, circumferential strain; SrC_S, systolic circumferential strain rate; SR, radial strain; SrR_S, systolic radial strain rate.

In table 3 results for diastolic strain rate measurements are listed. Previous studies have demonstrated a high variability of segmental diastolic measurements, therefore only averaged results are reported. When comparing all horses with AR to the control group, SrL_E was significantly lower and SrL_A was significantly higher in horses with AR. SrC_E was significantly lower in horses with severe AR. SrR_E and SrR_A showed no significant differences.

Table 3: Averaged diastolic peak values of 2DST measurements.

Different superscripts indicate significant (P<0.05) differences between groups (^{a,b}) or between control and the whole AR group (*). Variables with normal distribution are reported as mean ± standard deviation. Variables with non-normal distribution are reported as median (range).

(S ⁻¹)		Control	Mild	Moderate	Severe	All AR
SrL_{E}	average	1.13±0.13ª	1.02±0.17 ^{a,b}	1.04±0.12 ^{a,b}	0.93±0.18 ^₅	1.01±0.16*
					<i>P</i> =0.001	<i>P</i> <0.001
SrL_A	average	0.67±0.17	0.75±0.14	0.78±0.15	0.72±0.13	0.76±0.14*
						<i>P</i> =0.014
SrC_{E}	average	1.23±0.17ª	1.22±0.17ª	1.30±0.13ª	1.04±0.20 ^b	1.21±0.19
		<i>P</i> =0.007	P=0.016	P<0.001		
$\mathrm{SrC}_{\mathrm{A}}$	average	0.57±0.17	0.60±0.16	0.66±0.16	0.58±0.15	0.62±0.16
${\sf SrR}_{\sf E}$	average	1.78 (1.09-3.22)	1.83 (0.94-2.94)	2.00 (0.83-3.86)	1.71 (0.99-3.34)	1.87 (0.83-3.86)
SrR_{A}	average	1.49±0.68	1.52±0.47	1.93±0.56	1.85±0.74	1.74±0.60

SrL_E, early diastolic longitudinal strain rate; SrL_A, late diastolic longitudinal strain rate; SrC_E, early diastolic circumferential strain rate; SrC_A, late diastolic circumferential strain rate; SrR_E, early diastolic radial strain rate; SrR_A, late diastolic radial strain rate.

DISCUSSION

This study examines the ability of 2DST to detect altered myocardial function in horses with different stages of AR severity compared to control horses. Longitudinal strain rate was unaltered in systole, but in diastole significant differences were observed. Radial and circumferential systolic strain were significantly higher in horses with AR.

Speckle tracking is an ultrasound technique that allows quantification of the myocardial strain in two dimensions by tracking the speckles in the ultrasound image. The manually determined ROI is automatically divided in segments and the software calculates deformation of the wall for all segments separately and for the global ROI. Due to the size of the equine heart, the apex could not always be visualized throughout the cardiac cycle.¹¹ The apical segments were not approved for analysis, but for the global measurements, those segments were included by the software. Therefore, global values are not reported in this study. This technique allows evaluation of the longitudinal function of the LV and the feasibility of the technique in horses was demonstrated.¹⁰⁻¹² In human medicine, systolic longitudinal function is considered an important indicator of subtle myocardial dysfunction.^{8,18,19} Our results show no significant differences in longitudinal systolic strain and strain rate between control horses and horses with AR. The reason for this is not clear. It has been demonstrated that strain is related to stroke volume and is influenced by loading conditions. Strain rate is a better indicator of myocardial performance, but is not entirely independent of alterations in loading of the ventricle.^{20,21} In horses with AR stroke volume is increased⁴ and loading conditions are altered. Probably a complex interplay between altered myocardial function and altered hemodynamics is responsible for the lack of alterations in longitudinal function. Species-specific differences in LV response to volume and pressure overload might also explain the different findings in human and equine patients. Horses with moderate to severe AR had a larger longitudinal

displacement, however the difference was only significant in the IVS. This is probably a result of the increased ventricular length in horses with AR.⁴

Previous studies have demonstrated a moderate to high variability for diastolic segmental measurements with 2DST.^{10,11} 2DST images have a low frame rate (40 fps) and this might lead to undersampling of the fast diastolic movement of the myocardial wall. Averaged values of all segments have a low to moderate variability, therefore only averaged values were reported. Diastolic longitudinal function was altered in horses with AR. In horses with mild or moderate AR, SrL_E was not significantly different from control horses, probably due to the combined effects of age and AR. Horses with AR were significantly older than control horses and with increasing age the ventricle becomes stiffer and less compliant, resulting in a slower early filling (lower SrL_E).²²⁻²⁴ AR itself also has an effect on the ventricular filling. The AR jet causes a rapid rise in LV diastolic pressures and LV filling, resulting in a higher SrL_E.²⁵ In horses with severe AR, a significantly lower SrL_E was found and this might be an indication of onset of decompensation. SrL_A was significantly higher in horses with AR, probably also due to the effect of age. The reduced early diastolic filling is compensated by an increased late diastolic filling.

In horses with AR, systolic radial strain increased significantly in all segments, especially in horses with moderate AR. In horses with severe AR, a slightly lower strain was found. This is in accordance with the results from our study using tissue Doppler imaging to evaluate myocardial function.⁷ Systolic radial strain rate showed similar results, with the most pronounced effect in the LVFW. Circumferential strain was significantly higher in horses with AR, especially in the cranial LVFW. Again it was the moderate group that showed most significant results. For radial and circumferential strain and strain rate, it is probably again a complex interplay of different factors, including alterations in myocardial function and in loading conditions, that resulted in these findings. The results show a remarkable pattern, with an increase in horses with mild to moderate AR and a slight decrease in horses with severe AR. A similar pattern was found in our study

using tissue Doppler imaging.⁷ This may indicate that horses classified in the group of mild to moderate AR were in the compensated stage of the disease. Horses with severe AR had no clinical signs of heart failure, but these results may indicate the onset of decompensation, at least in some horses in this group.

Averaged diastolic radial strain rates showed no significant differences, however measurements of SrR_E and SrR_A have been demonstrated to have a moderate variability.¹⁰

This study was intended to screen for variables that might be of interest for detecting myocardial dysfunction in horses with AR. Therefore a large number of variables was included, which may lead to false positive results. A post-hoc Bonferroni correction was applied to correct for multiple comparisons, but results should be interpreted with caution and more research is needed to determine the clinical usefulness of the variables that showed significant differences in this study. Horses usually develop AR at older age and this is reflected in the significant difference in age between horses with AR and control horses. This may have confounded our results, since several indices of myocardial function might be age dependent.²²⁻²⁴ Further research on the effects of age on cardiac function in horses is needed. Horses were divided in groups of different AR severity partially based on subjective criteria. Currently, there is no gold standard for quantification of AR in horses. The criteria used in this study are commonly used in clinical practice⁴, but might not accurately reflect true AR severity. The last limitation is related to the technique of speckle tracking. Adequate image quality is paramount for accurate tracking of the speckles. The software also performs extensive smoothing of the curves.^{12,26} Visual assessment of image quality and tracking quality should always be performed before approving the results. 2DST depends on the ultrasound machine, transducer and off-line analysis software and settings, so results might not be interchangeable with those acquired by a different ultrasound machine or software.26

CONCLUSION

2DST is able to detect an altered myocardial movement in horses with AR. Radial and circumferential function show more significant differences than longitudinal function. Further research is needed to determine whether these findings contribute to a more accurate diagnosis and prognosis in clinical cases.

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Chapter 6: Left ventricular morphology and function in aging horses

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Chapter 6: Left ventricular morphology and function in aging horses

Abstract

BACKGROUND: Age-dependent changes of cardiovascular morphology and function have been extensively described in human medicine and small animals, but studies in horses are scarce.

OBJECTIVES: To evaluate the left heart morphology and left ventricular (LV) function in aged horses.

ANIMALS: 20 healthy aged horses (\geq 17 years) were compared to 20 healthy adult horses (4-10 years).

METHODS: LV, left atrial and aortic dimensions were assessed using 2D and M-mode echocardiography. These techniques were also used to evaluate LV function by calculating fractional shortening (FS), fractional area change (FAC) and ejection fraction (EF). In addition, systolic and diastolic LV myocardial velocities were measured using tissue Doppler imaging (TDI). LV longitudinal, radial and circumferential strain and strain rate were assessed by 2D speckle tracking.

RESULTS: LV and left atrial dimensions were slightly smaller in the aged group, but aortic dimensions were not different between groups. Aged horses showed alterations of LV diastolic function, characterised by decreased early diastolic velocity and strain rate, increased late diastolic velocity and strain rate and prolonged isovolumic relaxation time measured by TDI. LV systolic function showed significant differences between aged horses and the control group, but results were conflicting. Radial myocardial systolic velocity in the LV free wall and average circumferential and radial systolic strain were lower in aged horses. Systolic radial myocardial velocity, strain rate and strain in the interventricular septum and average longitudinal strain rate were significantly higher.

CONCLUSIONS AND CLINICAL IMPORTANCE: Aged horses showed alterations of LV function, predominantly during diastole. These should be considered when evaluating LV function in older horses with cardiac disease.

INTRODUCTION

Age-dependent cardiovascular changes have been extensively described in human medicine. With advancing age, the arterial walls develop increased wall stiffness due to elastin fragmentation and increased collagen and calcium deposition in the medial layer.¹ These arterial changes result in an increased systolic blood pressure.² While left ventricular (LV) systolic function is usually preserved, LV early diastolic filling rate declines with age. This has been attributed to age-related increases in myocardial wall thickness caused by increased cardiomyocyte size and alterations of the extracellular matrix with increased collagen deposition in the connective cardiac tissue.³ In order to preserve end-diastolic volume, LV late diastolic filling due to atrial contraction increases and the LV filling pattern shifts from predominantly early diastolic to late diastolic.^{3,4}

The age-related changes in LV function can be quantified by measuring transmitral flow by pulsed wave Doppler, or myocardial velocities using tissue Doppler imaging (TDI). In healthy asymptomatic individuals, aging causes a pronounced decrease of early diastolic myocardial velocities with an accompanying increase in late diastolic velocities.⁵ Similar changes of cardiac structure and function have been described in small animals.⁶⁻⁸ The transmitral flow pattern shows a shift towards more late diastolic filling in aged dogs.⁷ By 2D speckle tracking echocardiography (2DST), old dogs showed a significantly lower early diastolic and higher late diastolic myocardial strain rate while systolic LV function was unaltered.⁶ In cats, delayed LV relaxation causing alterations of the filling pattern and myocardial velocities can be expected in most healthy cats above 12 years of age.⁹

In horses, studies on the age-dependent alterations of cardiovascular function are scarce. However, this may be an important confounding factor when LV function is assessed in horses with cardiac disease such as valvular regurgitation. Valvular disease is common in aged horses, with murmurs detected in up to 20% of horses aged 15 years or older.¹⁰ The prevalence of left-sided valvular regurgitation in horses is 13.5% in horses between 15-23 years of age, and 14.8% in horses of 24

years and older.¹¹ In geriatric horses of \geq 30 years, left-sided diastolic murmurs could be detected in 19% and systolic murmurs in 17%.¹² Characterisation of left heart morphology and function in an aged population is needed to distinguish the impact of aging from the hemodynamic consequences of valvular regurgitation. Alterations of LV morphology and diastolic function have already been described in a population of horses with aortic regurgitation, where age may have been a confounding factor.^{13,14}

The aim of this study was to assess left heart morphology and LV function in aged horses without valvular regurgitation using 2D and M-mode echocardiography, tissue Doppler imaging and 2D speckle tracking.

MATERIALS AND METHODS

Study population

The study population consisted of 40 healthy warmbloods: 20 horses of \geq 17 years (22 ± 5 years) and 20 adult horses of 4-10 years (6 ± 2 years). The control group was matched for body size, with a body weight of 524 ± 75 kg in the aged group and 547 ± 30 kg in the control group (*P*=0.23). The aged group consisted of 8 mares and 12 geldings and the control group consisted of 10 mares, 8 geldings and 2 stallions. All horses underwent a general physical examination with thorough thoracic auscultation to exclude cardiac or respiratory disease. A standardised echocardiographic exam using 2D, M-mode, colour flow Doppler and pulsed wave Doppler was performed with simultaneous recording of an electrocardiogram. Horses with more than trivial valvular regurgitation were not included in the study. The study protocol was approved by the ethical committee of the faculty of Veterinary Medicine and Bioscience Engineering, Ghent University (EC2012_57).

Image acquisition

Echocardiography was performed with an ultrasound unit (GE Vivid 7 Dimension, GE Healthcare, Horten, Norway) with phased array transducer (3S Phased Array Transducer, GE Healthcare, Horten, Norway) at a frequency of 1.7/3.4 MHz (octave harmonics), with simultaneous recording of a base-apex ECG. LV, aortic and left atrial dimensions were evaluated using standardised right parasternal 2D and M-mode images.¹⁵ LV and left atrial size were assessed from a long axis four-chamber view and a LV short axis view at chordal level. A short-axis LV M-mode image was acquired at the level of the chordae tendinae for evaluation of LV diameters and calculation of fractional shortening (FS). The aortic dimensions and timing of valve opening and closure were measured from a 2D and M-mode long axis LV outflow tract view.

LV function was also assessed by TDI and 2DST. For TDI, the grayscale sector width was reduced to 30° and the velocity scale set at -32 to +32 cm/s. This resulted in a frame rate of 183 frames per second (fps). The LV walls were imaged from a right parasternal short-axis view at chordal level. Optimal alignment of radial wall motion to the ultrasound beam was achieved using the tilt function. The 2DST grayscale images were recorded at a frequency of 1.6/3.2 MHz (octave harmonics), using a sector width of 55° to achieve a frame rate of at least 40 fps. Longitudinal strain was evaluated from a four-chamber view which did not visualize the apex due to the decreased image width.¹⁶ Circumferential and radial strain were assessed from short-axis images of the entire LV, recorded at chordal level.

Off-line data analysis

Off-line analysis was performed using dedicated software (EchoPAC Software Version 201, GE Healthcare, Horten, Norway). Three cardiac cycles were measured and averaged per view for further analysis. From the short-axis M-mode image at chordal level, heart rate, LV end-diastolic internal diameter (LVIDd), LV free wall

thickness (LVFWd), interventricular septum thickness (IVSd) and relative wall thickness (RWT_{diast}) were measured.¹⁵ The end-diastolic LV volume (LVV_{bullet, diast}) was calculated by the bullet method from the LV area from the short axis view at chordal level and the LV length (LVL_{diast}) measured from the four-chamber view as the distance between apex and the middle of the straight line connecting the opposite sections of the mitral annulus.¹³ The end-diastolic LV area (LVA_{diast}), endsystolic left atrial area (LAA_{syst}) and end-systolic left atrial diameter (LAD_{syst}) were measured from the four-chamber view. LV dimensions were also measured at endsystole to calculate the fractional shortening (FS), fractional area change (FAC) and ejection fraction (EF_{bullet}).¹³ The aortic diameter was measured at the level of the sinotubular junction in early diastole (Ao_{Ix,Ediast}), late diastole (Ao_{Ix,Ldiast}) and in systole (Aoix,syst) as the maximal diameter in one of the first three frames after aortic valve opening. The diastolic decrease in aortic diameter per beat was calculated (Ao_{DiastDecr}).¹³ Pre-ejection period (PEP) and the ratio of pre-ejection period to ejection time (PEP/LVET) were measured from the LV outflow tract Mmode.13

TDI images were analysed in the Q-analysis mode of the EchoPAC software. From the right parasternal view, the interventricular septum (IVS) and LV free wall (LV) were evaluated.^{14,17} In each segment, a sample area was placed with an adapted length (range 11-12 mm) and width (range 5-6 mm) depending on wall thickness. The sample area was anchored inside the myocardium throughout the cardiac cycle. The cine compound function was used to convert the last cycle into the average of the three consecutive cycles per loop. Segmental peak radial systolic, early and late diastolic velocity (Vels, Vel_E and Vel_A), strain rate (SrRs, SrR_E, SrR_A) and strain (RS) were measured. For evaluation of diastolic function, the E/A ratio was calculated as Vel_E/Vel_A and the isovolumic relaxation time (IVRT) was calculated as the time between the end of the systolic velocity peak and the onset of the early diastolic velocity peak.¹⁴ For 2DST, the calculation of strain and strain rate was performed semi-automated using the "2D Strain" application of the ultrasound software. A region of interest (ROI) was drawn along the LV endocardial border in a

frame at end-systole and ROI width was adjusted to wall thickness. For longitudinal images, the ROI was set by tracing the endocardial border from the septal to the lateral mitral valve insertion. Speckle tracking started automatically, dividing the ROI into 6 segments. As the modified four-chamber view did not include the apical segments, these were discarded for the analysis. Tracking quality was evaluated automatically by the software and assessed visually by the operator, as described previously.^{16,18} In case of poor tracking quality, the ROI was adapted manually until the tracking was adequate or the segment was discarded for further analysis. Averaged peak values were calculated as the average of the peak values of the approved segments. Systolic, early diastolic and late diastolic values were measured for longitudinal strain and strain rate (SL and SrL), circumferential strain and strain rate (SC and SrC) and radial strain and strain rate (SR and SrR).^{16,18}

Data Analysis and Statistics

Statistical analyses were performed using dedicated computer software (SPSS Statistics 25, SPSS Inc, Chicago, USA). Normal distribution of the data was evaluated by visual inspection, the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Data are reported as mean \pm standard deviation, except if indicated otherwise. Aged horses were compared to the control group of young healthy horses using an independent samples *t*-test. The level of significance was $\alpha = 0.05$.

Results

Table 1 shows the values for LV, left atrial and aortic dimensions. Overall, the LV internal dimensions were slightly smaller in the aged horses, with a significantly lower LV length and LV volume as calculated by the bullet method. The relative wall thickness was slightly higher in the aged horses. The left atrial area was also significantly lower in the aged horses, although the maximal left atrial diameter was not different. No significant differences were found for aortic diameters or aortic diastolic decrease.
Measurement	Control horses (n = 20)	Aged horses (n = 20)	<u>P</u>
	(6 ± 2 years)	(22 ± 5 years)	
HR (bpm)	39 ± 7	39 ± 6	0.87
LVA _{diast} (cm ²)	144.7 ± 17.2	134.2 ± 19.0	0.08
FAC (%)	53.8 ± 5.4	56.9 ± 5.0	0.08
LVL _{diast} (cm)	16.8 ± 1.3	15.6 ± 0.9*	0.002
LVV _{bullet, diast} (mL)	1165 ± 166	1000 ± 147*	0.006
EF _{bullet} (%)	74.5 ± 3.7	76.8 ± 4.8	0.15
LVID _{diast} (cm)	11.1 ± 0.6	10.7 ± 0.9	0.14
FS (%)	43.6 ± 4.9	44.7 ± 5.3	0.48
RWT _{diast}	0.50 ± 0.04	0.54 ± 0.05*	0.016
LVFWd	2.5 ± 0.4	2.6 ± 0.4	0.45
IVSd	3.0 ± 0.2	$3.2 \pm 0.4^{*}$	0.038
LAA _{syst} (cm ²)	77.1 ± 8.5	69.6 ± 10.1*	0.016
LAD _{syst} (cm)	11.1 ± 0.6	10.9 ± 0.9	0.54
Ao _{lx,Ediast} (cm)	6.4 ± 0.6	6.5 ± 0.7	0.73
Ao _{lx,Ldiast} (cm)	6.1 ± 0.5	6.3 ± 0.7	0.55
Ao _{DiastDecr} (cm)	0.27 ± 0.13	0.22 ± 0.07	0.26
Ao _{lx,syst} (cm)	6.8 ± 0.5	6.6 ± 0.7	0.39
PEP (ms)	88 ± 14	95 ± 13	0.17
PEP/LVET	0.20 ± 0.03	0.22 ± 0.02	0.17

 Table 1: Left heart morphology and left ventricular function assessed by 2D and M-mode echocardiography in aged horses and control horses.

* = significantly different from control group (independent samples t-test)

HR, heart rate; diast, measured at end-diastole; syst, measured at end-systole; LVA, left ventricular area; FAC, fractional area change; LVL, left ventricular length; LVV_{bullet}, left ventricular volume calculated by bullet method; EF_{bullet}, ejection fraction calculated by bullet method; LVID, left ventricular internal diameter; FS, fractional shortening; RWT, relative wall thickness; LAA, left atrial area; LAD, left atrial diameter; Ao_{lx}, aortic diameter measured from the long axis view; Ediast, early diastole; Ldiast, late diastole; Ao_{DiastDecr}, aortic diastolic decrease; PEP, pre-ejection period; LVET, left ventricular ejection time

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LV systolic function assessed by 2D and M-mode parameters was not significantly different between aged horses and the control group. TDI and 2DST analysis yielded conflicting results for systolic function (Table 2). Aged horses showed a significantly lower radial myocardial velocity in the LV free wall and a lower average circumferential and radial strain. However, systolic myocardial velocity, strain rate and strain in the interventricular septum and average longitudinal strain rate were significantly higher.

	Measurement	Control horses	Aged horses	Sign
		<u>(n = 20)</u>	<u>(n = 20)</u>	
		(6 ± 2 years)	(22 ± 5 years)	
	R-TVI-LV _{ct} -Vel _S (cm/s)	9.1 ± 1.6	8.1 ± 1.3*	0.034
	-Vel _E (cm/s)	24.7 ± 4.1	17.2 ± 5.0*	<0.001
	-Vel _A (cm/s)	8.1 ± 2.6	9.7 ± 2.3	0.07
	-E/A (ratio)	3.3 ± 1.1	1.9 ± 0.7*	<0.001
	-IVRT (ms)	42 ± 14	74 ± 31*	<0.001
	$\text{R-TVI-IVS}_{\text{ct}}\text{-Vel}_{\text{S}} \text{ (cm/s)}$	3.7 ± 1.5	5.4 ± 1.8*	0.003
	-Vel _E (cm/s)	11.9 ± 3.1	11.9 ± 3.1	0.95
	-Vel _A (cm/s)	1.6 ± 1.2	4.2 ± 2.6*	<0.001
	R-SRI-IVS _{ct} -SrR _S (s ⁻¹)	1.2 ± 0.3	1.4 ± 0.3*	0.035
	-SrR _E (s ⁻¹)	2.9 ± 0.9	2.6 ± 0.9	0.25
	-SrR _A (s ⁻¹)	0.9 ± 0.3	1.6 ± 0.6*	<0.001
	R-SI-IVS _{ct} -RS (%)	39.4 ± 9.3	46.3 ± 11.5*	0.046
	2DST - SL average (%)	20.1 ± 2.6	20.6 ± 2.7	0.56
	2DST - SrL _S average (s ⁻¹)	0.89 ± 0.11	1.02 ± 0.13*	0.003
	- SrL _E average (s ⁻¹)	1.16 ± 0.12	1.26 ± 0.26	0.13
	- SrL _A average (s ⁻¹)	0.62 ± 0.14	0.85 ± 0.18*	<0.001
	2DST - SR average (%)	58.8 ± 20.7	42.3 ± 13.1*	0.005
	2DST - SrR _S average (s ⁻¹)	2.02 ± 0.62	1.58 ± 0.38*	0.011
	- SrR _E average (s ⁻¹)	1.84 ± 0.64	1.43 ± 0.39*	0.018
	- SrR _A average (s ⁻¹)	1.43 ± 0.65	1.80 ± 0.50	0.054
	2DST - SC average (%)	16.7 ± 3.1	14.8 ± 2.3*	0.045
	2DST - SrC _S average (s ⁻¹)	0.98 ± 0.13	0.98 ± 0.16	0.94
	- SrC _E average (s ⁻¹)	1.25 ± 0.16	1.20 ± 0.15	0.37
	- SrC _A average (s ⁻¹)	0.54 ± 0.15	0.68 ± 0.11*	0.003
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Table 2: Left ventricular function assessed by tissue Doppler imaging and 2Dspeckle tracking echocardiography in aged horses and control horses.

* = significantly different from control group (independent samples t-test)

R, right parasternal; TVI, tissue velocity imaging; LV_{ct}, left ventricular free wall at chordal level; Vel_s, peak systolic velocity; Vel_e, peak early diastolic radial velocity; Vel_A, peak late diastolic radial velocity; E/A, ratio of early to late peak diastolic radial velocity; IVRT,

isovolumic relaxation time; IVS_{ct}, interventricular septum at chordal level; SrR_s, peak systolic radial strain rate; SrR_E, peak early diastolic radial strain rate; SrR_A, peak late diastolic radial strain rate; RS, peak radial strain; 2DST, two-dimensional speckle tracking; SL, longitudinal strain; SrL_s, peak systolic longitudinal strain rate, SrL_E, peak early diastolic longitudinal strain rate; SrL_A, peak late diastolic longitudinal strain rate; SR, radial strain; SrR_s, peak systolic radial strain rate; SrR_E, peak early diastolic radial strain rate; SrR_A, peak late diastolic radial strain rate; SC, circumferential strain; SrC_s, peak systolic circumferential strain rate; SrC_E, peak early diastolic circumferential strain rate; SrC_A, peak late diastolic circumferential strain rate

LV diastolic function showed a decrease of early diastolic filling with a compensatory increase of late diastolic filling (Table 2). This could be detected both with TDI and 2DST by measuring myocardial velocities and strain rate, although these changes were not always significant. The E/A ratio of LV free wall myocardial velocity was significantly lower in aged horses, although complete reversal of the diastolic filling pattern (E/A < 1) did not occur in any of the horses.

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Figure 1: Tissue Doppler velocity curves from a right parasternal short axis view of the left ventricle (LV). A sample area is positioned in the LV free wall (yellow) and the interventricular septum (green). The peak systolic (S), early diastolic (E) and late diastolic (A) radial myocardial velocities are indicated. Panel A: young adult horse, large E peak; Panel B: old horse, decreased E peak and large A peak.

DISCUSSION

Horses show age-associated alterations in LV diastolic function which are similar to those found in humans and small animals. LV and left atrial internal dimensions were slightly smaller in the aged group, but aortic dimensions were not different between groups. Several indices of LV systolic function showed significant differences between aged horses and the control group, but some results were conflicting.

In the group of aged horses, the early diastolic myocardial velocity and strain rate were decreased while the late diastolic velocity and strain rate were increased (Figure 1). Similar results were found in a recent study investigating LV function by tissue Doppler imaging in 60 healthy warmblood horses aged 3-30 years.¹⁹ Physiologically, LV filling occurs in two distinct phases: the early diastolic rapid filling caused by LV relaxation, and the late diastolic filling caused by left atrial contraction. In humans, the filling pattern of the LV is predominantly early diastolic in young healthy adults, but this reverses with advancing age. The transmitral early diastolic E-wave declines by 30–50% from age 20 to 80 years, while the late diastolic A-wave velocity increases with age.² The underlying mechanisms include increased LV wall thickness and reduced LV diastolic compliance. A prolonged isovolumic relaxation (interval between aortic valve closure and mitral valve opening) has also been demonstrated in man and animal models.²⁰ This may be caused by an age-associated decline in intracellular Ca²⁺sequestration by the sarcoplasmic reticulum. The aged horses in our study population also showed a significantly longer isovolumic relaxation time compared to the control group. In horses with aortic regurgitation, lower early diastolic and higher late diastolic myocardial velocities have also been described.¹⁴ These alterations of diastolic function are probably the result of the effect of aging, as a ortic regurgitation is more common in older horses.

Remarkably, the early diastolic longitudinal and circumferential strain rate measured by 2DST was not lower in aged horses, although the late diastolic strain

rate was significantly increased. This is probably due to the technical limitations of the speckle tracking technique. The achieved frame rate for the 2DST grayscale images was 41 fps, which is insufficient to adequately track the rapidly moving myocardial wall during early diastole in horses. Poor tracking quality in early diastole has been described previously in studies evaluating the feasibility and reliability of 2DST in healthy horses.^{18,21}

Results regarding LV systolic function were conflicting in our study, with some variables indicating decreased systolic function and others indicating enhanced systolic function in the aged group. Similarly, two recent studies evaluating the effect of age on cardiac function by TDI and 2DST in warmblood horses demonstrated decreased systolic myocardial velocities by TDI and increased radial strain rate by 2DST.^{19,22} The conflicting results may partly be explained by the measurement methods. TDI and 2DST both provide indirect measurements of myocardial contractile function. TDI velocity measurements are influenced by several factors, such as overall heart motion and tethering by adjacent segments, the angle of insonation and loading (pre- and afterload).²³ 2DST deformation indices overcome the limitations of overall heart motion and are less influenced by insonation angle, but they are not load-independent and are also affected by factors such as heart size.²⁴ In human medicine, the impact of aging on LV longitudinal systolic function is a source of debate.⁴ The overall LV systolic function at rest does not change with healthy aging and EF is preserved.¹ However, older individuals do show an increased afterload due to vascular stiffening and alterations of the myocardial shortening velocities.

The LV and left atrial chamber dimensions were slightly smaller in the aged group compared to the control group, with a significantly higher relative wall thickness in the aged group. This can be explained by a decreased preload, as resting plasma volume is decreased in old horses.^{25,26} The lower plasma volume also affects diastolic function. Left atrial end-systolic pressure will be lower when plasma volume is decreased. This results in a slow opening of the mitral valve and thus

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early diastolic wall motion will be decreased. However, our results may also have been influenced by other confounding factors such as heart rate, training or body size. Heart rate was similar in both groups and thus probably did not influence the measurements. Training, especially endurance-type training, results in LV eccentric hypertrophy.²⁷ The control group and aged group were not matched for training status, and it is possible that the younger horses were trained more intensively. However, none of the horses in the study population was performing high-level endurance-type exercise. We could have corrected for the possible influence of body weight by allometric scaling, in which the chamber diameters, areas and volumes are normalised to a body weight of 500 kg. This can be useful if patients or study populations with different body weights are compared, as body size has a significant influence on cardiac dimensions.^{28,29} Scaled cardiac dimensions were not reported in this study as the aged horses and the control group were matched for body size. However, scaled dimensions have been calculated and the results were similar to the non-scaled variables. Therefore it can be assumed that differences in body size do not explain the findings of this study.

The aortic dimensions did not differ between the control group and the aged group. In human medicine, the ascending aortic diameter shows a linear association with age.³⁰ This is related to increasing aortic stiffness caused by increased collagen synthesis and decreased buffering of elastin in the aortic wall. Aortic root remodelling with aortic dilatation may be an adaptive mechanism to limit the increase in pulse pressure following increased aortic wall stiffness.³¹ In our study, the aged horses did not show significantly larger aortic diameters. In contrast, Endoh et al. (2017) demonstrated that the luminal diameter and thickness of the aortic arch at post-mortem examination increased with age in Thoroughbred horses. This was associated with a decreased percentage area of elastin and increased percentage area of collagen in the medial layer.³² Horses with aortic regurgitation also show aortic dilatation and this is attributed to the larger stroke volume stretching the aortic root.^{13,33} As aortic regurgitation is more common in older horses, age should be taken into account when evaluating aortic diameter in

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these horses. In addition, blood pressure, training or body size may also be confounding factors when assessing aortic dimensions.

The main limitation of this study is the low number of horses included in the study population. Recruiting a larger number of healthy old warmbloods was difficult due to the high prevalence of cardiac and respiratory disease in aged horses.³⁴ Furthermore, only young adult and old horses (\geq 17 years) were included. A larger study population including horses of different age groups would have allowed to determine the critical age at which the changes in LV function become detectable. Examining a large number of horses could also allow to model the effects of age, sex, body weight, breed and training status. Research in human medicine demonstrated gender-specific patterns of cardiovascular aging.³⁵

In conclusion, aged horses showed impaired early diastolic left ventricular filling with a compensatory increase of late diastolic filling due to atrial contraction. When evaluating horses with valvular regurgitation, age should be considered as a confounding factor.

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Chapter 7: Cardiac dimensions and function in matched untrained versus endurance-trained Arabian horses.

Adapted from a conference abstract for the International Conference on Equine Exercise Physiology, Lorne, Australia, November 2018

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CHAPTER 7: CARDIAC DIMENSIONS AND FUNCTION IN MATCHED UNTRAINED VERSUS ENDURANCE-TRAINED ARABIAN HORSES.

INTRODUCTION

Endurance training induces eccentric hypertrophy of the left ventricle (LV).¹ In horses, several studies have been published to describe the cardiac alterations in horses after training. In 2-year-old Thoroughbreds an increase in LV internal diameter, LV mass and relative wall thickness was observed after 18 weeks of training.² Buhl et al. (2005) followed 53 Standardbred trotters during the first 3 years of their training. They were examined by echocardiography every 6 months throughout their training, starting at 2 years of age until 5.5 years of age. They found a significant increase in heart size and prevalence of valvular regurgitation during that period.³ Another study in Thoroughbred racehorses demonstrated strong associations between racing performance and measures of LV size and systolic function.⁴ However, these studies all involved racehorses. Although held over relatively short distances, training involves mainly endurance work. In racing industry, horses start to train at a young age and they are not fully grown. This makes it difficult to separate the cardiac effects of training from cardiac alterations induced by growth.²⁻⁴ Endurance riding is an equestrian discipline recognized by the Fédération Equestre International (FEI). Races are held over distances of 100km to 160km in one day, comprising mainly aerobic work. Horses must be at least 6 years old to participate in 100km races. The aim of this study was to examine the effect of high-level endurance training on cardiac dimensions and function. To eliminate the alterations induced by growth, adult high-level endurance-trained Arabian horses were matched for age and bodyweight to a control group of untrained Arabian horses. Horses were examined by standard echocardiography and 2D speckle tracking (2DST) to detect differences in LV dimensions and function.

MATERIAL AND METHODS

The study was approved by the ethical committee of the Faculty of Veterinary Medicine and Bioscience Engineering (approval number EC2017_107). Echocardiography was performed in 13 untrained (11±3years, 448±24kg) and 13 high-level endurance-trained (11±3years, 434±53kg) Arabian horses. Horses were matched for age and bodyweight. For the trained horses, successful participation in at least 2 endurance races of ≥100 km in the preceding year was required to be included in the study. Data were collected at least 2 weeks after strenuous exercise. All horses were submitted to a thorough clinical examination and cardiac auscultation. Echocardiography was performed using a GE Vivid 7 Dimension ultrasound with 3S phased array transducer (GE Healthcare) with simultaneous recording of an electrocardiogram. Images were stored for off-line analysis using commercial software (EchoPAC Software version 11.2, GE Healthcare).

2D and M-mode images were collected to measure systolic (s) and diastolic (d) dimensions of the left heart (Figure 1). LV internal diameter (LVID) was measured from the short-axis M-mode image of the LV. Fractional shortening (FS) of the ventricle was calculated as:

$$FS = \frac{(LVIDd - LVIDs)}{LVIDd} * 100$$

LV area was measured from short axis image at chordal level (LVA). LV length (LVL) was measured from the four-chamber view as the distance between the middle of the straight line connecting the opposite sections of the mitral ring and the ventricular apex. LV volume was calculated by the Bullet method, using the formula:

$$LVV = \frac{5 * LVA * LVL}{6}$$

Chapter 7: Effect of endurance training on LV dimensions and function

Ejection fraction (EF) was calculated as:

$$EF = \frac{LVVd - LVVs}{LVVd} * 100$$

Left atrial dimensions were also measured from the four-chamber view. The maximal left atrial diameter at end systole (LADs) was measured parallel to the mitral annulus, one or two frames before mitral valve opening. In the same image, left atrial area was measured by tracing the inner edge of the atrium (LAAs). This was repeated at end-diastole (LAAd). Aortic diameter was measured from the right parasternal long-axis image of the LV outflow tract at the level of the sino-tubular junction in systole (Ao_{Ix,Syst}), in early diastole (Ao_{Ix,Ediast}) and in late diastole (Ao_{Ix,Ldiast}). The aortic diastolic decrease was calculated as:

$$Ao_{DiastDecr} = Ao_{lx,Ediast} - Ao_{lx,Ldiast}$$

Colour flow Doppler was applied to check for regurgitation of the cardiac valves. Horses with more than mild regurgitation from any cardiac valve were not included in this study.



Figure 1: Echocardiographic four-chamber image (right parasternal view) of an untrained Arabian horse (left panel) and a high-level endurance trained Arabian horse (right panel).

From a right parasternal view, long axis 2DST images of the LV were collected. Additionally, short axis 2DST images of the LV were recorded at the level of the chordae tendineae. During off-line analysis, the region of interest (ROI) was set by tracing the LV endocardial border. The width of the ROI was set to cover the myocardial wall but not the epicardium. The software automatically divided the ROI in segments and evaluated the tracking of each segment. If necessary, the ROI width or position were adapted to assure good tracking quality. From the long axis images, curves were generated for longitudinal strain (SL), strain rate (SrL) and displacement (DL). From the short axis images, radial and circumferential myocardial deformation were assessed. Strain (SR and SC respectively) and strain rate (SrR and SrC respectively) were calculated. From the strain curves, maximal strain (%) was measured. From the strain rate curves, systolic (S), early diastolic (E) and late diastolic (A) strain rate (1/s) were measured (Figure 2). Statistical analysis was performed using dedicated software (SPSS Statistics version 25, Chicago, IL, USA). Normal distribution of all variables was checked. Control horses and trained horses were compared by an independent samples *t*-test. Level of significance was set at 0.05.



Figure 2: Radial strain rate curves acquired by 2D speckle tracking. Different coloured curves depict the strain rate of different segments of the short axis image of the left ventricle.

Results

Results for the 2D and M-mode measurements are listed in Table 1. Systolic and diastolic LV dimensions (LVID, LVA and LVV) were significantly higher in trained horses compared to untrained animals. FS, aortic and left atrial dimensions were not significantly different between groups.

 Table 1: 2DE and M-mode parameters in untrained Arabian horses and in matched

 endurance trained Arabian horses.

	Untrained	Endurance trained
HR (beats/min)	39±6	40±6
LVIDs (cm)	5.8±0.8	6.6±0.9*
LVIDd (cm)	10.2±0.6	10.9±1.0*
FS (%)	43±6	39±4
LVLs (cm)	11.0±0.5	11.8±1.0*
LVLd (cm)	14.8±0.6	15.5±0.8*
LVAs (cm²)	28±5	33±7*
LVAd (cm²)	71±7	85±13*
LVVs (cm ³)	258±47	332±85*
LVVd (cm ³)	866±86	1097±200*
EF (%)	70±3	70±5
LADs (cm)	9.8±0.8	10.3±0.7
LAAs (cm ²)	64.5±8.3	69.2±7.2
LAAd (cm²)	42.5±5.2	45.5±5.2
Ao _{ix,syst} (cm)	6.9±0.5	7.0±0.4
Ao _{lx,Ediast} (cm)	6.1±0.4	6.1±0.4
Ao _{lx,Ldiast} (cm)	5.9±0.3	5.9±0.4
Ao _{DiastDecr} (cm)	0.27±0.06	0.30±0.14
RWTd	0.55±0.06	0.53±0.08

* indicates significant difference between groups (P<0.05)

HR, heart rate; LVID, left ventricular internal diameter; FS, fractional shortening; LVL, left ventricular length; LVA, left ventricular area; LVV, left ventricular volume; EF, ejection fraction, LAD, left atrial diameter; LAA, left atrial area; Ao_{lx,syst}, aortic diameter at systole; Ao_{lx,Ediast}, aortic diameter at early diastole; Ao_{lx,Ldiast}, aortic diameter at late diastole; Ao_{DiastDecr}, aortic diastolic decrease; RWT, relative wall thickness; s, systole; d, diastole

Table 2 lists the results from the 2DST measurements. A significantly (P<0.05) lower longitudinal, circumferential and radial strain rate during atrial contraction was seen in trained horses compared to untrained horses.

Table 2: 2DST parameters in untrained Arabian horses and in matched endurancetrained Arabian horses. The values displayed are the average values calculatedfrom all segments.

	Untrained	Endurance trained
SL _s (%)	20.0±3.2	19.0±2.5
SrLs (s ⁻¹)	1.00±0.16	0.92±0.15
SrL _E (s ⁻¹)	1.39±0.17	1.30±0.26
SrL _A (s ⁻¹)	0.83±0.13	0.63±0.16*
DLs (mm)	26.1±2.6	27.5±3.0
SR (%)	36.5±7.9	34.0±15.2
SrR _S (s ⁻¹)	1.58±0.32	1.37±0.34
SrR _E (s ⁻¹)	1.54±0.36	1.29±0.32
SrR _A (s ⁻¹)	1.70±0.54	1.32±0.23*
SC (%)	13.1±1.1	12.9±1.7
SrCs (s ⁻¹)	0.94±0.10	0.87±0.12
SrC _E (s ⁻¹)	1.37±0.15	1.22±0.16*
SrC _A (s ⁻¹)	0.68±0.08	0.54±0.12*

* indicates significant difference between groups (P<0.05)

SL, longitudinal strain; SrL, longitudinal strain rate; DL, longitudinal displacement; SR, radial strain; SrR, radial strain rate; SC, circumferential strain; SrC, circumferential strain rate; S, systole; E, early diastole; A, late diastole;

DISCUSSION

High-level endurance trained Arabian horses have larger LV dimensions than untrained Arabian horses. Additionally, 2DST was able to demonstrate alterations in myocardial wall motion during atrial contraction.

Exercise training induces adaptations throughout the body, including the cardiovascular system.⁵ The heart will adapt to the increased demand for oxygen supply to the working muscles. In human medicine, it has been described that LV eccentric hypertrophy occurs in response to endurance training.¹ Dilatation of the ventricle results in a larger stroke volume and an increased cardiac output. In horses, some studies have described similar effects, but most involved young horses where growth may have confounded the results.²⁻⁴ Michima et al. (2004) performed a study in 35 endurance horses, comprising both Arabian and Arabian crossbred horses.⁶ Cardiac dimensions and indices of cardiac function were measured. However, that study did not include a control group, so results could not be compared to untrained horses to detect alterations as a result of training. Another study compared echocardiographic measurements in elite and non-elite Arabian endurance horses.⁷ A significant larger LVIDd, LVIDs, LV mass and LV stroke volume were found in the elite horses compared to non-elite horses. Heart size as determined by echocardiographic dimensions was associated with performance in Arabian horses.

In this study, adult horses (\geq 6 years old) were selected and trained horses were matched for age and bodyweight to untrained horses. Similar to what has been reported in human medicine and in animals, an increase in LV dimensions in well trained horses was observed. At rest, LV internal diameter and length increase, both in systole and in diastole. A significant increase in LV volume was found, but this may be partly due to alterations in LV geometry and subsequent alteration in calculation error. The LV volume was calculated based on the Bullet method. This makes certain assumptions on LV geometry, which is based on human cardiology and may not be very accurate in horses. Additionally, LV dilatation probably also induces alterations in LV geometry and thus calculation of the volume in trained horses may be less (or more) accurate than in untrained horses.

FS was lower in endurance trained horses, although the difference did not reach statistical significance. This is probably due to the low number of horses involved in the study. A lower FS would be expected in trained horses. Since heart rate was not significantly different between both groups, a similar stroke volume should be generated to produce an equal cardiac output. In the dilated LV in trained horses, this requires less systolic shortening of the ventricle. Remarkably, EF did not show alterations in trained horses.

2DST is an ultrasound technique that is able to detect alterations in myocardial wall motion in horses.^{8,9} The technique has been applied in horses with lasalocid intoxication and in horses with atypical myopathy.^{10,11} In this study 2DST was used to evaluate alterations in myocardial wall motion due to endurance training. Several segments of the myocardial wall were analysed, both on short axis and long axis images. Longitudinal, radial and circumferential motion of the wall was evaluated. With 2DST, a significantly lower strain rate of the LV myocardial wall during atrial contraction was seen in trained horses. The mechanism responsible for the lower late diastolic strain rate is not clear. Dilatation of the LV results in alterations in myocardial wall tension, which may respond differently to the volume added by the atrial contraction.

This study also has some limitations. For the untrained horses it was not always clear why they were not trained for top-level endurance. This may result in a training bias, with the most talented horses being trained for the top-level and less talented horses are left untrained. However, the untrained horses were from similar bloodlines as the well trained horses, so it can be assumed that the potential for endurance racing was similar in both groups. Endurance training induces an expansion of the plasma volume.¹² As a result, loading conditions for the heart will be altered and this may influence our results. Deformation of the myocardial wall is not only a result of myocardial function, but is also influenced by

loading conditions. The alterations in plasma volume are part of the adaptations to strenuous training and cannot be separated from the cardiac adaptations.

CONCLUSION

This study investigated the cardiac effects of endurance training in adult age- and bodyweight-matched Arabian horses. Endurance training induced LV enlargement. In trained horses atrial contraction induced less pronounced deformation of the LV wall. Some pathological conditions induce similar alterations, so the level of training should be taken into account when evaluating cardiac function in sport horses.

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Chapter 8: Discussion

CHAPTER 8: DISCUSSION

In highly trained horses and in old horses, aortic regurgitation (AR) is guite common. In most horses it is slowly progressive and will not limit their performance. However, in some horses the disease progresses to severe regurgitation, resulting in left ventricular (LV) overload. This will have an important hemodynamic impact, with volume and pressure overload for the LV. Longstanding severe AR may lead to congestive heart failure and poor performance. Additionally, the risk for ventricular dysrhythmias increases in horses with AR, which may lead to sudden cardiac death and may make them unsafe to ride. Horses diagnosed with AR should therefore be monitored regularly. An accurate estimation of AR severity and prognosis is also important during the prepurchase exam of sport horses.¹ However, accurate assessment of AR severity in horses is difficult using the current echocardiographic techniques. Limited objective parameters are available and evaluation of severity and prognosis requires operator experience. The aim of this work was to search for new objective parameters that might be useful for diagnosing and monitoring AR severity in horses. Different echocardiographic techniques were used. From 2D and M-mode images, it was shown that horses with AR have an increased aortic diastolic run-off and decreased pre-ejection period (PEP) compared to control horses. Fractional shortening (FS) was unaltered in horses with compensated AR. Tissue Doppler imaging showed a significantly higher radial systolic and late diastolic myocardial velocity in horses with AR, however not in all segments of the myocardial wall the difference was significant. Alterations in myocardial wall motion between horses with AR and control horses could also be demonstrated by 2D speckle tracking (2DST). Radial and circumferential strain increased significantly in horses with AR. Surprisingly and in contradiction to what is seen in human medicine, longitudinal LV function was unaltered in horses with AR. This may be due to the influence of different mechanisms with counteracting effects on longitudinal strain.

The prevalence of AR is higher in well trained horses and old horses. Since both training and aging induce alterations in cardiac function, this may confound the results when investigating the effect of AR on the equine heart. A study comparing well-trained Arabian horses with untrained Arabians showed that endurance training induces LV eccentric hypertrophy. The level of training should thus be taken into account when evaluating horses with AR. To determine the effects of aging on cardiac dimensions and function, young adult horses were compared to old horses. Aging does not alter LV and aortic dimensions significantly, but it has an influence on LV diastolic function. Early diastolic LV filling is reduced in old horses and is compensated by an increased late diastolic LV filling. Therefore, age should also be taken into account when evaluating horses with AR.

Figure 1: Schematic overview of the hemodynamic consequences of aortic regurgitation (AR). The thin black arrows depict the mechanisms involved in the compensated stage of AR, the large white arrows represent decompensated/end-stage AR. The rectangular boxes show parameters that can be measured to quantify AR severity. All parameters involved have an influence on the myocardial wall motion, which can be measured with tissue Doppler imaging and 2D speckle tracking. LV, left ventricle; SV, stroke volume; FS, fractional shortening; PEP, pre-ejection period



1. HEMODYNAMIC CONSEQUENCES OF AORTIC REGURGITATION

The echocardiographic alterations in LV morphology and function in horses with AR can be explained by the important hemodynamic impact of the regurgitant flow (Figure 1). In horses with AR, part of the effective forward stroke volume (SV) flows back to the LV with every heartbeat. This regurgitant flow results in volume and pressure overload for the ventricle. If the onset of the regurgitation is slowly progressive, the heart will adapt to accommodate this overload. During diastole, the ventricle receives blood both from the left atrium and from the aorta through the leaking aortic valve. This results in a larger end-diastolic volume and pressure in the LV. The increased pre-load of the ventricle results in a higher contractility (Frank-Starling mechanism) to eject a larger SV. Ejection of this larger SV stretches the aorta and creates a higher systolic blood pressure and a higher afterload for the LV.^{2,3}

In chapter 3, an increase in end-diastolic LV dimensions and SV in horses with different degrees of AR severity was demonstrated. A study by Reef et al. (1987) also reported an increase in LV chamber size in horses with AR, although they did not differentiate between different grades of AR severity.² In human medicine LV internal diameter (LVID) is an important parameter to evaluate AR severity and to determine the best timing for surgical aortic valve replacement.^{4,5} The diastolic LV dimensions increase early during the development of AR. When systolic function deteriorates, this may indicate onset of irreversible myocardial damage. Ejection fraction (EF) and end-systolic LVID are commonly used to estimate the onset of systolic dysfunction and cut-off values have been established.⁶ Horses appear to respond differently to AR. In some horses in this research, diastolic LVID was only mildly increased, despite a large regurgitant jet and a very round apex as scored by subjective evaluation.

Based on the measured LV dimensions, the LV end-diastolic volume was calculated by the Bullet method and by the area-length method. The Bullet method uses the LV length and LV short axis area, whereas the area-length method uses the LV length and LV long axis area (Figure 2). End-diastolic LV volumes calculated by the Bullet method were somewhat larger than end-diastolic LV volumes calculated by the area-length method. However, for end-systolic volumes, the opposite was true and the Bullet method resulted in smaller volumes than the area-length method. These methods were developed for human medicine. They make assumptions about the LV geometric shape, which may not always be correct for the equine heart. AR induces LV dilatation and alterations in ventricular geometry, so it remains to be investigated which method is most accurate to calculate LV volume in these horses. Both methods demonstrated an increase in end-diastolic volume in horses with AR. The Bullet method showed an increase of 62% in horses with severe AR, the area-length method showed an increase of 74%. None of the methods applied was able to accurately differentiate between different degrees of AR severity.

LV dilatation results in a larger radius for the LV wall. Following the law of Laplace, this will result in a higher wall tension.

wall tension =
$$\frac{LV \text{ pressure } * LV \text{ radius}}{2 * LV \text{ wall thickness}}$$

Hypertrophy of the myocardial walls will increase the wall thickness and normalize the wall tension.^{5,7} Results from our study in chapter 3 demonstrate that horses with different degrees of AR severity did not show alterations in LV free wall thickness compared to control horses. With ventricular dilatation, thinning of the myocardial wall would be expected. Since thickness is unaltered, this indicates eccentric hypertrophy of the LV.



Figure 2: Comparison of the Bullet method and the area-length method for calculation of LV volume. A: area; L: length

Similar to what has previously been reported in horses and in humans, the aortic diameter increased significantly in horses with AR.^{2,8-10} This is probably due to the larger SV that stretches the aorta. During diastole, there was a larger diastolic runoff because blood flows forward to the systemic circulation and backwards to the LV. From the long axis image of the LV outflow tract, the aortic diameter in early and late diastole can be measured and the aortic run-off during diastole (Ao_{DiastDecr}) can be calculated (chapter 3). We demonstrated that horses with moderate to severe AR had a significantly higher Ao_{DiastDecr}. Due to the larger diastolic run-off, the end-diastolic pressure in the aorta will be lower and the end-diastolic pressure in the LV will be higher than in healthy horses. At the onset of systole, this will cause the aortic valve to open faster, resulting in a shorter PEP. In chapter 3, the Mmode image of the aortic valve with simultaneous ECG recording was used to measure the PEP and ejection time. A significantly shorter PEP was found in horses with AR. Opening of the aortic valve is a biphasic event.^{11,12} Initial opening of the valve is slow and occurs in the absence of aortic flow. It is hypothesized that this is the result of LV pressure elevation at the onset of systole, which stretches the aortic root. The commissures of the valve leaflets are separated and the valve opens about 8% of its maximum. With increasing LV pressure, blood is expelled and the valve opens rapidly. For this research, PEP was measured from onset QRS to

the second phase rapid opening of the aortic valve, since this indicates the start of ejection. However, due to LV dilatation, increased LV end-diastolic pressure and increased aortic diameter in horses with AR, it is possible that the initial opening of the aortic valve is also affected.

In chapter 4 PEP was measured by tissue Doppler imaging (TDI) as the time between onset QRS and the start of the systolic wall movement. This measures the wall movement as a result of blood flowing into the aorta, therefore results were somewhat larger than when measuring the time of opening of the aortic valve using M-mode. PEP can also be measured by pulsed wave (PW) Doppler as the time from onset QRS to onset of the aortic blood flow. However, good alignment of the ultrasound beam with the aortic flow is sometimes difficult to achieve in horses. When comparing results from different studies, the method of measuring PEP should be considered. Ejection time was unaltered in horses with AR and this probably results from the counteracting effects of increased preload (which would lead to a longer ejection time) and increased afterload (which would lead to a shorter ejection time). Although PEP and AoDiastDecr were not able to discriminate accurately between different stages of AR severity, including both measures in the echocardiographic examination of horses with AR may still be a valuable addition to estimate severity and prognosis as these parameters reflect the hemodynamic impact of AR.

To assess AR severity, most equine cardiologists currently combine quantification of the size of the regurgitant jet and evaluation of several parameters that relate to hemodynamic impact of the regurgitation. The size of the regurgitant jet is assessed subjectively from colour flow Doppler (CFD) images. LV dilatation is evaluated subjectively, LVID is measured throughout the cardiac cycle and FS is calculated. AR severity and hemodynamic consequences are estimated based on these criteria, but this requires operator experience as several parameters are assessed subjectively. Unfortunately, no gold standard is available to classify AR severity in horses. For this research a scoring system was developed to categorize
horses in groups of different degrees of AR (mild – moderate – severe). The scoring system aimed at mimicking and quantifying the subjective evaluation used by experienced cardiologists as much as possible.

All horses were scored for 3 criteria:

- LV dilatation was scored subjectively from the four chamber view and was mainly based on evaluation of apical rounding.
- From the CFD images, the regurgitant jet was scored based on size and area of the jet.
- End-diastolic LVID (M-mode, right parasternal short axis view) was measured at the level of the chordae tendineae and scaled for bodyweight using allometric scaling.

Subjective assessment of LV dilatation was given somewhat more weight in the scoring system because for most equine cardiologists this is an important parameter when evaluating AR severity. A total score for each horse was calculated and used to classify AR as mild, moderate or severe. When scoring the horses, operators were blinded to any previous classifications of the horse. The criteria used are not validated in horses as there is no gold standard and this may have resulted in misclassification of horses. However, it can be assumed that it was a good approximation of AR severity as assessed by an experienced clinician.

Correlation between diastolic LVID and the LV apical dilatation score proved to be poor. Apical dilatation gives the ventricle a very round and large appearance, resulting in a high score for the subjective evaluation of LV dilatation. This may indicate that the equine heart compensates for volume overload rather by dilatation of the apex than by dilatation of the basal parts of the ventricle where LVID is measured at the level of the chordae tendineae. Diastolic LVID might therefore not be the best indicator of AR severity in horses. Since LV dilatation and apical rounding are evaluated subjectively, it might be useful to develop methods to measure apical rounding objectively.

2. QUANTIFICATION OF THE REGURGITANT VOLUME

Currently, in equine cardiology, most parameters used to assess AR severity represent the hemodynamic consequences of the regurgitant jet on the heart. Quantification of the regurgitant volume itself is difficult to perform in horses. In human medicine, echocardiography is the most important non-invasive tool to diagnose and monitor AR.¹³ Images can be recorded from a parasternal, apical, suprasternal of transoesophageal view. Different echocardiographic methods have been developed to estimate the size of the regurgitant volume. Based on colour flow Doppler of the regurgitant jet several (semi-)quantitative methods calculate the size of the regurgitation. The vena contracta is measured as the smallest diameter of the regurgitant jet downstream of the regurgitant orifice. It is an estimate of the effective regurgitant orifice area (EROA). A small vena contracta (< 3 mm) is seen in patients with mild AR, whereas a large vena contracta (> 6 mm) is indicative for severe AR. For the PISA-method (proximal isovelocity surface area), the flow convergence is visualized and the radius is measured from the centre to the first aliasing signal. The EROA and the regurgitant volume can be calculated from the radius and the aliasing velocity. In humans, a regurgitant volume of less than 30 mL is considered mild AR, a regurgitant volume of 60 mL or more is considered severe AR. Using pulsed wave Doppler, the flow from a competent valve (mitral valve or pulmonary valve) can be measured. This allows calculation of the SV from a competent valve. The difference between the SV from the aortic valve and the competent valve provides an estimate of the regurgitant volume. Continuous wave Doppler of the aortic regurgitant flow can be used to measure the rate of deceleration of the regurgitation. The pressure half-time is a measure for the degree of regurgitation.

All these methods require a good alignment of the ultrasound beam with the blood flow. This is often difficult to achieve in horses, since apical and suprasternal and images cannot be recorded. Therefore, in horses it is difficult to accurately estimate the size of the regurgitant volume.

Chapter 8: Discussion

A study in 40 healthy horses demonstrated that CFD echocardiography is a sensitive technique for the assessment of intracardiac flow in horses.¹⁴ The same authors also investigated the use of CFD in horses with cardiac murmurs.¹⁵ Visualization of the regurgitant jet by colour flow Doppler allows evaluation of the size, direction and timing of the regurgitant jet. The size of the jet is assessed relative to the size of the receiving chamber. Absolute measures such as vena contracta width and the PISA method are not feasible in horses. In a study from Blissitt et al. (1995), horses with holodiastolic murmurs had a significantly larger length and size of the regurgitant area compared to control horses that had no pathological murmurs. However, it should be taken into account that several factors influence jet size.¹⁵ Machine settings, including gain of the colour signal and filter settings, have an important impact on the image displayed and the jet dimensions. Jets may also be judged differently according to their direction. Central jets can spread widely in the ventricle and may be overestimated. Eccentric jets sometimes spray close to the ventricular walls and may be underestimated. A subjective assessment of vena contracta width and proximal isovelocity surface area has been described to classify aortic valve regurgitation in horses but no studies validating these methods have been performed.^{16,17} Measurement of pressure half-time to evaluate AR severity is performed by some equine cardiologists, but no studies defining reference values are available.

3. EVALUATION OF MYOCARDIAL FUNCTION

Aortic regurgitation causes important hemodynamic alterations and this will lead to significant alterations in the movement of the myocardial walls with increasing AR severity. However, several mechanisms may have different, sometimes opposing, effects on myocardial walls. This should be taken into account when evaluating wall motion. In early stages of the development of AR, the regurgitation will increase ventricular preload and this will be compensated by increasing contractility of the myocardium. With increasing severity, LV dilatation will become evident. The

dilated ventricle needs less deformation to eject the same SV and this may mask the increased deformation due to increased preload. LV dilatation will also lead to alterations in LV geometry. Longstanding volume and pressure overload may eventually lead to decompensation and heart failure, resulting in a decrease in deformation.

FS and EF are generally considered to be good indicators of myocardial systolic function. For calculation of FS, LV dimensions are measured from short-axis Mmode images of the LV. These are one dimensional measures of the LV and only take into account the radial deformation of the LV. Longitudinal function of the myocardium cannot be assessed from these images. Calculation of EF is done based on LV volumes. These LV volumes are estimated based on measures of LV length and LV area, and thus ventricular deformation in both radial and longitudinal direction are taken into account.

Both FS and EF are indirect indicators of myocardial contractile function and they are influenced by loading conditions. An increased preload stretches the myocardial walls and this will increase contractility of the myocardium to eject the excessive volume. As a consequence, increased preload will result in a higher FS and EF. An increased afterload will hamper emptying of the LV into the aorta and end-systolic dimensions will increase, resulting in a decreased FS and EF. In horses with AR, both preload and afterload are increased. Both effects counteract each other and thus FS and EF will be normal. In equine scientific literature, it is sometimes stated that FS increases in horses with AR.¹⁸ A study by Reef et al. (1987) reported increased FS in 23 horses with AR, but no control group was included in that study and only limited information about AR severity in the horses was presented.² A study by Patteson (1994) reported 4 cases of horses with AR.¹⁹ Only in 3 of them FS was measured: one horse had a normal FS and one horse had an increased FS. The third horse showed a mildly increased FS, but this horse had acute AR due to endocarditis. With acute onset of severe regurgitation, hemodynamic alterations cannot be compared to alterations caused by chronic AR. Our study (chapter 3) did not demonstrate significant alterations in FS and EF between control horses and horses with AR. When the heart starts to fail and is no longer able to compensate for the regurgitation, the LV cannot dilate any further and the myocardium fails to increase contractility. Systolic dimensions of the heart increase rapidly and FS and EF decrease.¹⁰ This research did not include horses that had clinical signs of heart failure. However, in the group of horses with severe AR, FS and EF were lower than in the other groups although the difference was not significant. This may indicate the onset of myocardial failure in the group of horses with severe AR.

For a comprehensive evaluation of the 3D myocardial movement, all three directions (longitudinal, radial and circumferential) should be considered.²⁰ In human medicine, it has been shown that longitudinal function decreases early in the development of AR.⁴ It has been suggested that this may be related to the subendocardial longitudinal fibres being more vulnerable to damage from impinging regurgitant jets. In early stages of the disease, the decreased longitudinal function is compensated by an increase in radial function to preserve a normal ejection fraction.²¹ This may be a functional compensatory mechanism, however, it may also be the result of an altered LV geometry. When the shape of the ventricle changes to a rounder conformation, this will lead to more homogeneous strains. Longitudinal strain decreases and radial strain increases as a result of the altered morphology. When a decrease in radial function is detected, this may indicate the onset of myocardial failure.

Another factor to consider when assessing myocardial deformation is that deformation is not homogeneous over the thickness of the myocardial wall. In horses, dogs and humans, a deformation gradient from epi- to endocardial wall has been demonstrated.²¹⁻²³ During systole, the myocardial wall thickens in radial direction, reducing the ventricular volume. The endocardial part of the myocardial wall shows larger deformation than the epicardial part of the wall.

In horses, evaluation of myocardial wall motion is often done subjectively. A visual wall motion scoring system has been proposed, but this requires operator experience.²⁴ Short axis M-mode images of the LV provide a focal, one-dimensional image of radial myocardial wall motion. Fractional shortening is the most commonly used indicator of systolic myocardial function, but alterations in loading conditions of the LV may mask alterations in myocardial contractility. Since apical images cannot be recorded in horses, longitudinal motion cannot be evaluated with this technique. Tissue Doppler imaging and 2D speckle tracking are echocardiographic techniques that allow quantification of myocardial wall motion. Both techniques measure velocity and deformation of the wall. In human medicine, these techniques have been proven to detect alterations in myocardial wall motion in patients with AR. In chapter 4 (TDI) and chapter 5 (2DST) the ability of these techniques to detect abnormal myocardial wall motion in horses with AR was examined.

3.1 TISSUE DOPPLER IMAGING

Tissue Doppler imaging is an ultrasound technique based on the Doppler effect to measure the myocardial velocity. For accurate measurement of velocities, a good alignment of the ultrasound beam with the myocardial movement is required. TDI has been used extensively in human medicine.²⁵ In patients with AR, TDI was able to detect subclinical LV systolic dysfunction early in the development of the regurgitation, before it could be detected by standard echocardiographic parameters. Evaluation of longitudinal function was more sensitive than radial function.^{4,26} In small animals, TDI was also successfully applied to detect LV dysfunction in different conditions.^{22,27-30} In horses, TDI was validated³¹ and has been used in a number of clinical situations, including atypical myopathy³², after intoxication with lasalocid³³ and after application of clenbuterol.³⁴

In this research, TDI was used to evaluate ventricular myocardial motion in horses with AR (chapter 4). Left and right parasternal short axis images of the LV were

recorded to evaluate radial function. Evaluation of longitudinal function would require apical long axis images which cannot be obtained in adult horses. From short axis images, two myocardial wall segments were analysed at the level of the papillary muscles and two at the level of the chordae tendineae. TDI can be performed in two different modes: colour TDI and PW TDI. In this study, colour TDI was used. The myocardial movement is colour coded and superimposed on grayscale images. During off-line processing of the images, a sample area was placed on the myocardial wall. The size of the sample area should be adapted to the myocardial wall thickness. If the sample area is too big, the risk for inclusion of the epicardium or endocardium increases.³⁵ The resulting velocity and strain (rate) curves represent an average of the sample area. If the sample area is too small, artefacts will influence the results. The sample area was set to track the middle of the myocardial wall throughout the cardiac cycle. From this sample area, the radial velocity can be measured and radial strain and strain rate can be calculated. TDI can also be used in PW mode, with the sample volume located at a fixed distance of the transducer. The myocardial wall moves through this sample area. As a consequence, different parts of the myocardial wall (epicardial, midwall, endocardial) are measured during different phases of the cardiac cycle.

Colour TDI was able to detect alterations in radial function in horses with AR. An increase in radial systolic function became evident from several segments. The effect was most pronounced in horses with moderate AR. In human medicine, an increased radial systolic function to compensate a decreased longitudinal function has been described in patients with AR.²¹ However, using 2DST, no decrease in longitudinal function was found in horses with AR. In the group of severe AR, systolic function was often lower than in horses with moderate AR, although the difference was not significant. This may indicate the onset of decompensation in the horses with severe AR. Further research, with long-term follow-up of horses with AR and a larger number of horses per group may help to clarify this. Diastolic function also showed alterations in horses with AR, although the effect was not significant in all segments. Early diastolic velocity was lower in horses with AR. Even

horses with mild AR were significantly different from control horses and early diastolic velocity did not decrease further with increasing AR severity. This is probably due to the significant difference in age between horses with AR and control horses. In human medicine, it has been demonstrated that early ventricular filling decreases with increasing age as a result of reduced ventricular compliance.³⁶ Additionally, a higher late diastolic function was found in horses with AR, although the difference was not significant in all segments. This is probably also a result of the age difference between groups and is a compensation for the reduced early diastolic ventricular filling. The study in chapter 6 did indeed demonstrate a decrease in early diastolic function and an increase in late diastolic function in old horses. This may confound the results from the horses with AR and thus age should be considered when evaluating horses with AR.

Koenig et al. (2017) used PW TDI to assess LV function in horses with heart disease.³⁷ Twenty-five horses with AR were included in that study. In horses with AR a significantly decreased early diastolic wall velocity was found compared to control horses. This may also be age-related since horses with AR were significantly older than the control horses. When horses with AR were subdivided in groups of trivial-to-mild and moderate-to-severe AR, only the first group showed a significantly lower early diastolic velocity. The increased AR in horses with moderate-to-severe AR may cause an increase in early diastolic velocity, counteracting the effect of increased age. Indices of systolic function also showed some significant differences, but results were inconclusive.

In a recent study by Hopster-Iversen et al. (2014), both colour TDI and PW TDI have been applied in exercise stress echocardiography in horses.¹⁷ Healthy horses and horses with cardiac disease (valve insufficiencies or atrial fibrillation) were compared at rest and immediately after exercise. Compared to control horses, horses with AR had an increased early diastolic myocardial velocity at rest, but not after exercise. No alterations in systolic function were found. Given the small timeframe to collect data in stress echocardiography in horses, colour TDI is

recommended because different myocardial segments can be analysed off-line after collection of the images.

From two studies in healthy horses, PW Doppler appeared to produce more repeatable data.^{38,39} With PW TDI, results are immediately available. Nevertheless, colour TDI was chosen for our research because it provides better tracking of the wall throughout the cardiac cycle. It also allows simultaneous evaluation of different myocardial segments from one image. Additionally, strain and strain rate can be derived from the difference in velocity between two points in the myocardial wall. PW TDI does not allow calculation of strain and strain rate. Colour TDI and PW TDI are different techniques and results should not be used interchangeably.

This research demonstrated that TDI is able to detect alterations in myocardial radial function in horses with compensated AR. Radial systolic function increases, especially in horses with moderate AR. Diastolic function is also altered in horses with AR, however age is probably a confounding factor for these results. Unfortunately, longitudinal function cannot be evaluated by TDI in horses.

3.2 2D speckle tracking

Speckle tracking is another echocardiographic technique for quantification of myocardial function. Kernels of speckles are tracked throughout the cardiac cycle to monitor myocardial movement. With 2DST, speckles can be tracked in two dimensions and thus evaluation of myocardial function in radial, longitudinal and circumferential directions is possible. Both in human medicine and in small animals, the usefulness of 2DST has been demonstrated in a variety of cardiac diseases.⁴⁰⁻⁴² In horses, the feasibility of 2DST has been demonstrated^{43,44} and the technique was used to monitor myocardial function in various circumstances.^{23,34,45,46} In chapter 5, this technique was applied to detect alterations in myocardial motion in horses with different degrees of AR. Both short and long axis images were evaluated. The region of interest (ROI) was traced by manual lining of the endocardial borders of

the LV. The software then automatically divided the wall in segments and tracked the wall movement throughout the cardiac cycle and calculated velocity, strain and strain rate. A large ROI increases the number of speckles that can be tracked, but care should be taken not to include the epicardium in the ROI because this will result in poor tracking quality.

Similar to the results in the TDI study, radial strain increased significantly in horses with AR and the effect was most obvious in horses with moderate AR. In the TDI study, it was hypothesized that the increased radial systolic function in horses with moderate AR was a compensatory mechanism for a decrease in longitudinal systolic function, similar to what has been reported in human medicine.²¹ Surprisingly, no alterations in longitudinal systolic function were observed using 2DST in horses with AR. From our results, it was not clear why longitudinal systolic function was unaltered. AR induces important hemodynamic alterations, resulting in changes in loading conditions for the LV. The heart remodels to compensate for the regurgitation, with an increase in LV dimensions. The complex interaction of hemodynamic alterations and changes in LV geometry may be responsible for the lack of alterations in longitudinal function.

Diastolic function was also evaluated by 2DST. In longitudinal and circumferential direction, a lower early diastolic strain rate was found. This may also be an age-related effect, similar to what was found by TDI. No alterations in late diastolic function were detected. However, speckle tracking is performed at low frame rates (minimum 40 frames per second). This may lead to undersampling of the rapidly moving equine myocardium during diastole. Previous studies demonstrated a high variability in diastolic measurements, so results should be interpreted with caution.^{44,47}

3.3 COMPARISON OF TDI AND 2DST

2DST and colour TDI are both echocardiographic techniques that track the myocardial wall movement. Both techniques use different algorithms to calculate velocity and deformation of the ventricular wall. Therefore, results should not be used interchangeably. Each technique has its own advantages and limitations. Colour TDI measures velocity based on the Doppler principle. Good alignment of the ultrasound beam with the movement of the myocardial wall is mandatory for accurate measurement of velocities. If the insonation angle increases, this will lead to underestimation of myocardial velocities.²⁰ For evaluation of LV wall motion in horses good alignment can only be achieved in short axis images of the LV. In parasternal long axis images of the LV, the longitudinal movement of the wall runs perpendicular to the ultrasound beam. As a result only radial movement of the wall can be assessed by colour TDI in horses. Additionally, TDI measures velocity relative to a fixed point (the transducer). Velocity measurements are therefore affected by total heart motion. In human medicine images are recorded during breath hold to reduce the influence of total heart motion.⁴⁸ Unfortunately, horses are less cooperative and cannot hold their breath on request.

2DST tracks myocardial speckles and calculates the deformation and velocity of the ventricular wall from the frame-to-frame movement of these speckles. In contrast to TDI, 2DST is not influenced by total heart motion because movement of the speckles is measured relative to other speckles. Accurate tracking of the speckles depends on image quality. Poor image quality and artefacts may result in loss of speckles during tracking and this will affect the results.⁴⁹ During data acquisition, care should be taken to assure good quality images and to avoid artefacts. This is not always easy to achieve in horses. Speckles are tracked in two dimensions and this makes 2DST suitable to measure the longitudinal LV function from long axis images in horses. Two-dimensional tracking of the speckles also makes the technique less dependent on insonation angle. However, the technique is not entirely independent of insonation angle and image depth.^{50,51} Two-dimensional

echocardiography is anisotropic and differences in lateral and axial resolution create a certain degree of insonation angle dependence. AR induces LV dilatation and therefore insonation angle for the ventricular wall may be altered in a severely dilated ventricle and this may affect the results.

Frame rate, and consequently the temporal resolution, are also important technical parameters to consider when performing TDI or 2DST. TDI requires high frame rates.²⁰ Horses have large hearts and often the depth of the image needs to be set at 26-30 cm to fit the entire heart on screen. To achieve sufficiently high frame rates for TDI, 2D and TDI sector width need to be reduced and penetration depth set to a minimum. The high frame rate offers the advantage of a good temporal resolution and makes the technique suitable to accurately track the fast moving equine myocardium during diastole. 2DST images require a less high frame rate (50-60 frames per seconds is advised^{20,49}) and thus a wider sector can be used, allowing evaluation of the entire ventricle. In horses with AR and a severely dilated LV, the sector width may need to be increased, which will lower the frame rate. The low frame rate may lead to undersampling of the fast myocardial wall motion in diastole. Therefore, 2DST may be less suitable to accurately measure diastolic function in horses.

When choosing an ultrasound modality to monitor cardiac function, not only differences inherent to the technical aspects of TDI and 2DST should be considered. Practical considerations should also be considered. Both techniques require off-line processing of the images. With TDI, tracking of the myocardial wall is performed manually by the operator and from the resulting velocity and strain rate curves, peak values and timings can be measured. This makes post-processing of TDI images time-consuming. With 2DST, the ROI has to be defined manually once and the software performs automatic tracking throughout the cardiac cycle. Tracking quality is checked by the software and after approval by the operator, peak values and timing intervals are calculated by the software. Post-processing of 2DST images is less time-consuming. However, the software also performs smoothing of the

curves. The applied algorithms are developed for human medicine and may not be suitable for equine cardiology. The software makes assumptions on ventricular geometry, which may be different in horses compared to humans. Moreover, in horses with AR, LV geometry may change with increasing AR severity due to LV dilatation. Additionally, the algorithms are vendor specific and thus results may not be comparable to those acquired by another ultrasound machine or software.^{47,52}

Our studies with TDI (chapter 4) and 2DST (chapter 5) both demonstrated similar alterations in myocardial function in horses with AR. A consistent finding in both studies was that myocardial function showed a remarkable pattern with increasing AR severity. Different parameters were found to increase in horses with mild and moderate AR and decrease in horses with severe AR. The differences were not always significant, but the pattern was found in several myocardial wall segments and with different modalities (velocity, strain and strain rate). These findings may indicate that horses categorised as mild or moderate AR were in the compensated stage of the disease. The decrease in myocardial function in horses with severe AR may indicate the onset of decompensation. Further research with long-term followup of horses is needed to clarify this.

4. INFLUENCE OF AGE AND TRAINING

4.1 THE EFFECT OF AGING ON THE CARDIOVASCULAR SYSTEM

Aging induces alterations in the cardiovascular system. This has been investigated extensively in human medicine and in small animals.^{36,53-55} With increasing age, vascular stiffness increases, resulting in a higher systolic blood pressure and higher afterload for the heart. Myocardial wall stiffness also increases, impairing ventricular relaxation during diastole. Functionally, these structural alterations result in a reduction in early diastolic filling in older individuals. This reduced early diastolic filling is compensated by an increase in late diastolic filling.^{53,54} In horses the effects of aging on the cardiovascular system have not been investigated

extensively. A few studies have demonstrated structural alterations in the myocardial walls and the cardiac valves with increasing age.⁵⁶⁻⁵⁸ With increasing age, an increase in valvular collagen content and a reduction in contractile function of the aortic valve were seen. However, studies evaluating alterations in systolic and diastolic function of the geriatric equine heart were lacking.

For this research we compared horses with different degrees of AR severity to control horses. Since horses usually develop AR at older age, there was a significant difference in age between the control group (mean age of 8 years) and the horses with AR (mean age of 16-18 years). The significant age difference may confound the results and makes it difficult to separate the effects of AR from age-related alterations in cardiac function. Therefore, an additional study (chapter 6) was performed, comparing healthy adult horses, aged 4-10 years, to healthy old horses, aged > 17 years. LV, left atrial and aortic dimensions were measured by B-mode and M-mode echocardiography. Additionally, LV function was evaluated by Mmode, TDI and 2DST. Similar to what has been reported in human medicine and small animals, old horses had a reduction in early diastolic filling and a compensatory increase in late diastolic filling. Both TDI (chapter 4) and 2DST (chapter 5) showed a similar effect in horses with AR. This is probably a result of the confounding effect of age which differed significantly between both groups. Nevertheless, AR itself probably also has an effect on diastolic myocardial wall motion. In human medicine, it has been reported that severe AR itself may contribute to the rapid rise in LV diastolic pressures.⁵⁹ Mitral inflow velocity patterns have been used to evaluate the hemodynamic consequences of AR in human patients.⁶⁰ Mitral inflow, measured by Doppler echocardiography, is a consequence of the pressure difference between left atrium and LV. In patients with severe AR, LV end-diastolic pressure is increased and this is reflected in alterations in mitral inflow pattern. Patients with severe AR showed an increased early diastolic velocity. The high end-diastolic LV pressure impairs left atrial emptying, resulting in a high left atrial volume and pressure at the onset of diastole. When the mitral valve opens, this results in a fast early diastolic filling.

Additionally, the deceleration time of mitral early diastolic velocity was significantly shorter in severe AR, probably due to the rapid increase in LV diastolic pressure. In horses, good alignment of the ultrasound beam with mitral flow is difficult to achieve.

To demonstrate the effect of AR on diastolic wall motion, a study including age matched horses with and without AR should be performed. Since AR is age related, it may be difficult to find a sufficiently large study population.

In chapter 3 it was also demonstrated that horses with AR have a longer ventricle both in systole and in diastole, compared to control horses. However, the length of the ventricle did not increase with increasing AR severity. Horses with mild AR had a significantly longer ventricle than control horses, but it was not significantly different from horses with severe AR (despite a larger regurgitant volume). The effect cannot be attributed to the age difference, old horses even had a significantly shorter ventricle compared to younger horses. The increased ventricular length in horses with AR might be a consequence of the chronic ventricular overloading. However, mild AR only has limited hemodynamic impact and did not cause alterations in other LV dimensions. This raises the question if horses with a long ventricle are predisposed to the development of AR. The longer ventricle may represent an altered LV geometry, which also has an impact on aortic valve conformation. This research did not allow to determine whether the increased LV length was a cause or a consequence of AR.

Another finding from the study in chapter 3 is that aortic size is larger in horses with AR. We hypothesized that this is the result of a larger SV, stretching the aortic root. Comparing old horses to young horses did not reveal a difference in aortic diameter. This is in contradiction to what is found in human medicine, where an increase in diameter is found with increasing age.⁶¹ The old horses involved in the study in chapter 6 had a slightly lower bodyweight than the young horses, which may mask an increased aortic diameter with increasing age. Another possibility is that the study population was too small to demonstrate a difference.

4.2 The effect of training on the equine heart

Both in human and equine medicine, it is well known that training induces cardiovascular alterations. This is known as the "athlete's heart" and affects both the left and right heart.⁶²⁻⁶⁴ In human athletes, it has been described that cardiac morphology depends on the type of sports performed.^{65,66} The Morganroth hypothesis has been broadly accepted in the scientific literature. It hypothesizes that endurance training induces eccentric hypertrophy of the LV to accommodate the increased oxygen demand during longstanding aerobic exercise. Static strength training induces concentric hypertrophy. Recently, this theory has been questioned.^{63,66} Studies investigating the effects of static strength training have reported controversial results. The effects of strength training may not be as straightforward, probably as a result of variability in training stimuli.^{63,66} For endurance training, the hypothesis still stands. Numerous studies have demonstrated an increase in LV diameter and wall thickness with increasing endurance training.⁶⁶ A study in Thoroughbred racehorses competing in different race disciplines revealed a similar effect, as LV dimensions increased with increasing racing distance.⁶⁷ Similar to human athletes, these horses develop a cardiac morphology that is appropriate to the endurance component of the work performed.

In the horse racing industry, horses perform high-level, mainly aerobic work, making them suitable for investigating the cardiac effects of training. However, most Thoroughbreds and Standardbreds start training around the age of 2 years old, and most of them do not remain in active sports beyond the age of 6 years. This makes it difficult to examine the effect of training without the confounding factor of growth. The effects of growth on equine cardiac measures have been poorly investigated.^{68,69} At the age of 6 years horses are considered to be fully grown. Warmblood horses start their training at the age of 3-4 years old, so the confounding effect of growth on cardiac remodelling caused by training will be less pronounced than it is in racehorses. However, most Warmblood horses perform far less strenuous exercise and thus cardiac adaptations are less pronounced than in racehorses.⁷⁰ Arabian horses are bred and trained for long distance racing, which comprises almost exclusively aerobic work. According to the regulations of the Fédération Equestre International (FEI), horses have to be at least 6 years old to participate in long distance races (80 km or more). At this age, no effects of growth on cardiac parameters are to be expected and therefore Arabian horses were selected to investigate training-induced effects for the equine heart. High-level endurance trained Arabian horses were matched for age and bodyweight to untrained Arabian horses. Both groups had a mean age of 11 years. The results from that study confirm that high-level endurance training in Arabian horses induces LV enlargement, both in systole and diastole. Systolic LVID increased by 14% and diastolic LVID increased by 7% in well trained horses. A meta-analysis of studies in human long-distance runners revealed an increase of LVID by 10%.⁷¹ In sled dogs, an increase in diastolic LVID of 4% was reported after 5 months of endurance training.⁷² In that same study, an increase of left atrial diameter by 5% was found in well-trained dogs.⁷² This is probably due to the increased preload. Our study in endurance trained Arabian horses showed an increase in left atrial dimensions compared to control horses, but the difference did not reach significance. A larger number of horses may be required to demonstrate a significant difference.

Speckle tracking also demonstrated alterations in myocardial function in trained endurance horses. During atrial contraction, a lower strain rate in longitudinal, radial and circumferential direction was found in the trained horses compared to the control animals. The mechanism behind this phenomenon is not clear. Late diastolic atrial contraction adds a small volume to the ventricular filling. In a large, dilated ventricle, myocardial wall tension is altered and this may influence the response to atrial contraction. In human medicine, a study involving well trained male athletes demonstrated atrial remodelling compared to untrained individuals.⁷³ The study in chapter 7 did not look at atrial function. Left atrial dimensions were larger in endurance trained horses compared to untrained horses,

but the difference was not significant. More research is needed to elucidate the mechanisms involved.

Aortic regurgitation is more common in highly trained horses.⁷⁴ When evaluating the hemodynamic impact of AR in a sport horse, the level of training should be taken into account. Both training and AR induce LV eccentric hypertrophy. However, AR also has an influence on aortic dimensions (chapter 3). Systolic and diastolic aortic diameter are increased in horses with AR. Due to the regurgitation, Ao_{DiastDecr} is larger in horses with AR compared to control horses. Endurance training did not alter the aortic diameter and Ao_{DiastDecr} was similar for untrained and well trained horses (chapter 7). Additionally, in horses with AR, an increase in systolic radial and circumferential strain was demonstrated by 2DST (chapter 4). Comparing endurance trained horses to untrained horses using 2DST did not show alterations in systolic function (chapter 7). In well trained horses with AR, LV dilatation may not accurately reflect AR severity. Evaluation of other parameters may be necessary to assess the impact of the regurgitation.

5. FUTURE PROSPECTS

This research investigated the differences in cardiac dimensions and function between control horses and horses with different degrees of AR severity. Horses with AR need to be monitored to assess AR severity and to determine if they are safe to ride. In human medicine, well defined recommendations for the echocardiographic assessment of valvular regurgitation are available.¹³ A follow-up study in 558 dogs with mitral valve regurgitation identified clinical and echocardiographic variables that are able to predict a higher risk of death.⁷⁵ Unfortunately, in horses such data are not available. Objective parameters that accurately predict the evolution and outcome of AR are lacking. Currently, a combination of objective and subjective variables, including LV dimensions, Doppler echocardiography, electrocardiography, palpated pulse pressure and noninvasive blood pressure measurement is used to estimate AR severity, but this requires operator experience. In this research different echocardiographic techniques were used to screen for objective variables that quantify AR severity accurately. Significant differences were found in variables representing the hemodynamic impact of AR as well as in measures of myocardial function. The clinical usefulness of these parameters to determine AR severity and prognosis remains to be investigated.

All parameters were evaluated in different stages of AR severity. However, the different stages of AR severity were determined arbitrarily. Because there is no gold standard to determine AR severity in horses, a scoring system based on subjective and objective criteria was developed. Therefore, our results do not allow to conclude which parameters have prognostic value. Consecutive cardiac examinations should be performed to follow the evolution of these parameters during the development of the disease. Relating the results to the clinical outcome would allow to define prognostic parameters and reference values to predict the onset of clinically important cardiac problems. Unfortunately, for most horses in this study no information concerning the clinical outcome was available. Long-term yearly follow-up of horses diagnosed with AR would allow to follow the evolution of hemodynamic alterations and to define cut-off values that predict the onset of myocardial failure. However, such a long-term study is expensive, time consuming and it might be difficult to find a sufficiently large number of horses.

Since AR causes important hemodynamic alterations, with an increase in systolic blood pressure and a decrease in diastolic blood pressure, measurement of blood pressure is a good indicator of AR severity. However in horses, blood pressure measurement is not routinely performed during clinical examination. Non-invasive blood pressure can be performed by placing a cuff over the base of the tail. Invasive blood pressure measurement is performed routinely during general anaesthesia and blood pressure can be monitored throughout the cardiac cycle. However, this is more difficult to achieve in the unsedated standing horse due to

lack of patient cooperation. Comparison of blood pressure measured invasively and non-invasively demonstrated a good correlation between both measures. In standing horses, non-invasive blood pressure measurement had a low accuracy and precision. However, increases and decreases in blood pressure could be detected reliably.⁷⁶ Most equine cardiologists palpate the pulse pressure at the level of the facial artery to estimate the difference between systolic and diastolic pressure. Non-invasive blood pressure measurement could provide a more objective measure of pulse pressure. A pulse pressure of more than 60 mmHg indicates important hemodynamic alterations.¹ More research is needed to determine the usefulness of blood pressure measurement to estimate AR severity in horses and to establish reference values for different degrees of AR severity.

Serum biochemistry may also provide additional information about AR severity. In human medicine, cardiac troponins detect myocardial damage and the prognostic value has been demonstrated in various causes of cardiac injury, including myocarditis, congestive heart failure and septic shock.⁷⁷ The ability of cardiac troponins I and T to detect myocardial damage has been investigated in horses.^{78,79} Van der Vekens et al. (2015) demonstrated that cardiac troponin I is significantly increased in horses with moderate to severe valvular regurgitation.⁷⁸ The same authors also used a high sensitivity test for cardiac troponin T and demonstrated that this test could also detect myocardial damage in horses.⁸⁰ AR causes LV dilatation and this probably results in a release of cardiac troponins, which may be detectable in an early stage of the disease. Determination of cardiac troponins I and T in horses with different degrees of AR severity may result in reference values for the different stages of AR. This might be an additional parameter to estimate AR severity in horses and to monitor the evolution of the disease. Other cardiac biomarkers such as natriuretic peptides might also be useful to detect the onset of heart failure, but these have only been used in research settings.^{81,82}

Newly developed echocardiographic techniques, such as real-time 3D, also called 4D echocardiography, may also be useful to determine AR severity in horses.⁸³

Wide-angle full volume acquisition allows accurate evaluation of the threedimensional cardiac size and function throughout the cardiac cycle (Figure 3). Radial, circumferential and longitudinal LV function can be assessed simultaneously. In human medicine, the technique has been validated against magnetic resonance imaging and produces accurate and reproducible measurements of LV size, volumes and global function. While uni- and bidimensional techniques make geometric assumptions to calculate LV volume, 3D echocardiography does not require geometric assumptions, making it more accurate than 2D echocardiography and M-mode.⁸³ In dogs, the technique has also been validated against magnetic resonance imaging.⁸⁴ LV volumes in systole and in diastole measured by real time 3D echocardiography showed a good correlation with volumes measured by cardiac magnetic resonance imaging. Measurement of LV volumes by 3D echocardiography has not been performed in horses. Since apical images cannot be recorded in horses, automated calculation of the volume may be difficult.



Figure 3: Three-dimensional (3D) echocardiography of the left ventricle of a human heart.⁸⁵ The left panel demonstrates automated volume calculation of the left ventricle (LV) and the left atrium (LA). The right panel displays 3D apical images of the left heart.

3D echocardiography can also be performed in zoom mode to focus on smaller structures. Detailed anatomical information can be obtained and this might be useful to identify degenerative alterations of the aortic valve (Figure 4).



Figure 4: Three dimensional (3D) echocardiographic image of a human aortic valve. PV: pulmonary valve; TV: tricuspid valve; LA: left atrium; R: right coronary cusp; L: left coronary cusp; NC: non-coronary cusp.

In horses with AR, this might contribute to determination of AR severity and estimation of prognosis for the horse. In Figure 5, the long axis 3D image of the LV outflow tract of a horse is displayed. Hallowell et al. (2013) investigated criteria for evaluation of aortic valve prolapse in horses. Seven healthy horses without pathological murmurs were examined by 2D echocardiography to determine if aortic valve prolapse was present. Additionally, 3D echocardiography was performed to confirm the presence of aortic valve prolapse and to identify which valvular cusp was affected. In five horses aortic valve prolapse was confirmed, in all horses the non-coronary cusp was affected.⁸⁶ However, more research on the feasibility and reliability of real-time 3D echocardiography in horses is needed. To achieve a sufficiently high frame rate and spatial resolution, it is recommended to reduce the depth and sector width of the image as much as possible. Compared to humans, horses have large hearts, so it remains to be investigated if an acceptable frame rate can be achieved.



Figure 5: Three-dimensional echocardiographic image of the left ventricular (LV) outflow tract of a healthy horse.

Treatment of horses with AR is another topic that requires further investigation. Currently, most horses with AR are monitored regularly to evaluate disease progression without further treatment. If congestive heart failure develops, clinical signs become evident rapidly and horses are usually euthanized. In human medicine, the defective aortic valve can be repaired or replaced. Different techniques have been described for surgical repair of the valve.⁸⁷ For annular dilatation, circular annuloplasty, commissural plication and valve extension with pericardium have been described. Compared to valve replacement, valve repair offers the advantage that no foreign body is introduced and omits the need for lifelong anti-coagulation therapy. When repair of the valve is not possible, surgical or transcatheter (TAVR) aortic valve replacement can be performed. Surgical valve replacement requires median sternotomy, although less invasive surgical techniques, such as mini sternotomy and mini thoracotomy have been described.⁸⁸ TAVR is a minimally invasive technique. The new valve is inserted through a catheter introduced in the femoral artery and placed over the defective aortic valve.⁸⁸ Replacement of the valve introduces a foreign object in the body and this is not without consequence. Therefore, the optimal timing for surgery should be determined accurately. Onset of clinical symptoms is a clear indication for surgery in patients with AR, because this indicates the development of (irreversible) myocardial dysfunction. However, it has been demonstrated that a better clinical outcome is obtained if the aortic valve is replaced before the patient reaches this stage. Early echocardiographic signs of deterioration of systolic function in asymptomatic patients indicate the need for valve replacement. Valve replacement is indicated in asymptomatic patients with an ejection fraction below 50%. In asymptomatic patients with an ejection fraction larger than 50%, valve replacement should be considered if severe LV dilatation is found (end-diastolic LVID larger than 70 mm or end-systolic LVID larger than 50 mm).⁶ In horses, aortic valve repair or replacement has not yet been performed. Before such surgery can be performed in horses, some practical limitations need to be solved. The heart has to be stopped during surgery, but no equipment to support extracorporeal circulation in horses is available. Serial coupling of human equipment may be a solution, however it needs to be examined if this will provide sufficient flow and pressure for a heavyweight horse. The surgical procedure may also differ from what is done in human medicine, because a lateral approach of the equine heart will be required (due to their conformation) and it may be difficult to reach the aortic valve. TAVR is less invasive and may be feasible in horses. However, sufficiently large prosthetic valves need to be developed and more research is required before this technique can be applied in horses. The results from this research may be useful to determine optimal timing for valve replacement in horses. Both TDI and 2DST demonstrated a significant increase in radial systolic function in horses with moderate AR. In horses with severe AR, radial systolic

function decreased again, but the difference was not always significant. This may indicate the onset of myocardial failure in horses with severe AR before clinical symptoms of heart failure become visible.

In human patients for whom surgery is not recommended due to other cardiac or non-cardiac problems, medical treatment is the only option available. Additionally, in patients with severe regurgitation who do not (yet) meet the criteria for surgery, medical treatment delays the development of symptoms and the need for surgery.^{89,90} Medical treatment should focus on reducing the high LV loading due to AR to slow down the adaptive remodelling processes of the heart. Arterial vasodilators reduce the systemic blood pressure and the afterload, whereas venodilators and diuretics reduce the LV preload. In horses, medical treatment is usually initiated when clinical symptoms become evident, to alleviate symptoms and improve quality of life.⁹ Similar to human medicine, medical treatment in an earlier stage may delay the onset of symptoms and myocardial failure. However, research by Stevens et al. (2009) has demonstrated that horses with left sided heart murmurs have a normal life expectancy and thus the beneficial effect of medical treatment is guestionable.⁹¹ Nevertheless, in some individual cases of very valuable horses, medical treatment may be beneficial. In sport horses, administration of medication makes them unsuitable for competition due to doping regulations. More research is needed to determine if medication has beneficial effects for horses with AR and to define the optimal time to start the treatment.

6. CONCLUSION: HOW TO ASSESS AR SEVERITY IN HORSES.

Aortic regurgitation has an important impact on the cardiovascular system. Close follow-up of horses with AR is paramount to monitor progression of the disease and to assess the risk for sudden cardiac death. Cardiac auscultation, palpation of the pulse pressure and (non-)invasive measurement of the blood pressure contribute to assessing the clinical importance of the regurgitation. Echocardiography is currently the diagnostic tool of choice to examine a horse with AR. Different echocardiographic modalities are available to estimate the size of the regurgitation, the hemodynamic consequences for the heart and the ability of the myocardium to compensate for the regurgitation. Additionally, an electrocardiogram at rest and during exercise should be performed to detect ventricular arrhythmias and determine the risk for sudden cardiac death.

Quantification of the regurgitant volume is the best measure of AR severity, but this cannot be measured accurately in horses. Therefore, assessing AR severity in horses focusses on evaluating the hemodynamic consequences of the regurgitation and monitoring the progression of the disease. This research examined the usefulness of different echocardiographic techniques to determine AR severity and to detect onset of myocardial failure. By 2D and M-mode echocardiography, an increase in LV dimensions was seen in horses with AR. FS and EF were unaltered. This is probably due to altered loading conditions induced by AR. Only in the endstage of the disease, when the myocardium fails to increase contractility, these indices would be expected to fall. As a result of the hemodynamic impact of AR, PEP shortens and Ao_{DiastDecr} increases. These measures might be a valuable addition to the cardiologic exam in horses with AR because they are closely related to the size of the regurgitant volume.

In horses with AR, alterations in myocardial wall movement and deformation were demonstrated by TDI and 2DST. In systole, an increase in radial function was found, especially in horses with moderate AR. This may be a compensatory mechanism for decreased longitudinal function. Surprisingly, 2DST did not demonstrate a decrease in longitudinal function. The reason why longitudinal function is not affected in horses with AR is not clear. Several factors, including alterations in loading conditions and LV geometry, probably have an influence on the myocardial motion. Early diastolic ventricular filling was reduced and this was compensated by an increase in late diastolic filling. This is probably a result of a lower compliance of

the ventricle due to the significant difference in age between horses with AR and control horses.

Age and level of training have an important influence on myocardial function. An additional study comparing young and old healthy horses confirmed the shift in ventricular diastolic filling in older horses. The cardiovascular effects of training were examined by comparing untrained Arabian horses to endurance trained Arabians. Similar to what has been reported in human medicine, an increase in LV dimensions was found. Since age and level of training have an impact on echocardiographic parameters, these factors should be considered when evaluating horses with AR.

This research was intended to screen for variables that might be useful for classifying horses with different degrees of AR severity. To classify horses, a scoring system was developed, based on objective and subjective criteria commonly used by most equine cardiologists. Further research is needed to investigate the clinical usefulness of those variables that were significantly altered in horses with increasing severity of AR. The ultimate goal is to identify variables which can predict the progression of AR in the individual patient. To determine these prognostic parameters a follow-up study is needed in which echocardiographic results should be related to the progression and clinical outcome of the disease.

In conclusion, evaluation of horses with AR should be based on a wide variety of parameters. A thorough clinical examination, serum biochemistry, echocardiography and electrocardiography should be combined to estimate AR severity and clinical impact. Sequential examinations over time could provide more information on the evolution of the disease and may allow to assess prognosis more accurately.

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Summary-Samenvatting

SUMMARY

Aortic regurgitation (AR) is quite common in old horses and in well-trained horses and may have an important hemodynamic impact on the equine heart. The heart will compensate by different mechanisms when the development of regurgitation is slow and progressive. The left ventricle (LV) dilates and a larger stroke volume is generated to compensate for the regurgitant volume. In the compensated stage, no clinical signs of heart failure will become evident. However, the risk for ventricular arrhythmias increases and some horses may be at increased risk for collapse and sudden cardiac death due to ventricular fibrillation. When AR severity exceeds the compensatory capacity of the heart, clinical signs of heart failure become visible. Horses diagnosed with AR should be checked regularly to monitor the evolution of the disease and to determine if they are still safe to ride.

For a good follow-up of these horses, reliable parameters that accurately quantify AR severity are needed. In human medicine, several echocardiographic methods have been developed to measure the size of the regurgitant volume. These methods are more difficult to apply in horses because of their size and conformation. AR severity can also be determined by assessment of the hemodynamic consequences on LV size and function. Different echocardiographic modalities, including B-mode, M-mode, pulsed wave Doppler, tissue Doppler imaging (TDI) and 2D speckle tracking (2DST) can be used to measure the dimensions and function of the left heart. In human medicine, these techniques have been applied to determine AR severity.

In horses, AR severity is usually estimated from a combination of objective and subjective parameters. A more objective, quantitative assessment would allow for a more accurate estimation of prognosis and facilitate the follow-up of these horses. The aim of this thesis was to define objective parameters that can be used to determine AR severity in horses. Horses with mild, moderate or severe AR were compared to control horses by different echocardiographic modalities. A scoring system was developed to divide horses in groups of AR severity. The scoring system aimed at quantifying some of the subjective criteria that are used by experienced equine cardiologists to assess AR severity. Since AR is more common in old horses and in highly trained horses, a second aim of this thesis was to determine the influence of age and training on left heart morphology and function.

In chapter 3, standard 2D, M-mode and pulsed wave Doppler echocardiography were used to compare horses with and without AR. In horses with AR, an increase in LV and aortic dimensions was found. Stroke volume was also significantly increased, mainly due to an increase in diastolic dimensions. Systolic dimensions increased in horses with severe AR. LV free wall thickness was not significantly different between control horses and horses with AR, which indicates the development of eccentric hypertrophy in response to the regurgitation. Remarkably, LV length was significantly larger in horses with AR, but did not increase with increasing AR severity. Results of this research did not allow to determine if AR causes an increase in LV length or if the longer ventricle predisposes to the development of AR in horses. The increase in aortic diameter probably results from the larger stroke volume stretching the aortic root. Due to the backflow of blood to the LV with every heartbeat, the diastolic decrease in aortic diameter was larger in horses with AR. Fractional shortening and ejection fraction are frequently used as indices of LV systolic function. Both parameters did not show alterations in horses with AR and this is probably due to the opposing effects of an increased preload and an increased afterload in horses with AR. The pre-ejection period, measured from aortic M-mode images, was significantly shorter in horses with AR. The higher end-diastolic LV pressure and the lower enddiastolic aortic pressure cause the aortic valve to open faster at the onset of systole.

In chapter 4 colour TDI was used to quantify the motion of the LV free wall and the interventricular septum. TDI measures the velocity and deformation of the myocardial wall, based on the Doppler principle. Horses with AR showed an

Summary - Samenvatting

increase in radial systolic velocity and deformation. Especially horses with moderate AR had a larger systolic velocity of the myocardial wall, whereas horses with severe AR had a lower systolic function. In chapter 5, 2DST was used to demonstrate a difference in myocardial function in horses with and without AR. Speckle tracking is an echocardiographic technique that allows evaluation of the myocardial wall deformation and velocity. The software identifies patterns of speckles and tracks them throughout the cardiac cycle. This technique is less dependent on the insonation angle and allows evaluation of the longitudinal LV function in horses. Results were similar to what was found with TDI, with an increase in radial and circumferential systolic function in horses with AR. Again, it was the group of horses with moderate AR that had the highest values, while horses with severe AR had a somewhat lower deformation. The lower radial systolic function in horses with severe AR may indicate the onset of decompensation. In human medicine, it has been reported that the initial increase in radial systolic function may be a compensatory mechanism for a decreased longitudinal systolic function early in the development of AR. However, evaluation of the longitudinal function by 2DST did not reveal a decrease in longitudinal function in horses with AR. The reason for this is unclear, but it is probably the result of a complex interplay between several factors, including altered loading conditions and LV geometry.

Diastolic function was also significantly different in horses with AR compared to control horses. TDI showed a significantly lower early diastolic radial velocity of the myocardial wall. In late diastole an increased myocardial wall motion was found. However, these results may be confounded by the significant difference in age between control horses and horses with AR. 2DST demonstrated less significant differences in diastolic function. 2DST images are recorded at a relatively low frame rate, which leads to undersampling of the fast diastolic movement of the myocardial walls. As AR is more common in older horses, there was a significant difference in age between horses with AR and control horses. In human medicine it has been demonstrated that age has an important influence on the cardiovascular system. In chapter 6 healthy horses of different ages without valvular regurgitation were examined to determine the effect of increasing age on LV dimensions and function. The LV and left atrial dimensions were somewhat smaller in older horses, but aortic dimensions were not different from the control group. TDI and 2DST demonstrated alterations in myocardial diastolic function in older horses. Similar to what has been reported in human medicine, increasing age caused a decrease in early diastolic myocardial velocity and deformation in horses, probably as a result of increased ventricular stiffness. Late diastolic ventricular filling was increased to compensate for the reduced early diastolic filling. When evaluating diastolic function in horses with AR, age should be taken into account.

Training also has an important impact on the cardiovascular system. In chapter 7 the effect of endurance training on the heart of Arabian horses was studied. A group of well-trained Arabians was compared to a group of untrained Arabians, both groups were matched for age and bodyweight. Results demonstrated that endurance training induces eccentric LV hypertrophy. Horses with AR also develop eccentric hypertrophy. Therefore, when evaluating AR severity, the level of training of the horse should also be considered. Training did not cause alterations in left atrial and aortic dimensions and no alterations in systolic function were found with 2DST. Late diastolic wall deformation was significantly lower in well-trained horses, in longitudinal, radial and circumferential direction.

The final chapter, chapter 8, discusses all findings and the general conclusions. Several parameters measured by standard echocardiographic techniques reflect the hemodynamic impact and can be used to determine AR severity. LV dimensions, aortic diameter, aortic diastolic run-off and pre-ejection period are easy to measure variables that may contribute to the assessment of AR severity. Fractional shortening and ejection fraction are not expected to change due to

altered loading conditions for the LV. TDI and 2DST can track the myocardial movement throughout the cardiac cycle. TDI can measure radial function and the high temporal resolution makes it suitable to measure the fast diastolic movement of the walls. 2DST depends less on insonation angle and allows evaluation of the myocardial function in 3 directions.

Age and training affect the horse's cardiovascular system. Age causes alterations in diastolic function and training induces LV eccentric hypertrophy. Therefore, when evaluating horses with AR, the age and the level of training of the horse should be taken into consideration.

Finally, a few suggestions for future developments in the assessment of AR in horses are discussed. Real time 3D echocardiography is a promising technique which may allow accurate calculation of the ventricular volume and detailed assessment of aortic valve morphology in horses. Treatment of horses with AR is a topic that requires further investigation. More research is needed to determine which treatment may be beneficial for horses and to explore the feasibility of surgical interventions. Echocardiographic parameters that accurately define AR severity might be useful to define the optimal timing for treatment or surgical intervention.

In conclusion, assessment of AR severity in horses should be based on a combination of several parameters. Severity and prognosis of AR can be estimated from results of clinical examination, blood pressure measurement, biochemistry, echocardiography and electrocardiography. Different echocardiographic techniques are able to quantify the hemodynamic impact and alterations in myocardial function induced by AR. Sequential examinations over time are needed to monitor the evolution of the regurgitation and to assess the risk for heart failure or sudden cardiac death.

SAMENVATTING

Regurgitatie van de aortaklep (AR) komt vaak voor bij oudere paarden en bij paarden die intensief getraind worden. AR kan een belangrijke hemodynamische impact hebben. Aanvankelijk zal het hart in staat zijn om te compenseren voor de regurgitatie. Het linker ventrikel (LV) dilateert en het hart pompt een groter slagvolume om te compenseren voor het volume dat terugvloeit bij elke hartslag. Zolang het hart in staat is om voldoende te compenseren, zullen er geen uitwendige symptomen zichtbaar zijn. Het risico op ventriculaire aritmieën neemt echter wel toe, waardoor deze paarden een verhoogd risico hebben voor collaps of zelfs plotse hartdood door ventriculaire fibrillatie. Als de regurgitatie toeneemt, zal het hart uiteindelijk niet meer in staat zijn om te compenseren en zullen symptomen van hartfalen zichtbaar worden. Paarden waarbij AR werd vastgesteld moeten regelmatig gecontroleerd worden om de evolutie van de aandoening te volgen en te beoordelen of ze nog geschikt zijn voor sport of recreatieve doeleinden.

Voor een goede opvolging is er nood aan betrouwbare parameters die de ernst van de regurgitatie accuraat kunnen inschatten. Bij mensen werden verschillende methoden ontwikkeld om de grootte van het terugstromende volume te meten aan de hand van echografie. Praktische problemen als gevolg van hun grootte en conformatie maken deze methoden minder geschikt voor gebruik bij paarden. Daarnaast kan de ernst van AR ook ingeschat worden door na te gaan wat de hemodynamische impact ervan is op de grootte en functie van het LV, en dit opnieuw aan de hand van echocardiografie. Verschillende echocardiografische technieken zoals B-mode, M-mode, pulsed wave Doppler, tissue Doppler imaging (TDI) en 2D speckle tracking (2DST) laten toe om de afmetingen en functie van het linker hart te meten en werden in de humane geneeskunde al gebruikt om de ernst van AR te beoordelen. Bij paarden wordt de ernst van AR meestal beoordeeld op basis van een combinatie van objectieve en subjectieve parameters. Een objectieve, kwantificeerbare beoordeling zou een meer accurate inschatting van de prognose en betere opvolging van de paarden mogelijk kunnen maken.

Het doel van dit doctoraatsonderzoek was om objectieve parameters te definiëren die bruikbaar kunnen zijn bij het bepalen van de ernst van AR bij paarden. Paarden met milde, matige of ernstige AR werden vergeleken met controle paarden aan de hand van verschillende echocardiografische technieken. De paarden werden ingedeeld in de verschillende groepen aan de hand van een zelf opgesteld score systeem. Dat score systeem kwantificeert een aantal subjectieve criteria die door ervaren paardencardiologen gebruikt worden om de ernst van AR te beoordelen. Aangezien AR vaak voorkomt bij oudere en/of intensief getrainde paarden, was een bijkomende doelstelling van dit doctoraatsonderzoek het nagaan van de invloed van zowel leeftijd als training op de morfologie en functie van het linker hart.

In Hoofdstuk 3 werden paarden met en zonder AR onderzocht aan de hand van standaard 2D, M-mode en pulsed wave Doppler echocardiografie. Er werd een toename in grootte van het LV en de aorta gemeten bij paarden met AR. Het slagvolume was ook significant groter bij paarden met AR, aangezien vooral de diastolische metingen van het LV toenamen. De systolische afmetingen namen pas toe bij paarden met ernstige AR. De dikte van de LV vrije wand was niet significant verschillend. Deze resultaten weerspiegelen de ontwikkeling van excentrische LV hypertrofie. Opmerkelijk was dat de LV lengte significant groter was bij paarden met AR, maar dat het LV niet langer werd met toenemende AR. Uit de resultaten van dit onderzoek kon niet worden geconcludeerd of AR een toename in LV lengte veroorzaakt of dat een lang ventrikel predisponeert tot het ontwikkelen van AR bij paarden. De toename in aortadiameter is wellicht het resultaat van het grotere slagvolume dat de aorta oprekt. De diastolische leegloop van de aorta of 'aortic diastolic decrease' was groter bij paarden met AR door de terugvloei van bloed naar het LV. De LV functie werd beoordeeld aan de hand van de fractionele

verkorting en de ejectie fractie, beide parameters worden vaak gebruikt als indicatoren voor de systolische functie van het hart. Deze parameters waren onveranderd bij paarden met AR. Dit is waarschijnlijk het resultaat van de tegengestelde effecten van een toename in preload en een toename in afterload bij paarden met AR.

De pre-ejectie periode werd gemeten op de M-mode beelden van de aortaklep en was significant korter bij paarden met AR. De aortaklep opent sneller bij het begin van de systole door een hogere eind-diastolische druk in het LV gecombineerd met een lagere druk in de aorta.

In Hoofdstuk 4 werd gebruik gemaakt van colour TDI om de beweging van de LV vrije wand en het interventriculair septum te kwantificeren. Met TDI wordt de snelheid van het myocard gemeten, gebaseerd op het Doppler principe. Op basis van die metingen kan de deformatie van de wand berekend worden. Paarden met AR vertoonden een toename van de radiale systolische snelheid en deformatie. Vooral de paarden met matige AR hadden een grotere systolische snelheid van de myocardiale wand, terwijl bij paarden met ernstige AR de systolische functie weer afnam. In Hoofdstuk 5 werd 2DST gebruikt om een verschil in myocardiale functie bij paarden met en zonder AR aan te tonen. Speckle tracking is een echocardiografische techniek die speckles (beeldpunten) in het echografisch beeld volgt en daaruit de deformatie en snelheid van de wand berekent. Deze techniek is minder afhankelijk van de hoek waaronder het beeld wordt opgenomen en laat toe om ook de longitudinale functie van het ventrikel te beoordelen bij paarden. De resultaten waren vergelijkbaar met de resultaten van TDI, met een toename van de radiale en circumferentiële systolische functie bij paarden met AR. Opnieuw was het de groep van paarden met matige AR die de hoogste waarden vertoonde, terwijl de paarden met ernstige AR een iets lagere deformatie hadden. De daling van systolische radiale functie bij paarden met ernstige AR zou een eerste teken van decompensatie kunnen zijn. In de humane geneeskunde werd beschreven dat de initiële toename van de radiale systolische functie een

compensatie kan zijn voor een daling in longitudinale systolische functie vroeg in de ontwikkeling van AR. Evaluatie van de longitudinale functie aan de hand van 2DST toonde geen daling van de longitudinale functie bij paarden met AR. De reden waarom bij paarden met AR geen verandering van de longitudinale systolische functie werd gezien is onduidelijk. Waarschijnlijk is dit het resultaat van een complexe interactie van verschillende factoren.

De diastolische functie vertoonde ook significante verschillen tussen paarden met AR en controle paarden. TDI toonde een significant lagere vroeg diastolische radiale snelheid van de myocardiale wand. In late diastole werd een snellere beweging van de myocardiale wand beweging gemeten bij paarden met AR. Dit zou echter te maken kunnen hebben met het significante verschil in leeftijd tussen paarden met AR en controle paarden. 2DST toonde minder significante verschillen in diastolische functie. 2DST beelden worden opgenomen met een relatief lage frame rate waardoor de snelle diastolische bewegingen van de myocardiale wand moeilijk accuraat kunnen gemeten worden.

Doordat AR vooral voorkomt bij oudere paarden, was er bij de studies in Hoofdstukken 3, 4 en 5 telkens een significant leeftijdsverschil tussen de paarden met AR en de controle paarden. Bij de mens is aangetoond dat leeftijd een belangrijk effect heeft op het cardiovasculaire stelsel. In Hoofdstuk 6 werden gezonde paarden van verschillende leeftijden en zonder kleplekken onderzocht om na te gaan wat het effect is van toenemende leeftijd op LV afmetingen en functie. De LV en linker atriale dimensies waren een beetje kleiner bij oudere paarden terwijl de afmetingen van de aorta niet verschillend waren van de controle groep. TDI en 2DST toonden veranderingen in myocardiale diastolische functie bij oudere paarden. Vergelijkbaar met wat bij mensen werd beschreven, veroorzaakt toenemende leeftijd bij paarden ook een daling van de vroeg-diastolische myocardsnelheid en –deformatie, waarschijnlijk als gevolg van toegenomen stijfheid van het LV. Als compensatie voor de verminderde vroege ventriculaire vulling neemt de laat-diastolische vulling toe. Bij de beoordeling van de diastolische functie bij paarden met AR moet de leeftijd van het dier dus in overweging genomen worden.

Ook training heeft een belangrijke impact op het cardiovasculaire stelsel. In Hoofdstuk 7 werd het effect van endurance training op het hart van Arabische volbloeden bestudeerd. Een groep van goed getrainde Arabieren werd vergeleken met een groep van ongetrainde Arabieren. Beide groepen hadden een vergelijkbare leeftijd en lichaamsgewicht. De resultaten toonden dat endurance training bij paarden excentrische hypertrofie van het LV induceert. Ook paarden met AR vertonen excentrische hypertrofie, dus bij de beoordeling van AR moet rekening gehouden worden met de intensiteit van training. In tegenstelling tot AR veroorzaakt intensieve training geen veranderingen in de linker atrium en aorta dimensies. 2DST toonde geen verschil in systolische functie tussen getrainde en ongetrainde paarden. De laat-diastolische functie was wel significant lager bij goed getrainde paarden, zowel in longitudinale, circumferentiële als radiale richting.

Het laatste hoofdstuk (Hoofdstuk 8) bespreekt de algemene conclusies. Verschillende parameters die gemeten worden met standaard echocardiografische technieken weerspiegelen de hemodynamische impact en zijn een maat voor de ernst van AR. De LV dimensies, aorta diameter, de diastolische leegloop van de aorta en pre-ejectie periode zijn gemakkelijk te meten variabelen die kunnen bijdragen om ernst van AR in te schatten. Evaluatie van de myocardiale functie door fractionele verkorting en ejectie fractie wordt bemoeilijkt door de veranderde ventriculaire belasting als gevolg van AR. TDI en 2DST kunnen de myocardiale beweging volgen tijdens de hele cardiale cyclus. TDI kan worden gebruikt voor het beoordelen van de radiale functie en door zijn hoge temporele resolutie is deze techniek het meest geschikt om de snelle diastolische beweging te meten. 2DST laat toe om de systolische myocardiale functie in 3 richtingen te beoordelen.

In de discussie wordt ook kort besproken wat de effecten van leeftijd en training op het cardiovasculair stelsel zijn en wat hun belang is bij de beoordeling van paarden met AR. Tot slot worden een aantal suggesties voor toekomstige ontwikkelingen in

de beoordeling van AR bij paarden besproken. Real time 3D echocardiografie is een veelbelovende techniek die in de toekomst mogelijk zal toelaten om het ventriculaire volume en de morfologie van de aortaklep bij paarden accurater te bepalen.

Er is weinig informatie beschikbaar over de behandeling van paarden met AR. Er dient verder onderzocht te worden welke medicamenteuze behandelingen de ontwikkeling van AR stabiliseren of afremmen. Ook onderzoek naar de haalbaarheid van chirurgisch herstellen of vervangen van de aortaklep bij paarden kan misschien bijdragen aan een betere behandeling. Echocardiografische parameters die de ernst van AR accuraat kunnen bepalen kunnen bijdragen om het optimale moment van behandeling te bepalen.

Tot besluit kan gesteld worden dat de beoordeling van AR bij paarden best gebaseerd wordt op de evaluatie van meerdere parameters. De ernst en prognose van AR kan ingeschat worden aan de hand van resultaten van het klinisch onderzoek, bloeddrukmeting, biomarkers, echocardiografie en elektrocardiografie. Verschillende echografische technieken zijn in staat om de hemodynamische impact en de veranderingen in myocardiale functie door AR te kwantificeren. Regelmatige controle en opvolging is belangrijk om de evolutie van de regurgitatie te volgen en het risico op hartfalen of plotse hartdood in te schatten.

Curriculum Vitae

Curriculum Vitae

CURRICULUM VITAE

Sofie Ven werd geboren op 25 februari 1978 in Leuven.

Na het vervolledigen van het secundair onderwijs, richting Latijn-Wetenschappen, startte zij in 1996 met de studie industrieel ingenieur aan Groep T Hogeschool in Leuven. In 2000 studeerde zij af als ingenieur, optie elektronica en ICT, met onderscheiding. Meteen daarna ging zij aan de slag bij de dienst Informatiesystemen van de UZ Leuven als software ontwikkelaar voor het Elektronisch Patiënten Dossier.

In 2003 startte zij de studie diergeneeskunde aan de Universiteit Gent. Zes jaar later, in 2009 behaalde zij het diploma van dierenarts (optie paard) met onderscheiding. Na het afstuderen ging zij als assistent aan het werk bij de Dienst Heelkunde en Anesthesie van de grote huisdieren op de faculteit Diergeneeskunde. Aanvankelijk stond zij in voor de gehospitaliseerde patiënten met focus op wondzorg en oftalmologie, later legde zij zich toe op de anesthesie van grote huisdieren.

In 2012 werkte Sofie mee aan de opstart van de ORSI Academy van het OLV Ziekenhuis Aalst, waar zij verantwoordelijk was voor het op punt stellen van de anesthesie van varkens voor de opleiding humane robotchirurgie.

In 2013 begon zij als assistent aan haar doctoraatsonderzoek aan de vakgroep Inwendige Ziekten van de grote huisdieren onder leiding van professor Gunther van Loon, professor Annelies Decloedt en professor Piet Deprez. Naast haar onderzoek was zij ook betrokken bij de dienstverlening en verzorgde practica voor studenten.

Sofie Ven is auteur of mede-auteur van meerdere wetenschappelijke publicaties in internationale tijdschriften. Zij was ook spreker op verschillende congressen en symposia.

Naast haar activiteiten in de academische wereld heeft Sofie ook een praktijk als zelfstandig dierenarts.

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Dankwoord

DANKWOORD

Gedurende de voorbije 6 jaar hebben heel wat mensen mij geholpen en gesteund bij het maken van dit doctoraat. Een woordje van dank is daarom wel op zijn plaats.

Vooreerst wil ik mijn promotoren bedanken om mij de kans te geven om dit doctoraat te maken. Professor Deprez, tijdens mijn studie diergeneeskunde, aan het eind van mijn mondeling examen bij u, zei u dat ik eens moest nadenken over doctoreren. Ik heb toen geantwoord dat ik erover zou nadenken, maar eerlijkheidshalve moet ik bekennen dat ik toen bij mezelf dacht dat ik nooit aan zo'n zware opgave zou beginnen. Na enkele jaren groeide de interesse in wetenschappelijk onderzoek en hier sta ik vandaag dan toch. Bedankt voor het vertrouwen.

Gunther, met het cardioteam hebt u een groep van hele goede onderzoekers, maar vooral heel fijne mensen rond u verzameld en ik ben blij dat ik daar gedurende 6 jaar deel mocht van uitmaken. Bedankt om mij wegwijs te maken in de cardiologie en mij te begeleiden bij het schrijven van dit doctoraat.

Annelies, jij hebt heel wat van de praktische begeleiding op je genomen. Jij hebt mij wegwijs gemaakt in de wereld van tissue Doppler en speckle tracking. Tijdens de vele uren beelden uitmeten sprong je regelmatig eens binnen op mijn bureau om te informeren naar de vorderingen. Als de data er dan eindelijk waren, hielp je mij op weg met de statistiek en discussieerden we over allerlei hypotheses. Samen zijn we ook heel wat paarden gaan scannen, niet altijd in de meest comfortabele omstandigheden, maar de sfeer zat altijd goed ("ik was redelijk chill!"). Dankjewel voor de fijne tijd en de goede begeleiding.

Professor Rademakers, ook u hebt mij actief begeleidt bij het schrijven van dit doctoraat. Ondanks uw drukke agenda vond u telkens tijd om mijn artikels na te lezen en uw opmerkingen te formuleren. Met veel geduld hebt u me de principes van de fysiologie, het contractiepatroon van het hart, strain en strain rate uitgelegd. Uw inbreng heeft mijn doctoraat niet alleen inhoudelijk verbeterd, onze gesprekken waren voor mij telkens weer erg motiverend en vooral ook inspirerend. Bedankt dat u deel wilde uitmaken van mijn begeleidings- en examencommissie.

Ook de andere leden van de examencommissie, Charlotte Sandersen, Stijn Schauvliege, Pascale Smets en Edwin Claerebout, wil ik bedanken om de tijd te nemen mijn doctoraat te lezen en kritisch te evalueren. Voor jullie is het waarschijnlijk het zoveelste doctoraat, voor mij is het míjn doctoraat. Bedankt om hieraan te willen bijdragen.

Het cardioteam! De mensen waar ik het meest mee heb samengewerkt de voorbije 6 jaar. Dominique, Annelies, Lisse, Glenn en Nicky, jullie zijn een topteam en jullie gedrevenheid en enthousiasme werken aanstekelijk. Lisse, jouw positieve ingesteldheid is infectieus. Af en toe sprong je eens binnen met wat aanmoedigingen ('toi toi hé!') en dat deed deugd. Glenn, wat was ik blij dat jij 'het ingenieursproject' van me overnam! Ik wens jullie allebei heel veel succes met jullie doctoraat, maar ik ben er zeker van dat dat helemaal in orde komt. Laat me weten als het zo ver is, ik kom supporteren! Nicky, jij hebt me in het begin wegwijs gemaakt in het wat en hoe van een doctoraat. Maar ook voor een gezellige babbel kon ik altijd bij jou terecht. Dikke merci daarvoor.

Dominique, mijn bureau-maatje! Respect voor hoe jij erin slaagt om alles gecombineerd te krijgen: een gezin, een fulltime job, de nacht- en weekenddiensten, ... Je hebt me veel geleerd over cardiologie, bloedonderzoeken en inwendige ziekten in het algemeen. Verschillende cases waar ik niet uit raakte heb je samen met mij bekeken. Maar ook over allerlei andere onderwerpen hebben we hele fijne gesprekken gehad op ons bureau. Ik ga het missen! Bedankt voor alles!

Alex, mijn andere bureaugenoot en hele fijne collega. Je hebt geen talent voor stilzitten en meestal was je druk bezig in de kliniek. Maar je bent een topdierenarts en ik wens je heel veel succes met het diplomate-examen!

De dienst Inwendige Ziekten is meer dan alleen het cardioteam. Een hele hoop mensen houden de kliniek dag en nacht draaiende. Caroline, Barbara, Joke, Laurence, Lisa, Zoë, Ellen, Kirsten, ... op het einde hadden we wat minder contact, maar vooral in het begin heb ik veel van jullie geleerd! Ook alle interns waarmee ik nachtdiensten draaide, zowel op heelkunde als op inwendige ziekten, wil ik bedanken voor hun inzet en goede zorgen voor de patiënten.

Bart, Bonnie, Lieze, Linde, Laura, Jade, Katharina en Lisa (aka het runderteam), jullie zijn een fijne bende. Bedankt voor jullie steun! Linde, jij bent de volgende aan de beurt: veel succes morgen! Bart, voor jou ook een speciale dankjewel, je weet wel waarom.

En dan zijn er nog een hele hoop medewerkers die ervoor zorgen dat alles in het secretariaat, het labo, de apotheek en de stallen vlotjes verloopt. Hans, Sylvie, Sabrina, Elvin, Saar, Tony, Julien, Balder en Carlos, jullie werk wordt vaak onderschat, maar jullie zijn onmisbaar voor de dienst! Dankjewel voor al die praktische euvels die jullie gedurende al die jaren voor mij hebben verholpen!

Voordat ik aan dit doctoraat begon heb ik enkele jaren op de dienst Heelkunde gewerkt. Ook daar heb ik het plezier gehad te mogen samenwerken met fantastische mensen. Op de hospitalisatie werd ik meteen na afstuderen in het diepe gegooid, maar gelukkig hielp Annelies (VdE) me om te blijven drijven. Annelies, we zien elkaar niet zo vaak meer, maar af en toe lukt het toch om een gaatje te vinden in onze drukke agenda's om samen te gaan eten. Ik hoop dat we dat kunnen blijven doen!

Later kwam ik terecht in het team van anesthesisten: Stijn, Miguel, Barbara, Sanne en Tim. Ik heb heel veel van jullie geleerd en kwam elke dag met plezier naar het werk! Dikke merci voor de fijne samenwerking. Maar ook alle andere collega's op de dienst Heelkunde wil ik graag bedanken voor de fijne samenwerking: Caroline, Michèle, Kelly, Jeroen, Maarten H, Maarten O, Eva, Daphne, Ik vergeet er vast nog een paar: allemaal dankjewel! Om dit boekje er een beetje netjes te laten uitzien heb ik hulp gekregen van enkele mensen. Mia, dankjewel om de lay-out van de tekst te verzorgen, dat heeft mij heel wat uren uitgespaard. Goele, bedankt voor de mooie foto's van Diablo, ze geven dit boekje een persoonlijk tintje! De cover is het ontwerp van Pieter, die ook met veel geduld alle foto's bewerkt heeft tot een bruikbaar formaat.

Gelukkig was er ook nog leven naast het doctoraat. Wie mij een beetje kent, weet dat die tijd vooral gaat naar endurance rijden. De endurance in België is een kleine wereld. Toch is de lijst van vrienden te lang om iedereen apart op te noemen, ik ga me er niet aan wagen, want ik wil niemand vergeten. Velen onder jullie hebben de voorbije jaren hun paard naar de faculteit gebracht om mee te werken aan het onderzoek naar het effect van training op het hart. Een deel van de resultaten staan hier in dit boekje, dankjewel allemaal!

Daarnaast wil ik ook iedereen die heeft bijgedragen om de wandelingen, de trainingsweekendjes en de wedstrijden telkens weer tot een leuke ontspanning te maken hier ook bedanken. Afgelopen jaar was een moeilijk jaar voor de endurance, maar ik ben er zeker van dat we allemaal samen (ruiters, grooms, organisatoren, juryleden, dierenartsen, stewards en vrijwilligers) de endurance volgend jaar weer positief op de kaart kunnen zetten.

En dan is er nog één speciaal 'iemand' die ervoor zorgt dat endurance voor mij telkens weer een feest is. Diablo, mijn maatje, mijn once-in-a-lifetime paard! Samen hebben we al heel wat kilometers afgedraafd en ik had nooit gedacht dat we zo ver zouden geraken. Onvermoeibaar en altijd enthousiast. Merci maatje, voor al die fijne uren op training en op wedstrijd. En misschien wagen we ons toch nog eens aan een CEI***?

Uiteraard hebben mijn ouders ook een grote rol gespeeld om mij te brengen tot waar ik nu sta. Jan en Agnes, heel erg bedankt voor alle kansen die jullie me hebben gegeven! Jullie hebben zelf weinig affiniteit met paarden, toch kreeg ik op mijn 12^{de} een pony (al was dat misschien om van mijn gezeur verlost te zijn?). Dat

was vast niet zo vanzelfsprekend voor jullie: een pony kopen, een stal bouwen, inschrijven bij een ponyclub, Maar daarmee hebben jullie me wel alle kansen gegeven om te komen tot waar ik nu sta: 5 eigen paarden, een diploma dierenarts en nu dit doctoraat. Merci!

Zus, we zien elkaar niet zoveel, maar als we elkaar nodig hebben, dan weten we elkaar altijd te vinden en staan we er wel voor elkaar. Ik kan me geen betere zus wensen!

Pieter, wij maken al meer dan 20 jaar deel uit van elkaars leven. Weinig mensen weten hoeveel jij voor me doet. Niet alleen de talloze praktische zaken, maar zeker ook de morele ondersteuning. Je hebt me altijd gestimuleerd om mijn dromen waar te maken. Telkens als ik weer eens teveel aan mezelf twijfel, geef jij me net dat duwtje dat ik nodig heb om ervoor te gaan. Ik heb heel veel aan jou te danken, maar ik zeg het te weinig. Daarom: een hele dikke, welgemeende dankjewel voor alles!

Tot slot wil ik iedereen bedanken die ik hier vergeet te vermelden en die van ver of dichtbij heeft bijgedragen aan het schrijven van dit doctoraat.

Bedankt allemaal!

Sofie