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A Review of the Current Diagnostic Methods of Aortic Regurgitation in the Horse

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Abstract

Aortic regurgitation (AR) is an important condition in equine medicine. It is considered a clinically relevant cardiac disease in horses, and the prevalence of the disease changes according to the study population, type of sport, and breed. Aortic regurgitation can be acute or chronic, and the diastolic reversal of blood from the aorta into the left ventricle can be longstanding enough to develop congestive heart failure, exercise intolerance, poor athletic performance, dyspnea, ventricular arrhythmias, and sudden cardiac death. The diagnostic methods used in clinical practice are noninvasive pulse pressure measurement, two-dimensional and M-mode echocardiography, and most prominently, color flow, pulse wave, and continuous-wave Doppler echocardiography. Recently, tissue Doppler imaging and two-dimensional speckle tracking have been reported as diagnostic methods for horses with aortic regurgitation. The influence of this disease on athletic performance needs further investigation, mainly because it can have an economic impact on owners of athlete horses, and accurate diagnosis and classification are the cornerstones to understanding the effects of the disease. The diagnosis of aortic regurgitation must be made using a multi-parameter approach, especially if an association between aortic regurgitation and poor athletic performance is suspected. Therefore, this review aimed to integrate and create a compilation of current diagnostic methods for diagnosing and classifying aortic regurgitation in horses.

Keywords: Aortic regurgitation, aortic valve insufficiency, athletic performance, biomarkers, echocardiography, state-of-the-art review

Introduction

Aortic regurgitation (AR) has hemodynamic consequences that are of particular interest in equine medicine. It is considered a relevant cardiac disease in horses, with a prevalence that changes according to the study population, type of sport, and breed (Kriz et al., 2000a; Leroux et al., 2013). The prevalence of audible aortic murmur ranges from 2.2% in a mixed population, detected in up to 8.7% of referral hospitals, and up to 60% in racehorses (Stevens et al., 2009; Young et al., 2008; Zucca et al., 2010). In humans, age and male sex are clinically relevant predisposing factors for AR (Akinseye et al., 2018). Likewise, in horses, sex is a significant risk factor for AR, with stallions being overrepresented compared to geldings and mares (Kriz et al., 2000b; Leroux et al., 2013). It is well known that athletic training can often affect the aortic valve function without an audible murmur (Sessa et al., 2018; Young, 1999; Young & Wood, 2000). Hence, the clinical task is twofold. First, to determine which valvular regurgitation has minimal impact on horses and their quality of life, and second, to determine whether there is a tangible impact on the athletic performance or safety of riders when horses are involved in equestrian sports (Marr, 2019; Reef et al., 2014).

In practice, clinicians need to identify and classify AR, recognizing the consequent cardiac remodeling that creates a risk for horses involved in training or athletic sports (Leroux et al., 2013). Frequently detected in routine clinical examinations in animals older than 10 years (Austin, 2023; Leroux et al., 2013; Reef et al., 2014), AR is commonly mild and perhaps of no clinical importance, with average athletic performance and life expectancy in horses (Stevens et al., 2009; Young et al., 2008). However, other individuals develop severe regurgitation with cardiac remodeling, such as left ventricle (LV) dilation, ventricular arrhythmias, and congestive heart failure (CHF) (Marr, 2019; Ven et al., 2018). The most prominent clinical signs include dyspnea, exercise intolerance, poor athletic performance, ataxia, and sudden cardiac death (Ellis et al., 2023; Marr & Bowen, 2010; Reef et al., 2014). Therefore, this critical information must be consolidated for practical and educational purposes, and this manuscript aims to create a review of the current diagnostic methods of AR available, promoting a multi-parameter approach.

Etiology and Pathophysiology of Aortic Valve Regurgitation

Veterinary practitioners must consider that AR can be detected in healthy individuals during routine echocardiography. This regurgitation is often physiological and does not accompany an audible cardiac murmur (Stevens et al., 2009). Widely accepted, regurgitant jets detected on color flow Doppler (CFD) echocardiography that occupy a small area behind the valve leaflets and are of brief duration are considered clinically insignificant (Blissitt & Bonagura, 1995; Marr & Reef, 1995). In a study evaluating thoroughbred horses using CFD echocardiography, small AR was detected in 80% of horses with physiological regurgitant jets, reported as the most common valve with physiological regurgitation (Young et al., 2008). Hence, it is critical to accurately assess horses with AR despite the absence of clinical signs. The economic impact of the disease on athlete horses and the

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potential safety hazards for riders have made this necessary (Hövener et al., 2021; Reef et al., 2014). For example, 33% of warmblood horses diagnosed with a murmur during a pre-purchase examination were not sold in the study by Verdegaal et al. (2002), and 20% were sold at a lower price. Therefore, it is disastrous for horses with insignificant AR if poorly classified (Keen, 2016).

A fundamental definition of AR is the reversal of blood flow from the aorta to the LV during diastole (Akinseye et al., 2018; Flint et al., 2019). In humans, AR can be caused by primary disease of the aortic valve or abnormalities of the apparatus and structures associated with the aortic root and ascending aorta (Flint et al., 2019). Aortic regurgitation can have an acute onset or develop over time, and both presentations differ in etiology, clinical progress, and consequences. Acquired AR can sometimes be associated with the destruction of valve leaflets or interference with the proper coaptation of valves with vegetation in infectious endocarditis (Fröhlich et al., 2004; Henderson et al., 2020; Maxson & Reef, 1997; Porter et al., 2008). In some horses, aortic aneurysm, ventricular septal defects, or coexisting malformations can be associated with AR, as described by previous authors, who reported clinical and echocardiographic findings in a horse with a quadricuspid aortic valve and ventricular septal defect (Michlik et al., 2014).

Currently, what is known regarding primary valve damage has been reported in a study that observed 1557 equine hearts, concluding that the aortic valve was the most common site of valvular lesions (Else & Holmes, 1972a), with the histopathological characteristics of these lesions described as myxomatous degeneration (Else & Holmes, 1972b). During the same period, Bishop and collaborators used 30 specimens of equine hearts to describe the histopathological features of the valvular lesions (Bishop et al., 1966). The aortic valve is the most common site of valvular pathology, and these are usually nodular lesions or bands with no similar distribution between the valve leaflets. The histopathological interpretation of the valve lesions was predominantly related to different degrees of fibrous thickening with cellular infiltrates of mixed origin, predominantly fibrocytes, and rich in mucopolysaccharides, leading to possible myxomatous valve disease (Bishop et al., 1966; Else & Holmes, 1972b). This degenerative process is mainly due to an increase in the extracellular matrix with glycosaminoglycans and proteoglycans, valvular endothelial, and valvular interstitial cell abnormalities, with a consequent loss of the collagen fibrosa layer and altered expression of matrix metalloproteinases and their inhibitors; however, this condition has been studied in detail in dogs and humans only (Fox, 2012; Richards et al., 2012).

Inflammatory reactions do not play a critical role in myxomatous degeneration of the valve structure, neither in humans or dogs (Aupperle & Disatian, 2012; Fox, 2012; Orton et al., 2012). Heart valves are dynamic structures affected by mechanical and functional behaviors that create biochemical and structural reactions (Aupperle & Disatian, 2012). Indeed, a study proposed the horse as a model for AR in humans, with an in vitro evaluation of the aortic valve contractile response (Bowen et al., 2004). The main findings were associated with valve dependence on different endocrine, paracrine, and neuronal mediators, such as thromboxane, endothelin 1, serotonin, and alpha-adrenoreceptor agonists (Chester, 2005; Chester et al., 2000, 2001). These mediators critically affect valvular cell density and lead to collagen remodeling and elastin loss in mammals. Serotonin and growth factor-beta are principal mediators in the pathogenesis of valvular degeneration (Aupperle & Disatian, 2012). However, the serotonin theory involved in the pathogenesis of valve damage has been applied to humans and dogs. These factors have not yet been elucidated in horses and are probably speculation, despite the evident influence on the valve contractile response (Marr, 2019).

Acute Aortic Regurgitation

Acute AR is frequently caused by acute infectious endocarditis associated with bacterial or fungal infection of the valvular apparatus or endocardium (Henderson et al., 2020; Pierce et al., 2012; Porter et al., 2008). This condition is more common in young animals, and males have been overrepresented in some studies (Porter et al., 2008). Endothelial damage and bacterial colonization are the initial stages; secondarily, a biofilm is built at the same time as possible platelet aggregation starts with more bacterial colonization, and finally, the formation of vegetation attached to the endothelium (Akinosoglou et al., 2013). The most prominent organisms associated with endocarditis are *Pasteurella* spp., *Actinobacillus* spp., *Pseudomonas* spp., and *Rhodococcus equi* (Church et al., 1998; Kelmer & Hayes, 2009; Maxson & Reef, 1997; Porter et al., 2008). Dental diseases, medical procedures, or thrombophlebitis are frequently reported as potential routes of infection (Akinosoglou et al., 2013; Kern et al., 2017).

The acute onset of AR develops a significant regurgitation, which becomes a hemodynamic emergency, causing volume overload in a normal LV that cannot adapt to the sudden increase in volume and pressure from the simultaneous regurgitant jet and the atrial inflow (Marr & Bowen, 2010). The sudden increase in LV end-diastolic pressure (EDP) rises above the left atrial pressure, resulting in the premature closure of the mitral valve in diastole. As the LV develops elevated EDP, it leads to mitral regurgitation and the onset of high pulmonary venous pressure, leading to acute pulmonary edema (Flint et al., 2019). Furthermore, the initial inability of the LV to adapt to increased volume affects the total stroke volume, and a consequent decrease in cardiac output triggers compensatory tachycardia (Ross, 1985). Impairment of coronary flow is also involved in the pathophysiology of acute AR due to elevated LV pressure at the end of diastole and shortened diastole due to tachycardia. Hence, if no intervention is performed, hypotension and cardiogenic shock develop (Flint et al., 2019).

Chronic Aortic Regurgitation

In contrast to acute AR, diastolic regurgitation in the LV over time allows compensatory remodeling of the chamber (Flint et al., 2019). The LV end-diastolic volume increases, and the wall tension increases at the same time as per Laplace's law, which states that wall tension is related to the product of the intraventricular pressure and radius divided by the wall thickness, creating eccentric hypertrophy with chamber dilation (Flint et al., 2019; Marr & Bowen, 2010). This allows LV function to prevail, preventing a sudden increase in EDP (Popović et al., 2018). Consequently, by the time the AR worsens, the LV dilation reaches its dimensional limits, increasing interstitial fibrosis, thereby decreasing LV compliance, creating a rise in the end-systolic volume and EDP with a decline in systolic function and subsequent elevation of the pressure in the left atrium and pulmonary artery wedge pressure, finally developing CHF (Rigolin & Bonow, 2006).

Clinical Diagnostic Methods

Auscultation

During clinical examination, AR is auscultated with a characteristic decrescendo holodiastolic murmur, often musical and louder over the aortic valve region on the left side or in both hemithoraces (Marr, 2019; Stevens et al., 2009). The accuracy of auscultation has been widely discussed in the literature, and various studies have arrived at different conclusions regarding the impact and accuracy of auscultation findings (Marr & Bowen, 2010). For example, a study described the accuracy of detecting the cardiac pathology underlying the murmur as highly dependent on the examiner's experience. The results showed that veterinary internists came to the correct diagnosis based on auscultation alone in 53% of all cases, general equine practitioners came to the correct conclusion in 33% of cases, and students in 29% (Naylor et al., 2001). In other studies, the specificity of auscultation for the precise diagnosis of regurgitation was described as up to 100% (Young & Wood, 2000). Other studies also found a significant association between a murmur over the mitral, aortic, or tricuspid area and regurgitations detectable in CFD echocardiography in the same valve (Kriz et al., 2000b).

In a study by Hövener et al. (2021) with a population of warmblood horses examined with auscultation and echocardiography, the agreement between auscultated murmur and CFD regurgitation jet was 94% if one murmur and one regurgitation were present. Individually, the agreement between auscultation and echocardiography is 81% for AR (Hövener et al., 2021). However, a confounding factor was added once two murmurs were auscultated in the same horse, and the agreement dropped to 76% in these cases. There is a belief that veterinary cardiologists have not yet elucidate if cardiac dimensions and pathological remodeling of the heart can modify murmur intensity over time. It also occurs with the aortic valve and left-sided diastolic murmur because no direct correlation exists between the grade of enlargement and murmur intensity (Gehlen et al., 2003). Therefore, considering these factors, definitive diagnosis and hemodynamic evaluation are recommended when an incidental aortic diastolic murmur is present (Hövener et al., 2021).

Non-invasive Pulse Pressure Measurement

When AR is severe, there is a decrease in diastolic arterial pressure (DAP) as a result of the diastolic recoil of blood from the aorta into the LV through the insufficient valve and an increase in systolic arterial pressure (SAP) due to increased preload and activation of cardiac compensatory mechanisms (Marr & Bowen, 2010). The difference between SAP and DAP is the pulse pressure (PP). In clinical situations, one can appreciate the increased amplitude of PP by palpating a hyperkinetic pulse, and some authors consider this a clinical marker of AR severity (Asmar et al., 2001; Marr & Bowen, 2010). The evaluation of pulse pressure through noninvasive blood pressure (NIBP) is an objective measurement of PP (Asmar et al., 2001; Blacher et al., 2000). Boegli et al. evaluated NIBP in horses with AR and mitral regurgitation and compared them with those of healthy horses. Horses with AR according to echocardiographic severity were assigned to a scoring system and classified as mild, moderate, or severe AR, respectively (Boegli et al., 2019).

The results of NIBP showed significantly decreased DAP and increased PP in the group of horses with AR compared to healthy individuals. In addition, when a correlation was performed between subjective pulse quality and severity score according to echocardiographic results, a hyperkinetic pulse was significantly associated with severe AR. However, it is not superior to the evaluation of PP using NIBP, considering the practical limitations of using these devices in horses. The authors concluded that PP correctly classified and detected the severity of AR in horses. Hence, the cutoff set that PP <38 mm Hg most probably indicates a healthy horse, and PP >61 mm Hg highly indicates AR (Boegli et al., 2019).

This parameter is of clinical significance because monitoring disease progression is a determinant for identifying unsafe horses for riders at risk of collapse or sudden cardiac death (Reef et al., 2014; Young, 2003; Young et al., 2008). Indeed, PP monitoring over time helps to detect the progression of AR and, together with abnormal pulse quality and frequent ventricular premature depolarization as a part of the clinical characteristics associated with the onset of exercise intolerance or risk of death (Marr, 2016; Reef et al., 2014; Ven et al., 2016). Nonetheless, as a predictor for the progression of the disease, the sensitivity of PP is low and still in dispute if helpful in classifying horses at different stages of the disease. Although PP is specific for recognizing severe AR, it is evident that it is not very sensitive to differentiate horses with mild to moderate AR from those with severe AR (Boegli et al., 2019).

Two-dimensional and M-mode Echocardiography

Echocardiography is still the recommended method for assessing the severity and clinical relevance of AR in horses despite some limitations in diagnosis (Vitale et al., 2023). It also helps identify abnormalities in valve morphology, such as cusp deformation or coaptation defects (Flint et al., 2019; Reef et al., 2004). Findings such as valve thickening with a fibrous band-like lesion parallel to the free edge of the left coronary leaflet and prolapse into the outflow tract are commonly reported with the non-coronary cusp often involved. However, these conditions are the beginning of valvular disease in dogs and humans, and aortic valvular prolapse is a risk factor for valvular disease in horses (Hallowell & Bowen, 2013). In addition, diastolic fluttering or vibrations affecting the aortic valve leaflets are frequently observed in long-axis parasternal views when there is a mild-to-moderate eccentric AR jet directed toward the mitral valve leaflet (Reef et al., 2014). In some situations, diastolic fluttering of the aortic valve prevents complete opening of the anterior mitral valve leaflet. In fact, this phenomenon can be recognized as premature closing of the mitral valve; in some situations, it is considered a sign of increased LV EDP and is part of the criteria to consider severe AR, especially if there is LV dilation (Marr & Bowen, 2010; Reef et al., 2014; Reef & Spencer, 1987).

The evaluation of surrounding structures such as the aortic root and ascending aorta is essential, considering the presence of significant aneurysms or dilation, which can cause an increase in regurgitation volume and may occur with long-standing moderate-to-severe AR (Marr & Bowen, 2010). In a study performed by Ven et al., evident enlargement in the left atrium and LV was due to volume overload related to advanced ventricular enlargement, volume overload, or concurrent mitral regurgitation. The latter is apparently caused by AR-induced dilation of the LV and mitral annulus (Ven et al., 2016). In this study, the time of events was also evaluated, and a short pre-ejection period (PEP) and small pre-ejection period/ejection time ratio (PEP/ET ratio) were reported in horses with moderate to severe AR (Ven et al., 2016). Pre-ejection period and ET are essential

measures of systolic function in horses with AR, with the typical finding being a shorter PEP due to decreased aortic EDP and increased LV EDP. On the other hand, ET is commonly not different between horses with different AR severities. However, the interpretation of these parameters cannot be isolated, and precautions should be taken considering these parameters are volume overload and heart rate dependent (Ven et al., 2016).

The most commonly used echocardiographic parameters to evaluate a horse's heart condition are fractional shortening and ejection fraction, which are essential for recognizing systolic heart function (Koenig et al., 2017). However, these parameters are not helpful in the compensated stage of AR, where systolic function is frequently preserved (Decloedt et al., 2020; Ven et al., 2016), providing less value in the pre-clinical stages. Previous studies have confirmed that no significant differences in fractional shortening and ejection fraction are evident between the different stages of AR (Ven et al., 2016). Indeed, the preload in AR causes an increase in these parameters. The AR is easily confirmed during routine echocardiography, and the severity is commonly evaluated using the regurgitant jet flow size. However, the size of the jet is a measure that is not repeatable or reproducible. Ven and collaborators proposed a scoring system that included LV diameter and subjective LV appearance, combining subjective and objective assessments of LV size, shape, and regurgitant jet size and bullet-method-derived stroke volume (Ven et al., 2016).

Color Flow Doppler

The AR is visualized in the parasternal long-axis and short-axis views of the horse (Flint et al., 2019). Color jet area or jet width can be measured from these views. Considering the limitations and pitfalls of this mode, using proper gain settings and Nyquist limits to avoid overestimation or underestimation of AR severity by CFD is needed to perform an accurate interpretation (Flint et al., 2019). The limitations of CFD in assessing AR become more evident in the presence of eccentric jets, diffuse jets, and jets originating along the entire coaptation line (Zoghbi et al., 2017). In many situations, AR severity is based on the jet size in the left ventricle outflow tract (LVOT), which is often performed using visual estimation rather than direct quantitative measurement. In humans, the vena contracta (VC) width is a semiquantitative parameter derived from CFD for evaluating AR severity (Flint et al., 2019). This parameter is practical because it is easy to measure, considering the narrowest portion of the AR jet just before it expands into the LV during diastole.

The VC estimates the effective regurgitant orifice (ERO), considering a circular regurgitant orifice. In humans, a VC wider than 6 mm is considered a severe ERO (Tribouilloy et al., 2000); however, this parameter is still not widely implemented in horses, and its utility in classifying the severity of AR is still unclear (Keen, 2016; Marr & Bowen, 2010). In contrast, the proximal isovelocity surface area (PISA) method allows the quantification of AR by evaluating the zone of flow convergence. Proximal isovelocity surface area is obtained in the parasternal long-axis view, assuming a hemispherical shape of proximal flow convergence, and using the conservation of mass principle, the effective regurgitant volume (RVol) and ERO can be calculated (Tribouilloy et al., 1998). The use of PISA is reliable in humans with AR (Flint et al., 2019). Currently, no data exist on horses, and its utility in classifying horses with AR at the time of this publication is unknown (Keen, 2016).

Pulse Wave and Continuous Wave Doppler

Pulse wave (PW) Doppler and continuous wave (CW) Doppler are helpful for AR classification using subjective and objective approaches (Ven et al., 2016). Subjective analysis of the Doppler signal can provide beneficial information; for example, a dense Doppler signal represents more regurgitant red blood cells and, thus, larger regurgitant volumes. In contrast, a less dense signal suggests only mild regurgitation (Mcconachie et al., 2013; Stadler et al., 1994). The pressure half-time (PHT) method is also helpful for objectively evaluating the severity of AR (Flint et al., 2019). After the AR jet is interrogated with CW Doppler, the PHT identifies the rate of velocity deceleration, which is directly correlated with the rate of diastolic pressure equalization between the aorta and LV. The more severe the AR, the faster the equilibration of the aortic and LV diastolic pressures (Flint et al., 2019). A PHT of <200 ms is associated with a rapid rise in LV diastolic pressure, most commonly associated with a large regurgitant volume, and supports a diagnosis of severe AR in humans (Flint et al., 2019; Teague et al., 1986). However, this parameter lacks a foundation in equine cardiology, and no current investigation is available testing its clinical utility in this species, sensitivity, specificity, or reliability, and a reference range is not currently available (Marr, 2019).

Pressure half-time values should be interpreted cautiously, as they are dependent on numerous technical and physiologic factors such as whether AR is acute or chronic, AR jet characteristics such as eccentric jets or central jets, the size and positioning of the sampling signal, angle of alignment to the blood flow, diastolic blood pressure, and compliance of the LV (Flint et al., 2019). Pulse wave Doppler allows evaluation of the aortic arch or descending aorta, and a brief diastolic flow is expected; however, holodiastolic flow reversal is commonly associated with more advanced AR in humans (Flint et al., 2019). This approach has limitations in horses because of anatomical reasons and limitations of ultrasound. In human cardiology, the retrograde regurgitant volume through the aortic valve is quantified,using a continuity equation with a spectral Doppler signal (Flint et al., 2019). This method assumes that the forward flow through the aortic valve equals that through the other valves. This approach is commonly used for mitral valve regurgitant volumen measurement in humans mitral valve disease (Ross, 1985). Thus, the regurgitant volume equals the LVOT flow minus mitral flow. Assuming that both the mitral and aortic annuli are circular, and no significant mitral regurgitation is present. A regurgitant volume of >60 mL in humans is considered a severe AR (Teague et al., 1986).

Tissue Doppler Imaging

This ultrasound modality objectively quantifies LV myocardial wall motion and deformation (Decloedt et al., 2013a). Tissue Doppler imaging-derived measurements are more sensitive for detecting subtle myocardial dysfunction (Schwarzwald et al., 2009). Strain imaging measures the deformation of the myocardial wall and is less affected by the total heart motion and tethering artifacts (Koenig et al., 2017). A study by Ven et al. used tissue Doppler imaging (TDI) in an attempt to recognize abnormal LV radial function in horses with AR compared with healthy horses. In this study, the wall velocity and strain were different in horses affected by AR (Ven et al., 2018). Indeed, Chronic AR causes LV diastolic enlargement, and in the compensated stage, the end-systolic dimensions increase; hence, the myocardial wall causes a more significant

displacement during ejection, resulting in increased stroke volume and wall velocity, especially in moderate-to-severe AR (Koenig et al., 2017; Ven et al., 2018).

Other parameters were inconclusive, and the effect of age was involved in some parameters, with an unclear difference between the healthy and AR groups. Another limitation is related to the inability to obtain left parasternal longitudinal views in horses and to measure the LV longitudinal function in horses, which can only be evaluated with speckle-tracking, but not with TDI (Koenig et al., 2017). In conclusion, radial strain increases in mild to moderate AR; however, in horses with severe AR, the radial strain is lower, indicating the onset of myocardial failure in this advanced stage (Ven et al., 2018). Other studies have suggested that the use of TDI may be limited in AR because of a lack of consistent changes in the variables in the different severity groups (Koenig et al., 2017). Nevertheless, TDI is operator-dependent and not widely available, not to mention the limitations related to equine thorax anatomy and standard views that are difficult to obtain in different breeds and sizes of horses compared to humans and dogs.

Two-dimensional (2D) Speckle Tracking (2DST) Echocardiography

This technique tracks speckles in the myocardial wall and helps to evaluate radial, circumferential, and longitudinal left ventricle wall deformation, showing good reproducibility and reliability in horses (Decloedt et al., 2011, 2013b). Decloedt et al. (Decloedt et al., 2020) investigated 2DST in horses with AR at different stages of severity. For those with moderate AR, the radial and circumferential strains were higher, and in horses with severe AR, the same values did not change compared to the healthy control group. A surprising result was that longitudinal wall deformation was associated with LV longitudinal function (Decloedt et al., 2020). This parameter is essential in humans, with AR as an indicator of subtle myocardial dysfunction (Ewe et al., 2015; Olsen et al., 2011). However, no significant differences in longitudinal systolic strain and strain rate were evident between control horses and horses with AR. This is because a high stroke volume in the compensated stage falsely increased LV longitudinal function, counteracting the impaired ventricle longitudinal function due to AR in the affected horses, generating a confusing effect (Decloedt et al., 2020). Hence, 2DST is not recommended for determining subtle dysfunction in horses with AR.

Circumferential strain was higher in horses with compensated AR associated with volume overload, which is considered a hyperdynamic motion of the interventricular septum and left ventricle-free wall (Decloedt et al., 2020). Horses with severe AR did not show increased strain values compared with the control groups, indicating the onset of systolic dysfunction (Decloedt et al., 2020). In addition, it is unclear whether the commercial setting of the software is a limitation for its use in horses because the algorithm is designed to analyze images obtained from human patients. In particular, apical images and anatomical orientations differ from equine heart anatomy. Hence, further investigation is required to validate these results and confirm their use in clinical practice (Decloedt et al., 2020; Schwarzwald et al., 2009).

Cardiac Biomarkers

Atrial natriuretic peptide (ANP) concentrations in horses with heart disease were previously evaluated in Warmblood horses in a study by (Gehlen et al., 2007), including horses with AR. In this study, there was no correlation between left atrial size and plasma levels of ANP in horses with valvular regurgitation, in contrast to humans, where ANP concentrations correlated with left atrial diameter and pulmonary capillary wedge pressure (Lang et al., 1995). However, horses with advanced heart disease and evidence of congestive heart failure may have higher plasma ANP concentration; the results in the mentioned study can be justified because the AR in the horses admitted were not severe at the moment of evaluation (Gehlen et al., 2007). In a recent study, there was a positive correlation between ANP, brain natriuretic peptide (BNP), and endothelin 1 and ventricular parameters associated with abnormal dimensions in horses with different types of cardiac diseases, especially those that cause abnormal enlargement of the ventricles (Chaleshtori et al., 2023). Nevertheless, the study included horses with various manifestations of heart valve disease and not specific diseases. Thus, these biomarkers can be helpful in the diagnosis of congestive heart failure and advanced disease states, and possibly in the follow-up of horses with confirmed cardiac disease (Chaleshtori et al., 2023).

N-terminal pro-brain natriuretic peptide (NT-proBNP) is the most widespread biomarker in human and small-animal cardiology (Harr et al., 2022). Critical, especially for differentiating dyspnea of unknown origin before echocardiographic evaluation is available. In a recent study evaluating NT-proBNP concentration in healthy horses and horses with LV dilation, values were significantly different, independent of the etiology, and it was considered an important diagnostic biomarker in the follow-up of horses with structural disease (Demeyere et al., 2023). However, it is necessary to establish cutoff values to estimate the importance of prognosis, and the study was not specific for any disease in particular. Therefore, regarding AR only can be stated that due to the cardiac remodeling associated with long-standing diastolic regurgitation, LV dilation plays an important role in the evolution of the disease and NT-proBNP can be of help in the follow-up of the progression of the disease. However, further investigation is needed in a larger group of horses (Demeyere et al., 2023).

Troponin-C, -I, and -T are components of the tropomyosin-troponin complex associated with actin filaments in the skeletal muscles of the heart and striated muscle (Apple, 1999; Sidebotham & le Grice, 2007). cTnI and cTnT have different N-terminal amino acid sequences. Hence, the capacity to differentiate between cardiac and skeletal muscle troponin isoforms is important (O'Brien et al., 1998). A small fraction of cardiac cTnI isoforms is free in the cardiac myocyte cytosol, and damage to the myocardium results in an early increase in serum concentrations of cTnI that can persist for several days as a result of additional release from degenerating myofilaments (van der Vekens et al., 2015).

The immunoassay commonly used is a commercially available ELISA kit for humans because there is still a lack of purified equine cTnI, and cTnI appears to be highly similar among mammalian species (Schwarzwald et al., 2003). To date, no studies have reported cTnI immunoassays as diagnostic aids in the different stages of AR. Only one report of a horse with rupture of the septal wall of the LV associated with an endocardial jet lesion and tract formation into the ventricular septum was published using sequential measurements of the cTnI using a human cTnI immunoassay (Cornelisse et al., 2000). cTnI can be increased in the bloodstream of horses with severe AR with LV

enlargement or if the AR jet is severe enough to create lesions in the endocardium. However, this hypothesis still needs to be tested, and cannot be confirmed at the time this review was published.

Athletic Performance, Standard Exercise Testing, and AR

Poor performance is commonly described as a reduced ability to perform physical tasks that should be within the capabilities of a healthy individual with similar characteristics and training, less-than-expected performance, or reduced performance (Hodgson et al., 2014). The etiology of AR, abnormalities of the leaflets, the size and volume of the regurgitant jet, irradiation into the chamber, degree of remodeling of the LV, and severity of volume overload are critical aspects to understand if AR has a real impact on athletic performance (Zucca et al., 2010). Several studies have attempted to analyze the implications of valvular regurgitation and its effect on racing performance in clinically normal horses (Buhl et al., 2005b; Fraipont et al., 2011; Kriz et al., 2000b; Young et al., 2008). However, the shared conclusion was that a direct association between murmur or regurgitations and performance evaluated considering racing time, money won, percentage of victories, or race type-specific time for rating could not be established appropriately (Zucca et al., 2010). The main reason for not establishing an appropriate conclusion was the logistic and technical difficulties out from the authors' control. No definitive studies have proven that intensive sports participation could worsen the progression of AR in horses, and limited information on this topic in athletic horses is available. However, once moderate to severe AR is found, athlete horse owners are usually discouraged from competition participation owing to the risk of riders and cardiac events (Zucca et al., 2010).

Regarding AR and athletic performance in humans, some patients with preserved ventricular function can have good functional capacity and even reach high levels of sporting performance (Stern et al., 2014). Currently, no conclusive information exists about the worsening of AR and intensive sports, and limited data exist on human athletes with AR (Cavarretta et al., 2020). During exercise, the severity of AR diminishes, this hypothesis in humans is related to a shorter diastolic time with a consequent decrease in the time of regurgitant jet and less regurgitant volume (Popović et al., 2018). Hence, the study by Forteza Albertí et al. (2017) showed that the diastolic regurgitation fraction becomes smaller with exercise in asymptomatic humans with AR. However, no information is available on horses with these characteristics, and the possibility that this occurs in this species is hypothetical.

In general, poor performance related to cardiovascular disease in horses is often difficult to prove because of the lack of consistent objective criteria on which to reach a diagnosis (Lightowler et al., 2004). Multimodality imaging cannot be used alone in the evaluation of horses with AR that are training or practicing a specific sport; instead, it is recommended to perform a complete functional exam and stress test to reveal the presence of cardiac events during effort (Cavarretta et al., 2020; Ellis et al., 2023). Standard exercise tests with endoscopy, telemetric echocardiography, and Holter electrocardiography can provide information about cardiopulmonary and metabolic functions (Reef et al., 2014). A treadmill is an excellent tool for analyzing these animals during a standard exercise test, evaluating different parameters that can be altered, which may predispose them to exercise intolerance and poor performance (Buhl et al., 2005a).

The intensity of a standard exercise test should exceed that of a horse's normal activities or training. Although not standardized, the use of drugs may be necessary for sympathetic stimulation to identify inappropriate HR, aberrant conduction, or ectopy associated with adrenergic stimulation (Reef et al., 2014). Evaluation of exercise tolerance, peak oxygen consumption, and peak exercise oxygen pulse are promising parameters that need to be standardized in horses, especially in asymptomatic horses without evident cardiac remodeling (Forteza Albertí et al., 2017). What is widely analyzed during exercise testing is the effect of exercise on auscultation, heart rate, rhythm and murmur severity, peak HR during the procedure, and rhythm during the different phases of the test, including during the recovery time and stress echocardiography before and after exercise, focusing on systolic function, presence or worsening of murmurs and diastolic and volumetry of the LV (Buhl & Ersbøll, 2006; Reef et al., 2014). In addition, dynamic airway endoscopy, arterial blood gas tension, and basic laboratory tests are performed (Marr & Bowen, 2010). Clinically significant structural lesions, intermittent premature complexes, or isolated atrial fibrillation can be assessed during exercise testing to determine if the HR is appropriate for the work performed or if arrhythmia worsens during exercise (Sandersen et al., 2006). For safety reasons, patients with CHF should not perform exercise testing, as well as horses with severe valvular regurgitation with secondary atrial fibrillation, pulmonary hypertension, or impaired systolic function with ventricular arrhythmias (Reef et al., 2014).

Conclusion and Recommendations

Aortic regurgitation is an important disease in equine medicine, with a relatively high prevalence among all types of horses practicing different sports, especially after a certain age. The impact of the disease in athletes and elderly horses requires more information and accurate diagnosis and classification. Therefore, a consensus statement and an update of the recommendations are needed to standardize the classification of AR. Initially, different authors stated that auscultation is the cornerstone in identifying horses with AR that require further evaluation. Considering the confounding factors, such as changes in intensity in the murmur after chamber remodeling, auscultation alone is not recommended for further evaluation, and to monitor the progression of the disease, it is possible that in some horses, the severity of the murmur does not correlate with the severity of the real regurgitation. In contrast, PP is a sensitive parameter for identifying horses with advanced AR, considering that PP >60 mm Hg suggests that disease progression is likely. However, classifying the severity of regurgitation as PP is not recommended and should be interpreted cautiously in conjunction with other results.

Echocardiography is the principal method used to identify the etiology or underlying mechanism of AR and to classify AR in horses. Diastolic fluttering of the aortic valve, premature closure of the mitral valve, and post-valvular dilation of the aortic root are subjective features to be considered in the criteria to identify the severity of AR. In contrast, the LV dimensions and volumes are the most critical objective parameters in this disease because they can help to guide the therapy and are the most representative cardiac remodeling after long-standing diastolic regurgitation and volume overload caused by AR. The main objective parameters to classify the severity of AR may be the left ventricle indexed diameter and the left ventricle volumes derived from the Simpson's method of discs. A small PEP/ET

ratio is also an important parameter but needs further investigation to confirm its utility to be considered in the AR and heart rate influence on this parameter. To the authors' knowledge, the sphericity index is not reported in this disease, but is a measurable method that future investigations can explore instead of the conventional subjective evaluation of the LV and apex appearance.

Doppler signal analysis is practical when subjectively interpreted. Initially, the color jet area and width can be helpful but require experience and proper settings. The limitations associated with different types of jets make it difficult to standardize the results, mainly because it is a visual measure, inter- and intra-observer variability is high, and ultrasound settings influence the results. However, the PHT as the rate of deceleration of the AR can be an objective method to classify the severity of AR that in horses has not been well studied using spectral Doppler. Hence, reference intervals need to be created for horses, and measurement and interpretation are performed based on empirical results and assumptions transferred from human cardiology. Proximal isovelocity surface area is another non-novel method that requires investigation in horses, and its use is not recommended to classify the severity of AR at this time due to the lack of clear information. The continuity equation is commonly used in mitral valve regurgitation, but its utility can be implemented in AR and is an easy method to quantify the regurgitant volume that can be useful in analyzing the progression of the disease or increased regurgitation over time.

The limitations of TDI have been reported. Its use can be limited because there are no consistent changes across different AR severity stages, and the results can be confusing if interpreted alone. The same happened with 2DST considering that in some parameters, no significant changes exist between affected horses and healthy groups, and it is currently used when the disease is in an advanced stage but not in the pre-clinical stage. Cardiac biomarkers are of limited use or are at least only relevant if CHF is present. Considering the recent novel microRNA or proteomics, AR does not have a report of possible miRNA expression patterns. The authors consider that this is an unexplored trail that will expand the knowledge of AR in the upcoming years, and proteomics can have an exciting impact on athletic performance studies.

Finally, the diagnosis of AR must be made using a multiparameter approach, and no isolated information is considered conclusive. Besides, the severity in one patient with AR can be classified as moderate or severe according to the approach used, and standardization is needed. As stated, athletic performance and AR still need a conclusive answer, and a multi-parameter interpretation should aid in confirming the actual association between AR and poor athletic performance. A systematic review and meta-analysis can help us resolve this clinical question.

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