Diagnosis and Treatment of Atrial Arrhythmias in Horses

Diagnose en behandeling van atriale ritmestoornissen bij het paard

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Table of Abbreviations

95% CI = 95% confidence interval of the population estimate

2LL = 2 log likelihood

AERP = atrial effective refractory period

AF = atrial fibrillation

AFCL = atrial fibrillation cycle length

AFCL4CH = atrial fibrillation cycle length measured in the left atrial free wall from the four chamber view

AFCLRA = atrial fibrillation cycle length from the right atrial dorsal wall at the level of the tuberculum intervenosum in a right parasternal view

AFCLLLA = atrial fibrillation cycle length measured in the left atrial free wall from the left parasternal long-axis view

AIC = Akaike information criterion

APD = atrial premature depolarization

APD90 = monophasic action potential at 90% repolarization

AUC = area under the curve

AVB = atrioventricular block

AVB2 = second degree atrioventricular block

BIC = Bayesian information criterion

Bid = two times daily

BPM = beats per minute

BW = body weight

Bwt = body weight

Cl = clearance

Cmax = maximal plasma concentration

Cp_{ss} = plasma concentration at steady state

Cp_{ss} av = average plasma concentration at steady state

Cp_{ss} max = maximal plasma concentration at steady state

Cp_{ss} min = minimal plasma concentration at steady state

CV = coefficient of variation

ECG = electrocardiogram

Emax = maximal response achievable

ERP = effective refractory period

F = oral bioavailability

FFT = fast Fourier transform

FOCE-ELS = first-order conditional estimation with extended least squares

FS = fractional shortening

HF = high frequency band

HR = heart rate

HRM = heart rate monitor

HRV = heart rate variability

IV = intravenous

Ikr = rapid component of the delayed rectifier potassium current

LC = liquid chromatographic

LF = low frequency band

LOD = limit of detection

LOQ = limit of quantification

LS = Lomb-Scargle perdiodogram

LVET = left ventricular ejection time

LVPEP = left ventricular pre-ejection period

LVIDd = left ventricular internal diameter at end-diastole

LVIDs = left ventricular internal diameter at end-systole

k_a = absorption rate constant

$k_{el\alpha}$ = distribution rate constant

$k_{el\beta}$ = elimination rate constant

MAP = monophasic action potential

MS/MS = mass spectrometric system

NIBP = non-invasive blood pressure

PCL = pacing cycle length

PK = pharmacokinetic

PO = per os

OR = odds ratio

QTc = corrected QT interval

RA = right atrium

RMSSD = root mean squared successive differences in RR intervals

ROC = receiver operating characteristics curve

RV = right ventricle

SA node = sinoatrial node

SD = standard deviation

SD1 = standard deviation of the Poincaré plot of the RR intervals perpendicular to its line of identity

SD2 = standard deviation of the Poincaré plot of the RR intervals along its line of identity

SDNN = standard deviation of the RR intervals

SR = sinus rhythm

SVPD = supraventricular premature depolarization

$T\ 1/2_a$ = absorption half-life

$T1/2_{el\alpha}$ = distribution half-life

$T1/2_{el\beta}$ = elimination half-life

TI = triangular index

Tid = three times daily

TINN = triangular interpolation of the RR interval histogram

Tmax = time to maximal plasma concentration

TVEC = transvenous electrical cardioversion

$Tv\theta$ = population typical value of the fixed effect parameter

UPLC = ultra-performance liquid chromatography

V_c = volume of distribution of the central compartement

V_p = volume of distribution of the peripheral compartement

VERP = ventricular effective refractory period

VLF = very low frequency band

VPD = ventricular premature depolarization

VT = ventricular tachycardia

ω = variance of the interindividual variability

GENERAL INTRODUCTION

Arrhythmias, both physiological and pathological, are fairly common in horses. Atrial ectopic beats are often harmless, but predispose to the development of atrial fibrillation (AF), a condition with an important impact on athletic performance that may occasionally lead to collapse or rarely even sudden death during strenuous exercise. Horses with AF can usually be converted into normal sinus rhythm (SR) by means of pharmacological or electrical cardioversion but relapse may occur. A rapid diagnosis of AF is important in order to maximize treatment success and minimize rider risk and risk for relapse. In some horses, post-conversion, long-term pharmacologic treatment may be indicated in order to suppress premature depolarizations and prevent recurrence of AF while reverse remodeling takes place. The first part of this PhD research aims to improve diagnosis of supraventricular arrhythmias, with a special focus on atrial premature depolarizations (APD) and AF. In a second part of this PhD thesis the pharmacokinetics and electrophysiological effects of the antiarrhythmic drug sotalol are explored in order to use this drug in horses with atrial arrhythmias.

Atrial Arrhythmia and Atrial Fibrillation: Origin and Clinical Importance

Atrial Premature Depolarizations

Atrial premature depolarizations (APDs) are caused by abnormal impulse formation in the atrial myocardium, outside of the sinoatrial node. They can be associated with underlying external causes such as autonomic imbalance, hypokalemia, certain drugs (catecholamines, anesthetics), infections, fever, anemia, hypoxia and colic. Numerous APDs, however, can be indicative of cardiac abnormalities, such as myocardial disease, atrial enlargement due to atrioventricular valvular disease, endocarditis, or congenital heart disease [1]. APDs can be found in healthy horses with normal performance and may be clinically insignificant [2]. Clinical symptoms, mostly poor performance, are more likely when APDs are frequent at rest, associated with runs of atrial tachycardia or structural heart disease [3]. However, the greatest concern of APDs lies in their potential to incite atrial tachycardia, atrial flutter and AF [4].

Atrial Tachycardia

Atrial tachycardia is defined as 4 or more consecutive APDs and may be either sustained or nonsustained (paroxysmal). Atrial tachycardia in horses is uncommon and if it occurs, underlying structural or myocardial disease should be suspected. If atrial tachycardia occurs unrelated to AF treatment, it may be clinically insignificant or may lead to a loss in performance, but the most important clinical concern of atrial tachycardia is the potential to induce AF. At high sustained rates, the differentiation with atrial flutter may be difficult or impossible.

Atrial Flutter

Atrial flutter is relatively rare in horses. Most often it is found during pharmacological treatment of AF with quinidine sulphate as a transitional rhythm between AF and SR [5]. Atrial flutter represents a form of atrial circuit movement or macro-re-entry [4]. Re-entry is caused by abnormal impulse propagation in a region with different electrophysiological properties, such as different refractoriness and conduction velocity. It occurs when an impulse is conducted through cells with a short refractory period but is blocked by cells with a longer refractory period. If such an impulse is conducted over a path of excitable tissue until it can loop back to its origin and re-excite the initial area, a re-entry loop is formed and the depolarization wave

can continue to turn around in the atrium and act as a continuous source of electrical activity. The size or path length of the loop is determined by the length of the depolarization wave and the amount of excitable tissue in between the 'head' and 'tail' of the depolarization wave, the so called excitable gap [3] (Fig.1). During atrial flutter a single re-entry loop continuously turns around over a fixed pathway in the atrium. The clinical circumstances, importance and assessment of atrial flutter are comparable to those of atrial tachycardia and differentiation between atrial flutter and high rate, sustained atrial tachycardia may be difficult or even impossible. Atrial flutter is believed to be easier to treat compared to atrial fibrillation.
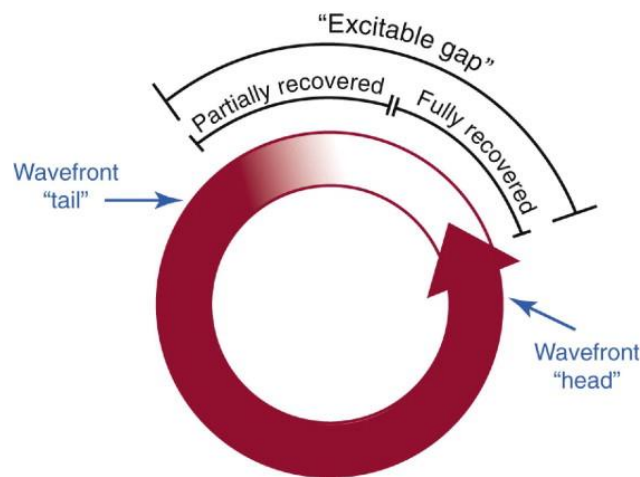
Figure 1: Schematic drawing of a re-entry loop. After passing of a depolarization wavefront (arrow head), the myocardial tissue is brought in a refractory state for a time equal to the refractory period (red). After that, the tissue gradually recovers until it has regained full excitability (white). A re-entry loop can only propagate as long as a small area of excitable tissue (excitable gap) precedes the depolarization wave front [3].

Atrial Fibrillation

AF is the most common clinically important dysrhythmia in horses with a prevalence ranging between 0.3% and 2.5% of the population [6,7]. Horses are predisposed to the development of AF compared to other species because of the large size of the atria and because high vagal tone results in a relative inhomogeneity of refractoriness within the atrial tissue. Large horses, such as draft horses, warmbloods and Standardbreds are predisposed and a genetic heritability has been identified in Standardbred horses [6,7]. AF can be acute or chronic and it can be paroxysmal (short period of AF, disappears spontaneously, usually within 24-48 hours), persistent (terminates only after treatment) or permanent (resistant to therapy). AF often develops in the absence of underlying heart disease – in this case it is termed lone AF – but can also be a consequence of atrial enlargement or congestive heart failure due to congenital

or acquired cardiac disease. Electrolyte derangements, especially potassium depletion, which may occur due to excessive sweating during exercise or administration of furosemide, are also thought to predispose for AF development [1].

The anatomical and pathophysiological basis for the development of equine AF is not completely understood but different theories have been proposed, including the presence of ectopic foci, multiple re-entry waves as a continuous source of electrical activity, the leading circle concept and the presence of rotors and spiral waves [8-14]. A first theory explaining the initiation of AF suggests the presence of a rapidly firing focus in the atrium. The high frequency of these impulses prevents the organized conduction over the atria, because of differences in the refractoriness of the myocardial tissues, leading to a disorganized, fibrillatory atrial activity. In human patients it has been shown that ectopic activity usually originates from parts of atrial myocardial tissue that extend into the wall of the pulmonary veins, the myocardial sleeves [15]. A second important theory regarding the maintenance of AF after initiation is the multiple re-entry wavelet hypothesis [14]. According to this theory, several small re-entrant circuits randomly turn around over the atria. Wavelets may break up at anatomical obstacles or may die out due to collision. If a critical number, typically 5 or 6 in a human heart, of these re-entry loops circle chaotically through the atria, AF becomes self-sustained [3]. The more wavelets coexist in the atria, the more stable AF becomes. Recently, in human medicine, there has been increasing interest in spiral waves or rotors, which are specific, organized forms of functional re-entry that may be important in order to sustain AF[14]. Finally, AF itself induces atrial electrical, structural and functional remodeling which are also important factors in the progressive, self-perpetuating and recurrent nature of AF [16,17]. Since, in human patients, atrial changes may become irreversible as time progresses, prompt diagnosis of AF is important to maximize treatment outcome and minimize risk for recurrence.

Clinical signs of AF depend on the degree of underlying heart disease and the level of exercise expected from the horse. Lone AF in low-performance horses is usually an incidental finding without overt clinical signs, but when (sub-) maximal performance is required, exercise intolerance and poor performance are observed [4,18]. In horses with lone AF, cardiac output at rest is usually not affected. During exercise, however, when the atrial contraction is an important contributor to the ventricular filling, maximal cardiac output is limited. Furthermore, horses with AF can reach extremely high heart rates, often exceeding 300 beats per minute (BPM) at maximal exercise. Exercise, especially in combination with stress, may

lead to R-on-T phenomenon and may occasionally be associated with collapse or rarely even lead to sudden death [4,19,20]. Other symptoms that have been described with AF are epistaxis, acute pulmonary edema, respiratory distress, incoordination and prolonged recovery after exercise.

Atrial Dysrhythmias and Atrial Fibrillation: Diagnosis

Auscultation

APDs can be detected during auscultation as premature beats interrupting an otherwise regular underlying rhythm and may or may not be conducted to the ventricles. On auscultation, APDs are usually preceded by a short diastolic interval and are followed by an either normal or prolonged diastolic interval. However, interlaced beats may not be detected on auscultation.

Both atrial tachycardia and atrial flutter usually lead to an irregularly irregular heart rhythm at rest. They are often indistinguishable on auscultation and may mimic AF. Despite the rapid and regular atrial rate, the ventricular response rate is often irregular due to variable physiological blocking at the level of the atrioventricular node. At increasing heart rates, however, 4:1, 3:1, 2:1 or 1:1 atrioventricular conduction may yield a regular heart rate [3].

Auscultation of horses with AF is typically characterized by an irregularly irregular heart rhythm with frequent long pauses and early beats. The rhythm remains irregular at increased heart rates. The first heart sound (S1) is usually loud while the fourth heart sound (S4) is absent. Horses with lone AF usually have normal resting heart rates, but during increased sympathetic tone, e.g. due to exercise, stress or pain, heart rates increase excessively. During strenuous exercise rates of 300 BPM and more are no exceptions in otherwise healthy horses. Loud cardiac murmurs or signs consistent with congestive heart failure are indicative of an underlying, probably predisposing, heart disease. Increased resting heart rates (>50 BPM) may indicate underlying heart disease, but heart rates of 50-60 BPM can be present in horses with no underlying cardiac abnormalities.

Although auscultation is a valuable diagnostic tool and may allow to make a probability diagnosis, some abnormalities sound alike or are even indistinguishable on auscultation. Confirmation with an electrocardiogram (ECG) is always necessary.

Electrocardiography

Technique

Electrocardiography is the technique of recording cardiac electrical activity over a period of time using electrodes placed on the skin. It is the golden standard method for diagnosing cardiac arrhythmias. The normal conduction process follows a rather fixed pathway through the heart starting from the sinus node through the atrial myocardium, over the atrioventricular node and via the His and Purkinje fibers towards the ventricular myocardium (Fig.2). As the electrical impulse spreads through the heart, the ECG shows successively a P wave (atrial depolarization), a QRS complex (ventricular depolarization) and a T wave (ventricular repolarization).
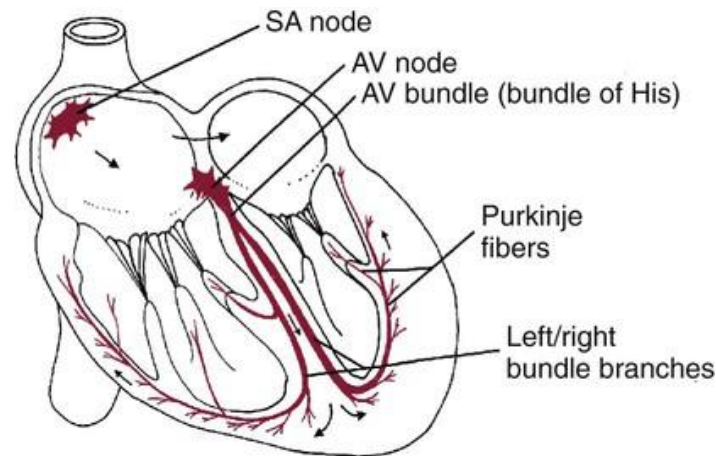
Figure 2: Conduction pathway in the equine heart (SA: sino-atrial; AV: atrioventricular).

Equipment

Performing and interpreting an equine ECG requires specialized equipment and a certain amount of expertise. The basic equipment needed for an ECG are electrodes, a recording device (Fig.3) and a software program to display the ECG. Specific equine self-adhesive electrodes are available containing more contact gel and stronger glue than those used in human medicine in order to improve skin contact and to better remain in place (Fig.3). Using a small, battery-powered ECG recording device allows to attach the device on the horses back and allows for long-term recordings or recordings during exercise (Fig.4).
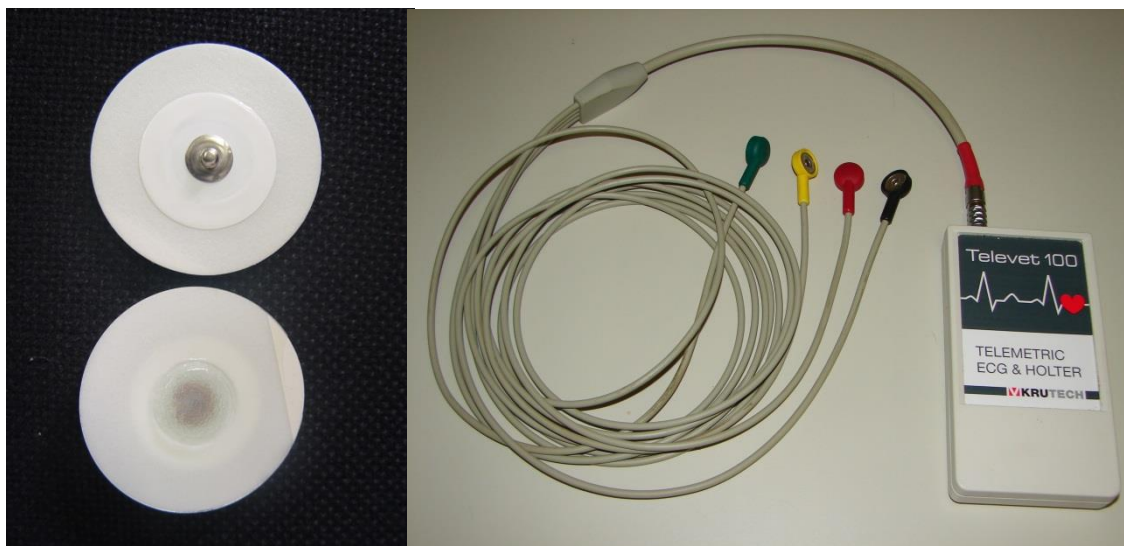
Figure 3: The basic equipment needed to record an equine ECG: electrodes (left) and a recording device (right). Afterwards the recordings have to be imported and analyzed in a special software program.

Subsequently, the recorded ECG needs to be imported into computer software for automatic or manual ECG trace analysis. Manual analysis requires expertise and may be time-consuming. Automatic analysis by the ECG software is very often prone to error (more in horses compared to small animals or human patients), especially for recordings during exercise.

Figure 4: ECG recording equipment for lunging (left) and ridden (right) exercise. The electrodes are protected by a girth during lunging and can be placed under or before the saddle during ridden exercise.

Electrode Configurations

Several electrode configurations have been described in horses. Usually electrodes are positioned along the mean electrical axis, which is directed from the apex of the heart towards the base and slightly cranial and to the right. This means that one electrode should be placed on the ventral part of the thorax near the cardiac apex and a second electrode should be positioned more dorsal, towards the cardiac base somewhere in the region between the lower neck and the withers. This base-apex configuration is often used in equine medicine. However, no universal method for recording an ECG in horses is available. A number of semi-orthogonal lead systems has been evaluated experimentally in horses but are rarely used in clinical practice and cannot be applied during exercise [21,22]. A modified Einthoven's lead system is applicable for ECG studies in resting horses and several modified base-apex leads have been described for continuous, ambulatory ECG monitoring or exercise ECG in horses [22-27]. Recently, a new 12-lead ECG method for horses was described, but the technique still needs validation in clinical cases [28]. Since the electrode position influences morphology, amplitude and duration of the different ECG leads, the positioning of the electrodes must be taken into account and lead-specific reference values should be used [24,29]. A combination of multiple leads can be displayed in ECG software programs. Each lead measures the cardiac depolarization from a different angle, so minor electrical changes can sometimes be identified more easily in one lead compared to the other. Therefore using multiple lead recordings is advised.

In horses, ECG is important for assessing heart rate and rhythm. The fact that ECG vector analysis is not applicable in horses, makes that the exact electrode positioning is of limited importance, as long as the electrodes are positioned along the mean electrical axis of the heart. The most commonly used electrode configuration is (a variation on) a classical base-apex configuration. In horses, on a base-apex configuration, the P wave is often bifid and the Q wave is usually absent, leading to a QRS complex with an rS morphology. The T-wave morphology is variable and can be positive, negative or biphasic (Fig.5). At increased heart rates, the T wave is usually positive, opposite to the QRS complex.

Figure 5: Normal ECG of a horse in a base-apex electrode configuration.

Of special interest for the diagnosis of APDs is a good visualization of the P wave. Most configurations focus on registering ventricular activity and large QRS complexes. This may result in suboptimal electrode positioning for P wave registration, since the atrial activation has a more cranial to caudal direction compared to the ventricular electrical axis. In addition, an increase in heart rate often results in a P wave that is buried in the preceding QRST, so P wave morphology is difficult or even impossible to assess during exercise. Compared to a base-apex configuration, a significantly higher P wave amplitude has been described with the negative electrode on the right side of the withers and the positive electrode 15 cm behind and above the left olecranon, as this position is more along the electrical axis of the atrium [29].

Diagnosis of Atrial Arrhythmias

<u>Atrial Premature Depolarizations</u>

APDs are usually characterized by a premature P wave with normal or abnormal morphology that may or may not be followed by a normal QRS-T complex and is followed by a non-compensatory pause (Fig.6).

Generally, the electrical activity of the APD will enter the sinus node and reset its timing, leading to a non-compensatory pause. This differentiates APDs from ventricular premature depolarizations (VPDs). When the ventricle discharges prematurely, the sinus node is not affected and will continue firing at its regular time intervals. If the next sinus complex after the VPD finds the atrioventricular nodal tissue still refractory (and thus not conducting), this results in a compensatory pause. However, in some cases the electrical activity of an atrial premature complex probably does not enter the sinus node due to refractoriness of the sinus node or due to electrical block to enter the sinus node (as in sinus nodal disease) and the sinus

node will not be reset. In these cases, a compensatory pause will be present and differentiation with a VPD may be hampered.

Whether an APD is conducted through the atrioventricular node, depends on its refractoriness. If the atrioventricular nodal tissue is not refractory, the APD will be conducted to the ventricle. Since APDs arise from the atrial myocardium, ventricular conduction is generally not affected and QRS complex and T wave morphology remain unchanged [1,4,30,31]. This differentiates APDs from VPDs. The latter are not related to a preceding P wave and usually have a QRS complex with abnormal morphology, due to abnormal intraventricular conduction. However, for some APDs the premature P wave is buried in the preceding T wave or QRS complex and thus difficult to identify. In addition, premature atrial impulses can also be conducted aberrantly through the ventricle as a result of incomplete repolarization or persistent refractoriness of ventricular conducting tissues. This may slightly alter the QRS-T complex and can make differentiation with premature depolarizations from a ventricular origin challenging.

Figure 6: ECG of an APD (arrow). Note the normal QRS morphology and the non-compensatory pause after the APD.

Atrial Tachycardia and Atrial Flutter

During sustained atrial tachycardia rapid but often regular atrial activity may lead to regular P waves especially at a rate between about 120 and 300 BPM [3] (Fig.7). In between P waves iso-electric lines are often present. Atrial flutter, on the other hand, is also characterized by a very rapid, abnormal, but regular atrial activity that is often visible as a saw-toothed ECG baseline (Fig.8). Each undulation represents a macro-reentry rotation of the depolarization wave, usually at an atrial rate between about 170 and 300 BPM [4]. Isoelectric lines between P waves may also be present during atrial flutter, hampering the differentiation between atrial tachycardia and atrial flutter. Atrioventricular conduction in both arrhythmias is usually variable, resulting in a ventricular rate response that is usually irregular at rest but often becomes regular during periods of increased sympathetic tone. Patterns of 4:1, 3:1, 2:1, or 1:1

atrial-to-ventricular conduction can be observed [4]. QRS morphology is usually not affected, except in cases of incomplete ventricular repolarization. Depending on the atrial rate, the origin and the conduction pathway, atrial tachycardia and atrial flutter may lead to the same changes on the surface ECG. Exercising the horse usually leads to slightly higher atrial rates probably due to the influence of sympathetic tone on impulse formation and/or impulse conduction. If strenuous exercise leads to a markedly increased atrial rate, the underlying cause is supposed to be atrial tachycardia instead of atrial flutter as the sinus node impulses are unlikely to entrain the reentry circuit. However, especially when the atrial rate is high, distinguishing correctly between both arrhythmias may be impossible from the surface ECG and other electrophysiological techniques, such as multiple intracardiac electrocardiograms or 3D electroanatomical cardiac mapping may be necessary [32,33].

Figure 7: Base-apex ECG of a horse with atrial tachycardia at an atrial rate of 148 per minute.

Figure 8: Base-apex ECG of a horse with atrial flutter. The two vertical green lines indicate the flutter cycle length which is the time (ms) between two flutter waves. The first part of the ECG (dotted arrow) shows a 2:1 atrioventricular conduction, while conduction is more irregular in the remaining part.

Atrial Fibrillation

The ECG of horses with AF is characterized by the absence of P waves; instead, rapid baseline fibrillation "f" waves are present (Fig.9). These f waves may be large or small and the number of atrial impulses per minute is difficult to count but often exceeds 300 BPM. Ventricular response rate is irregular, with irregularly irregular RR intervals and normal QRS

morphology. Abnormal QRS complexes, either originating from the ventricle or aberrantly conducted, are present in 10-60% of horses with AF during exercise [1,20]. In otherwise healthy horses in AF at rest, vagal tone is usually high and the ventricular rate will be close to normal or slightly increased. Ventricular response rate increases with increased sympathetic tone (exercise, stress, pain), decreased vagal activity (as with certain antiarrhythmic drugs) or underlying heart disease.

Figure 9: ECG of a horse with AF. Note the fibrillation 'f' waves instead of normal P waves and the irregular RR intervals.

Challenges in the Diagnosis of Atrial Arrhythmias

Visual ECG screening is time consuming and requires an experienced observer. Sometimes, especially for exercise ECGs or in the case of low ECG trace quality, interpretation can be challenging, even for an experienced cardiologist. Therefore, after importing ECG recordings into ECG analysis software, programs using complex algorithms for the automatic analysis of the different waves, are often used. However, most of these algorithms are designed for human and small animal medicine and fail to interpret the horse's ECG correctly. The different QRS polarity and especially the large T wave with variable polarity of the equine ECG may lead to software confusion. Furthermore, movement artifacts complicating automatic ECG analysis are common in horses, especially during exercise. Therefore, the visual screening of the horse's ECG usually remains necessary.

Both APDs and VPDs are characterized by premature complexes, but a correct differentiation is important because of a difference in clinical relevance. While APDs are often harmless and not an indication to stop riding the horse, frequent VPDs are a known risk factor for the induction of ventricular tachyarrhythmia, which may lead to cardiovascular collapse and even sudden death [4]. VPDs, as opposed to APDs, are the consequence of abnormal impulses arising from the ventricular myocardium. This leads to a change in ventricular conduction and consequently changes in QRS and T wave morphology and duration [1,4,31]. However, depending on the site of origin, changes in ventricular conduction and morphology can

sometimes be minimal. This can make differentiation from APDs with a hidden P wave difficult, especially when QRS morphology following the APD is atypical. Using multiple leads and adapted electrode configurations is often helpful. Furthermore, as was explained before, differentiation between an APD and a VPD based on the presence of a non-compensatory or fully compensatory pause, is not always possible since the sinus node may sometimes not be reset in case of an APD.

Heart Rate Variability

Heart rate variability (HRV) describes and quantifies the beat-to-beat variability and the long-term variation in heart rate [27]. In order to maintain normal blood pressure, the heart is under the influence of both the autonomic nervous system and the neuroendocrine system. Therefore, heart rate, even in the resting, healthy horse, is not static but cyclical and beat-to-beat variation in heart rate occurs. HRV is a measure for the functioning of the autonomic nervous system, especially the balance between sympathetic and vagal activity [27,34]. In SR, higher variability represents a healthy heart. In healthy horses, an increase in parasympathetic tone or a decrease in sympathetic tone results in a larger beat-to-beat variation and an increased HRV.

Technique

There are numerous methods and parameters available for describing the variations in heart rate. The methods most frequently used are time-domain analysis, frequency-domain analysis and non-linear methods. A summary of these methods and their frequently used parameters is displayed in Table 1.

Time-domain parameters are relatively easy to obtain. The most commonly used parameters are SDNN, the standard deviation of the RR intervals and RMSSD, the square root of the mean squared differences of successive RR intervals. Furthermore, geometrical methods, converting these RR intervals into a geometrical pattern and applying formulas to quantify these patterns, can be used. Commonly used examples are the Triangular Index; the integral of the density distribution (the histogram of the RR intervals) divided by the maximum of the density distribution, and the triangular interpolation of the RR interval histogram or TINN, which is the baseline width of the distribution measured as a base of a triangle approximating the RR interval distribution (Table 1).

A second method to describe HRV is the frequency-domain or spectral density analysis method. Spectral density analysis breaks the HRV into the frequency components that compose the overall HRV and thus provides the basic information of how power (i.e. variance) distributes as a function of frequency [35,36]. This technique has been adapted from astronomy where the overall power of a star is measured from the different frequencies of the light emanating from it. In case of HRV, it is performed by taking a series of numbers along the time axis (in this case RR intervals), applying computationally efficient algorithms, assigning bands of frequency and then counting the number of RR intervals that match each

band. The most commonly used algorithm is the fast Fourier transform (FFT), alternative techniques being the Lomb-Scargle (LS) periodogram or wavelet entropy scales. Normal values for the different frequency bands cannot be extrapolated from human medicine or between algorithm techniques, so when spectral density analysis is used in horses, care should be taken to use normal values designed for horses and specific for the algorithm used [27].

Table 1. Most commonly used methods for heart rate variability analysis.		
Time-domain methods	SDNN	Standard deviation of all NN intervals
	SDANN	Standard deviation of the averages of NN intervals in all 5 min segments of the entire recording
	RMSSD	The square root of the mean squared differences between successive NN intervals
	SDNN index	Mean of the standard deviations of all NN intervals for all 5 min segments of the entire recording
	NN50	Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording
	pNN50	NN50 count divided by the total number of all NN intervals
	Triangular Index	Total number of all NN intervals divided by the height of the histogram of all NN intervals measured on a discrete scale with bins of 7·8125 ms (128 Hz)
	TINN	Baseline width of the minimum square difference triangular interpolation of the highest peak of the histogram of all NN intervals
Frequency-domain methods (FFT, LS or wavelet entropy)	VLF	Power in the very low frequency range (0.003 - 0.04 Hz*)
	LF	Power in the low frequency range (0.04 - 0.15 Hz*)
	HF	Power in the high frequency range (0.15 – 0.40 Hz*)
	LF/HF	Ratio LF (ms^2)/HF (ms^2)
Non-linear methods	SD1	Standard deviation of the Poincaré plot of the RR intervals perpendicular to its line of identity
	SD2	Standard deviation of the Poincaré plot of the RR intervals along its line of identity

*Adapted from Camm and Malik, 1996 [35]. * Values from Bowen, 2010 [27]*

A third method for HRV analysis are the non-linear methods. Numerous parameters to measure non-linear properties of HRV have been used, including Poincaré sections, low-dimension attractor plots, single value decomposition and attractor trajectories [35]. Currently

the most commonly used non-linear methods for HRV parameters are SD1 and SD2, the standard deviation of the Poincaré plot of the RR intervals perpendicular to and along its line of identity, respectively (Fig.10).
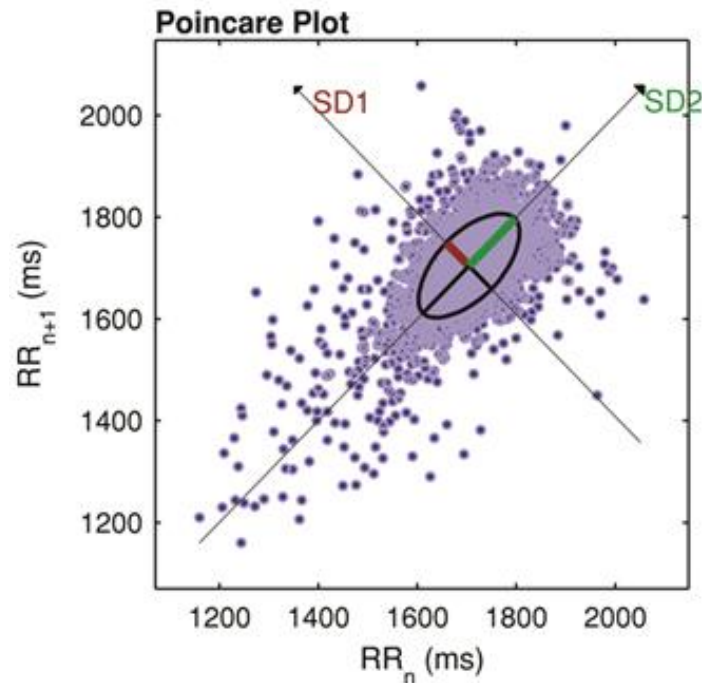
Figure 10: Poincaré plot of the RR intervals demonstrating the calculated SD1 and SD2.

Use in Human Medicine

Exercise, Training and High-Level Performance

Based on several scientific studies, HRV is being increasingly used by professional athletes, trainers and everyday sportsmen as a monitoring tool to evaluate athletic training, overtraining and fatigue [37-39]. Training is a stressor with an important impact on HRV and HRV serves as an estimate for the recovery of the body. In HRV guided training, the daily amount and type of training is based on individual HRV measurements [37]. It appears to be more effective for developing aerobic performance than pre-planned training [37]. Over the last decades, numerous HRV devices and applications have been developed to guide sportsmen to improve their performance.

Marker for Cardiac and Non-Cardiac Disease

There is an increasing amount of literature concerning HRV in human medicine over the last decades (>4,000 publications in the last decade). Data suggest that abnormalities in HRV during SR are risk markers for all common causes of cardiac death: both arrhythmic, vascular

and hemodynamic [39]. The most important mechanisms that influence HRV are neurohumoral activation and altered sympathovagal interaction. Since these mechanisms are important contributors to arrhythmogenesis, atherogenesis and the progression of heart failure, it is not surprising that altered HRV measurements predict various causes of cardiac death. More specifically, altered (usually lower) HRV has been associated with congestive heart failure, cardiomyopathy, arterial hypertension, atherosclerosis, ventricular and supraventricular arrhythmias, cardiac arrest and sudden death. It is used as a predictor of risk of mortality for a number of conditions, including acute myocardial injury, progressive heart failure and heart transplantation [35,39,40].

A variety of non-cardiac conditions has also been associated with changes in autonomic tone and thus HRV. For instance, decreased HRV is used as an early marker for diabetic neuropathy and as a predictor of survival of premature babies in an intensive care unit [35]. Furthermore, HRV, and especially spectral density analysis has proven to be useful as a follow-up marker in the treatment of Alzheimer's Disease, chronic obstructive pulmonary disease, renal failure, leukemia, epilepsy, chronic migraines and obstructive sleep apnea [41,42].

Psychophysiology

There is a growing interest in the use of HRV in the field of psychophysiology. Under conditions of stress, acute time pressure, emotional arousal and elevated anxiety, HRV decreases, probably due to increased attention or vigilance and increased sympathetic tone together with vagal motor inhibition [43,44]. HRV is also reduced in individuals reporting a greater frequency and duration of daily worry and in individuals suffering from post-traumatic stress disorder and depression [40,45].

Diagnosis of Arrhythmia

HRV parameters are expected to be increased with cardiac arrhythmias, especially in the case of frequent premature depolarizations and AF, which lead to an increased variation in beat-to-beat intervals. Higher variability in this case represents more arrhythmias. For this reason, HRV is also being used for the detection of several arrhythmias, among which AF, in human medicine [46-50]. Duverney et al.[48] used wavelet analysis of heart rate intervals in order to detect paroxysmal and chronic AF with a high sensitivity and specificity (>90%), whereas Park et al.[49] used three parameters derived from the Poincaré plot of the RR intervals with a sensitivity of 91.4% and a specificity of 92.9%. A computer algorithm based on the time-domain parameters of HRV was developed by Yaghoubi et al.[50] and could differentiate AF

from SR in 680 ECGs (340 AF en 340 SR) with a sensitivity and specificity around 99%. Gilani *et al.*[46] studied 146 different HRV parameters to differentiate AF from SR on a 1-minute ECG recording in 105 AF cases and 96 SR cases. Most parameters could differentiate between SR and AF with sensitivity and specificity above 98% and the authors proposed the use of commercial monitors with automatic HRV calculations for AF detection. Based on the results of these experimental studies, several automatic home monitoring devices using algorithms based on HRV parameters, have been marketed especially for AF diagnosis [47,51]. These devices allow rapid arrhythmia detection by the patient, after which confirmation with electrocardiography is required.

Use in Horses

Exercise, Training and High-Level Performance

Based on the experience in human training, HRV is becoming a well-known tool in equine studies evaluating the effects of different levels of exercise, training and high-level performance [52,53]. Due to the increasing availability of HRV devices and applications for use in horses, the use of HRV as a training tool is rapidly gaining popularity, especially in endurance horses. However, although the equipment and hardware is often adapted for horses, the settings, detections algorithms and HRV calculations are generally set for use in humans and not validated for use in the horse.

Marker for Cardiac and Non-Cardiac Disease

HRV has been used sporadically to study the autonomic regulation of the horse's heart [34,54] and has recently been introduced as a prognostic indicator in horses suffering from ischemic gastrointestinal disease [55]. Furthermore, a few studies suggest the use of HRV parameters as an indicator of pain in the horse [56,57]. There are, however, no studies evaluating its prognostic or diagnostic use in horses suffering from other diseases.

Psychophysiology

The most common use of HRV in equine (and other animal) studies is as a marker for stress and anxiety in welfare studies [34,58-60]. Over the last decade a large number of studies have been published using HRV to estimate stress in horses during different handling or exercising procedures. In these studies higher HRV is supposed to indicate a lower stress level and a happier horse.

Diagnosis of Arrhythmia

There are currently no studies available about the potential of HRV to diagnose arrhythmia in horses. One study used spectral domain analysis in a horse with AF to estimate the role of the autonomic nervous system and the modulation of the intrinsic behavior of the atrioventricular node during AF [61]. This study showed a large increase in frequency-domain analysis HRV measurements in this AF horse compared to horses in SR.
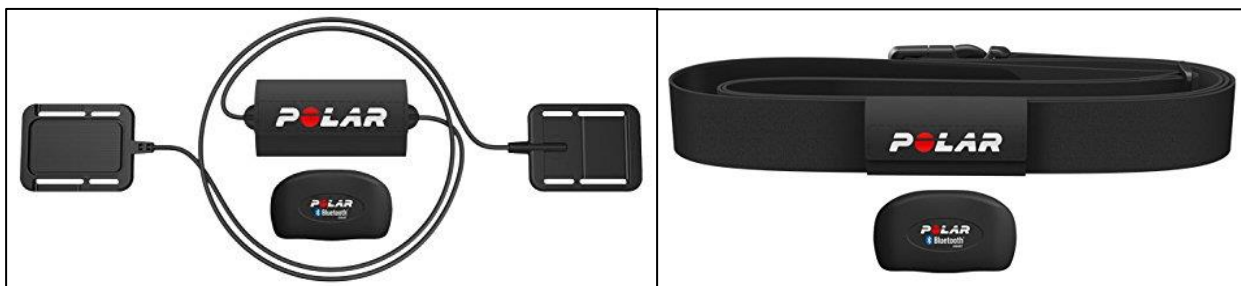
Heart Rate Monitors

A heart rate monitor (HRM) is a device that displays heart rate in real-time and often also records heart rates for later study. HRMs for horses are identical to or have been adapted from human models and can be used for recordings at rest and during exercise.

Technique

HRMs usually consist out of an electrode set and a heart rate sensor (Fig.11). The sensor automatically detects the R peaks on the ECG by looking for sharp deflections from the baseline. Subsequently, the sensor sends these data in real-time to a device displaying and/or recording the heart rate. Special plastic electrodes or an electrode belt which can be placed around the chest have been designed for horses (Fig.11). In general, HRMs are affordable, easy-to-use systems, which can be used by veterinarians in the field and by horse owners.

Figure 11: Heart rate monitors for horses. Special plastic electrodes (left) or an electrode belt have been designed for horses.

Use in Horses

Current Use

HRMs are increasingly used in sports horses to assess their level of fitness, to adjust workload and to monitor training progress. They have long been used in disciplines such as endurance and racing, and more recently also in other sports disciplines. Changes in heart rate, maximal heart rate and heart rate during recovery can be observed at different levels of exercise and training programs are adjusted based on the results.

In veterinary medicine HRMs are also gaining popularity, both for clinical and for experimental purposes. Where studies using HRV formerly were performed with RR intervals derived from surface ECGs, RR data obtained from HRMs are now increasingly being used[58,60,62].

Pitfalls

The most important pitfall of HRMs is correct RR interval detection. Both in humans and in horses, a few recent studies objectively assessing the performance of HRMs have shown significant differences between results from HRMs and ECG recordings [63-65]. First, contact between the horse's skin and the electrode has to be sufficient to register electrical activity. For equine ECGs, special adhesive electrodes, containing more gel and stronger glue, are available, but HRM electrodes are usually non-adhesive and contain no gel nor glue, so skin contact may be suboptimal. The performance of different HRM electrodes has not been compared in horses. A second problem is heart rate detection by the HRM software program. In horses, the T wave can be very pronounced and systems based on detecting R waves by looking for sharp deflections from the baseline, can erroneously register T waves. Furthermore, movement artifacts are common, especially during exercise, and may also lead to erroneous R peak registrations. Most HRMs have not been validated for use in horses, so studies assessing the correctness of RR detection with an equine HRM are necessary [63].

Another pitfall in using HRMs is the use of variable artifact correction methods by some devices or software programs. Artifact correction omits outliers, i.e. RR intervals that strongly differ from other RR intervals or replace them by interpolated values. Although this filtering helps to get rid of artifacts, it also blunts the beat-to-beat variation caused by arrhythmias, possibly hampering the differentiation between SR and pathological arrhythmias. Different software programs may use different artifact correction algorithms. No studies have been performed on how this artifact correction influences HRV in horses and it is currently unknown which level of correction should be used.

Atrial dysrhythmias and Atrial Fibrillation: Treatment and Prognosis

Treatment of Atrial Dysrhythmias in horses

Antiarrhythmic Drugs

Based on the human Singh-Vaughan Williams classification, antiarrhythmic drugs can be divided into categories according to their mode of action (Table 2). Although many antiarrhythmic drugs have multiple action mechanisms, they are classified based on the primary mechanism of action and the effect on the different phases of the cardiac action potential.

Class	Mechanism	Examples	Comments	Side effects
Ia	Na^+ channel block (intermediate association/dissociation) and K^+ channel blocking effect	Quinidine	Affect QRS morphology, prolongs QT interval	Colic, diarrhea, arrhythmia, stridor, ataxia, seizures
		Procainamide		Arrhythmia, vasodilation
Ib	Na^+ channel block (fast association/dissociation)	Lidocaine	Overdose prolongs QRS complex	Central nervous system excitement
		Phenytoin		Lethargy, colic, seizures, arrhythmia
Ic	Na^+ channel block (slow association/dissociation)	Flecainide		Arrhythmia, depression, hypotension, agitation, sudden death
		Propafenone		Arrhythmia, bronchospasm
II	β- blocker	Propanolol	Non-selective (β_1 and β_2), also some class I action	Weakness, lethargy, may worsen heart failure and bronchospasms
		Esmolol	β_2-selective	
		Atenolol	β_2-selective	
III	K^+ channel blocker	Sotalol	Also non-selective β-block, prolongs QT interval	Weakness, lethargy
		Amiodarone	Also class I, II and IV activity, prolongs QT interval	Diarrhea, colic
IV	Ca^{2+} channel blocker	Diltiazem		Weakness, lethargy
V	Other, unknown mechanisms (direct nodal inhibition?)	Magnesium sulphate		Rare
		Digoxin		Anorexia, depression, colic, arrhythmia

Table 2. Singh-Vaughan Williams classification of antiarrhythmic drugs used in horses.

APDs

Treatment of APDs depends on the underlying cause. Sometimes idiopathic APDs resolve following a period of rest and corticosteroid therapy. If APDs are infrequent, treatment is often not necessary. In rare cases of rapid supraventricular tachycardia, long-term antiarrhythmic therapy may be beneficial to reduce the ventricular response rate [66-68]. Also in case of APDs after cardioversion of AF, long-term antiarrhythmic drug administration may be beneficial to attempt reduction in early recurrence of AF and in order to allow for reverse electrical remodeling to take place.

Atrial Tachycardia, Atrial Flutter and AF

Management strategies for atrial tachycardia, atrial flutter and AF include either no treatment, or conversion to normal sinus rhythm, while hardly any information is currently available for a 'rate control' strategy. Atrial fibrillation has been shown to be often associated with abnormal ventricular complexes and R-on-T episodes, both representing a potential risk for collapse or even sudden death, while this has so far not been shown for atrial tachycardia and atrial flutter. Clinical decision making is influenced by the underlying condition and the intended level of activity of the horse.

Horses with severe cardiac disease or congestive heart failure are not candidates for cardioversion because AF recurrence is very likely. For horses in lone AF, however, the clinical consequences of AF can be difficult to estimate. Despite general consensus on the poor performance caused by AF in high level exercising horses, clinical consequences of AF generally abate when exercise demands decrease. Therefore the level of intended activity and the owner's expectations influence clinical decision making. Some horses are able to perform well when used for less intense athletic work, while others experience reduced performance even at low level exercise. Cardioversion is recommended when performance issues are present, when the average maximal heart rate during exercise is greater than 220 BPM at an intensity at, or slightly exceeding the horse's normal activities, or whenever abnormal ventricular depolarizations are present [4]. In horses where treatment is not possible or declined, an exercise ECG should be performed to identify potential risk factors for collapse or even sudden death, such as extremely high heart rates or abnormal ventricular responses [4,20].

Restoration of SR can be obtained either by electrical or pharmacological cardioversion. Both techniques are generally safe and effective with reported success rates ranging between 60 and 95%, provided that no significant underlying heart disease is present. Factors likely to

influence treatment success are the presence of atrioventricular valvular regurgitation, atrial enlargement and primary myocardial disease [69]. If concurrent congestive heart failure or grave other cardiac abnormalities are present or the horse is aged or retired, cardioversion may be either of little benefit or unsuccessful. In these cases long-term pharmacological rate control therapy may be attempted.

Pharmacological Cardioversion

Quinidine sulphate is the standard drug for the pharmacological treatment of equine AF. It is effective in many horses and several treatment plans can be followed. Other drugs that have been described include flecainide, amiodarone and propafenone (Table 3).

Quinidine

Quinidine sulphate (22 mg/kg every 2h until adverse reactions or max 4 treatments) remains the drug of choice for the pharmacological AF treatment in horses. It is a class Ia antiarrhythmic drug that blocks the fast inward sodium channels in the myocardium. It prolongs the effective refractory period and, as such, slows down the electrical activity of cardiac tissues. However, it can cause dangerous ventricular arrhythmias and has a wide range of cardiovascular and noncardiac side-effects, so close monitoring during treatment is necessary. Quinidine sulphate is administered via the enteral route, but intravenous treatment with quinidine gluconate (1.0- to 1.5 mg/kg every 10 minutes until max 11 mg/kg) has also been described. Because of its irritating effect on mucous membranes it has to be administered by nasogastric tube. Several treatment protocols have been described, consisting of multiple consecutive doses with close clinical and echocardiographic monitoring until successful conversion to SR. Cardiovascular adverse effects, such as hypotension, proarrhythmia and sudden death may occur, as well as neurological (ataxia, aggression, collapse) and gastrointestinal (diarrhea, colic) side effects ranging from mild to lethal. Treatment with quinidine is often successful, especially for recent onset AF, and the treatment of choice in many veterinary institutes. However, quinidine's toxicity and its limited availability in many countries have stimulated research into alternative therapies.

Other Drugs

Amiodarone, a class III antiarrhythmic drug with β-adrenergic properties that increases the refractory period by blocking potassium and sodium channels, has a good conversion rate and few cardiac side effects in human medicine. In horses, amiodarone has a low oral bioavailability (6-33%) [70]. Different intravenous treatment protocols have been reported with

success rates between 50 and 65% [71,72]. Side effects may occur after long-term treatment (>56 hours) and include hindlimb weakness, depression and diarrhea [71,73]. Amiodarone is not advised as a first option treatment of AF but can be tried in horses refractory to quinidine treatment.

Flecainide is a class Ic antiarrhythmic drug that slows intracardiac conduction and reduces excitability in cardiac tissues due to its potent sodium channel blocking effects. Flecainide (0.2mg/kg/min for 10 minutes) has been used successfully in an experimental acute AF model [74-76], but showed dangerous, potentially lethal side effects in horses with naturally occurring AF [77]. These side effects included depression, agitation, mild colic, hypotension, prolongation of QRS complex and QT intervals and wide-QRS ventricular proarrhythmia [74,76,77]. Several case reports have described sudden death during both intravenous and oral flecainide treatment [78,79]. Due to a high risk for sudden death, flecainide is currently not advised for AF treatment in horses [16,80].

Propafenone, another class Ic antiarrhythmic drug used in humans for cardioversion, was studied as an alternative treatment for horses with AF. In 6 horses with AF, intravenous propafenone treatment starting with a 2 mg/kg bolus and thereafter 7 µg/kg/min for 2 hours, resulted in an increase in AF cycle length but cardioversion to SR was not obtained [80].

Table 3: Drugs studied for cardioversion in horses with AF							
Class	Drug	Route	Dose	Studied population	Efficacy	Adverse effects	Refs
Ia	**Quinidine gluconate**	IV	1.0- to 1.5 mg/kg every 10 minutes until max 11 mg/kg	Naturally occuring AF	65-80%	Proarrhythmia, colic, diarrhea, ataxia, stridor, seizures	81,82
Ia	**Quinidine sulphate**	NGT	22 mg/kg every 2h until adverse reactions or max 4 treatments	Naturally occuring AF	80-95%	Proarrhythmia, colic, diarrhea, ataxia, stridor, seizures	1,69
Ic	**Flecainide**	IV	0.2 mg/kg/min for 10 minutes	7 horses with acutely induced AF	100%	QRS and QT prolongation	83
				6 horses with acutely induced AF	100%	Agitation and QT prolongation	84
				10 horses with naturally occuring AF	10%	Dangerous ventricular proarrhythmia	77
Ic	**Propafenone**	IV	2 mg/kg bolus thereafter 7 µg/kg/min for 2h	2 horses with naturally occuring AF	0%	Sinus brachycardia, bronchospasms	80
III	**Amiodarone**	IV	5 mg/kg/h for 1 h followed by 0.83 mg/kg/h for 23h and 1.9 mg/kg/h for 30h	6 horses with chronic natural AF	66%	Diarrhea, hind limb weakness	71
			6.52 mg/kg/h for 1 h followed by 1.1 mg/kg/h for 47 h	6 horses with chronic natural AF	50%	Colic, diarrhea	73

NGT: nasogastric tube ; Refs: references

Transvenous Electrical Cardioversion

Electrical cardioversion appears to be the most viable alternative to quinidine sulphate treatment in horses, and is becoming increasingly important because of its high success rate (>95%) even in quinidine refractory cases. By means of direct current shock delivery, depolarization of the whole atrial myocardium is obtained, thereby terminating the arrhythmia[85,86] (Fig.12). During this procedure one catheter is placed, through the jugular vein, in the right atrium while a second catheter is positioned in the left branch of the pulmonary artery. Furthermore, a temporary pacing catheter is placed in the right ventricle to perform temporary pacing if post-shock asystole due to temporary AV-block should occur [5]. R wave synchronized biphasic shocks are delivered with increasing energy levels (mean

cardioversion energy is around 160 joule) until restoration of SR [86]. Apart from occasional mild post-anaesthetic myopathy, this technique is safe and has shown to be effective in 98% of lone AF horses [85,87].

Figure 12: Transvenous electrical cardioversion procedure in a horse.

Future Prospects

Despite the high efficacy of current treatment options, recurrence of AF is common. In human medicine, recurrence is often due to the presence of ectopic foci originating from the myocardial sleeves in the pulmonary veins. Therefore, catheter ablation techniques have been developed in order to destroy these foci or prevent them to enter the atria, thereby preventing AF recurrence [9,11,88]. Ablation is performed using radiofrequency or cryo-ablation in order to destroy a small amount of myocardial tissue, which leads to conduction block, preventing the ectopic beats from entering the atria. In 95% of the human cases, the focus is located within the pulmonary vein but other possible sites include right atrium, left atrium, coronary sinus, superior vena cava or vein of Marshall [9]. Success rates in human medicine are relatively high but because of the presence of multiple foci or restoration of conduction, several treatment attempts are sometimes required to obtain permanent rhythm control.

Catheter ablation requires registration of the activation and conduction patterns in the atria for an exact determination of the location of the ectopic foci and a very precise positioning of the ablation catheter in the heart. In horses, ablation has never been performed. Positioning of the catheter would be challenging, because of the large heart and the superposition of lung tissue, hampering ultrasonographic guidance or fluoroscopic techniques. Promising research results facilitating the development of this technique in horses, have recently been published. One study revealed the complex anatomy of the pulmonary veins in horses [89] and also intracardiac electroanatomical mapping techniques are currently being studied and have been successfully used in horses [33].

Recurrence of AF

Recurrence of AF is relatively common in horses with reported recurrence rates around 35-45% [90]. Reported recurrence rates are variable due to differences in horse population, previous AF duration, presence of underlying heart disease and follow-up time.

Risk Factors

Many risk factors for AF recurrence have been described. Several experimental studies have demonstrated extensive atrial remodeling during AF [16,86,91] and the likelihood of successful conversion decreases and recurrence rates increase with increased AF duration. In one study in horses with AF of less than 3 months duration, the recurrence rate was 15%, whereas 65% if AF had been present for a longer period [69]. A recent large multicenter study, however, was not able to identify a correlation between AF duration and recurrence [90]. Atrial size and horse breed have also been previously determined as risk factors for AF recurrence [3,92,93], but were not significantly different in a more recent study [90]. It is possible that recurrence rates do not increase linearly with increased AF duration or increased atrial size, but that a threshold-based difference is present. Treatment modality (electrical or pharmacological cardioversion) was not related to AF recurrence [90]. Previous episodes of AF or previous unsuccessful treatment attempts, suggesting differences in atrial electrophysiology or the presence of underlying atrial myocardial disease, were identified as risk factors for AF recurrence [90]. Other suggested risk factors are mild to moderate mitral valve regurgitation [90] and the ratio of the AF cycle length to the atrial size [93] before cardioversion. After cardioversion, a decrease in active left atrial fractional area change, which can be an indicator of atrial remodeling or underlying myocardial disease, was also reported as a risk factor for AF recurrence [69,90].

Prevention of AF Recurrence

In horses, little is known about prevention of AF recurrence and preventive measures are based on information from human medicine.

AF recurrence is also common in humans and prevention is mainly attempted by pharmacological antiarrhythmic therapy [94]. Many patients eventually need prophylactic antiarrhythmic drug therapy to maintain SR, suppress symptoms, improve exercise capacity and hemodynamic function, and prevent tachycardia-induced cardiomyopathy due to AF. A large variety of antiarrhythmic drugs have been studied and are used in human medicine with variable results. Recommendations support the use of propafenone, flecainide, dofetilide, dronedarone or sotalol in patients treated for lone AF with minimal underlying heart

disease[94,95]. While amiodarone prevents AF recurrence better than the above antiarrhythmic drugs, guidelines advise its use only in cases refractory to other drugs because of its important extra cardiac side effects [101]. In a meta-analysis of 44 randomized controlled trials comparing antiarrhythmic drugs against control (placebo or no treatment), the overall likelihood to maintain SR after cardioversion was approximately doubled by the use of antiarrhythmic drugs [96].

In horses, prevention of AF recurrence is attempted with a combination of management changes and antiarrhythmic therapy. After cardioversion a period of rest before resuming exercise activities is advised in order to allow for atrial reverse electrical and contractile remodeling to take place [97]. Long-term antiarrhythmic treatment in horses has poorly been described. One study described the use of phenytoin, a drug with class 1b antiarrhythmic properties, in two horses after successful cardioversion [98]. Another study did not find a statistical difference in AF recurrence in horses treated with KCl supplementation (n=39), ACE-inhibitors (n=34), dexamethasone (n=33) or digoxin (n=6) after cardioversion compared to untreated horses (n=83) [90]. Various antiarrhythmic drugs are empirically being used by veterinarians after cardioversion but no further studies on the pharmacological prevention of AF recurrence in horses, nor studies on their pharmacokinetic properties in horses have been published.

Sotalol

Before the start of this PhD research, scarce information about sotalol in horses was available. An important part of this PhD thesis studies the pharmacological profile and clinical effects of sotalol in horses. Sotalol has a straightforward pharmacokinetic profile and a high oral bioavailability (F) in humans and small animals. Furthermore, sotalol is used in human medicine in patients with various cardiac rhythm disorders as an effective antiarrhythmic and anti-fibrillatory agent [99-102]. Although it can be used for cardioversion of AF patients it is mostly used in the prevention of AF recurrence [101,103]. Guidelines for the management of patients with AF recommend sotalol for the prevention of AF recurrence in outpatients in SR with little or no heart disease, prone to paroxysmal AF, if the baseline uncorrected QT interval is less than 460 ms, serum electrolytes are normal, and risk factors associated with class III drug–related proarrhythmia are not present [94].

In dogs and cats, sotalol is the most commonly used long-term treatment for ventricular arrhythmias, but its efficacy in the prevention of recurrence of atrial flutter and AF also has been shown [104-106].

Mechanism of Action

Sotalol, or 4-(2-isopropylamino-1-hydroxyethyl) methanesulfonanilide, is a water-soluble, racemic mixture of its stereoisomers, D- and L-sotalol [107] (Fig.13).
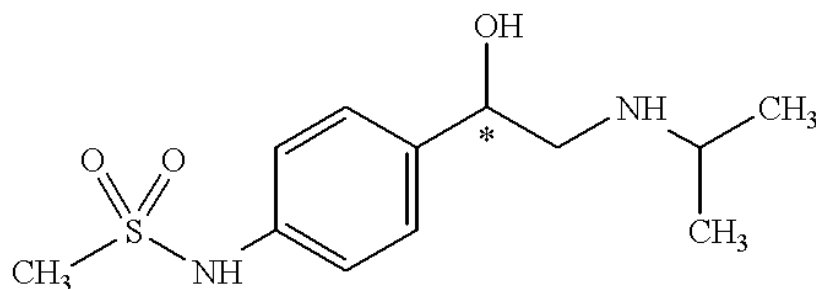

Figure 13. Structure of sotalol [108].

Sotalol has potent, non-cardioselective β- blocking (class II) effects, blocking both β_1- and β_2-adrenoreceptors. β-blockers are competitive antagonists of the endogenous catecholamines, such as adrenaline and noradrenaline, on the β-receptors of the sympathetic nervous system. β_1-adrenergic receptors are located mainly in the heart and in the kidneys, while β_2-adrenergic receptors are located primarily in the lungs, gastrointestinal tract, liver, uterus, vascular smooth muscle, and skeletal muscle. By blocking β_1-adrenoreceptors, sotalol prevents the activation of the inward calcium channels in the cardiac muscle cells [108]. This will result in a decrease in intracellular Ca^{2+}, and a lower excitability of the cell. Important cardiac effects of β-blockers are a decrease in heart rate and blood pressure but also a decreased cardiac contractility, possibly exacerbating congestive heart failure. Sotalol, however, unlike other, selective β-blockers, is devoid of intrinsic sympathomimetic actions and exerts no or very little depressant effect on myocardial contractility, because of its additional class III antiarrhythmic properties [109]. Some studies even report positive inotropic effects [107]. Class III antiarrhythmic function primarily results in a lengthening of the cardiac repolarization phase. By blocking outward potassium channels, sotalol inhibits the rapidly activating component of the delayed rectifier potassium current IKr, thereby slowing the repolarization phase of the cardiac tissues and enhancing cardiac contractility [99,108]. In human electrophysiology studies, sotalol prolongs the duration of action potentials recorded in cardiac tissue, increases the refractory period and lengthens the QT interval on the surface electrocardiogram [99,107,108].

However, sotalol-induced prolongation of the QT interval is more pronounced at slow heart rates and may not be apparent during exercise-induced tachycardia. This heart rate dependence, also referred to as reverse use dependence, has also been reported for other agents with class III action, but its clinical importance is unknown [108].

Intravenous sotalol is not available in many countries, but oral tablets, either containing sotalol or sotalol hydrochloride, are available for use in humans at concentrations between 40 and 160 mg per tablet. Sotalol is not registered for use in the horse. However, since no other antiarrhythmic drugs are registered for horses, off label use, following the cascade system, is allowed.

Pharmacokinetics and Pharmacodynamics

Unlike many other antiarrhythmic drugs, pharmacokinetics of sotalol in humans show a good oral F, no drug-drug interactions, no biotransformation and an entire renal excretion (Table 4) [108]. Sotalol pharmacokinetics conform to a first-order, linear two-compartmental model, in which all processes of drug absorption, distribution and elimination occur by first-order kinetics [107]. Higher doses of sotalol are necessary to prolong cardiac repolarization than to achieve β-blocking activity. Significant β-blockade in humans occurs at plasma sotalol concentrations as low as 0.8 µg/mL, while class-III activity starts from 1.2 µg/mL [110]. Plasma concentrations aimed for in humans are 1-3 µg/mL [111]. The minimally effective antiarrhythmic dose of orally administered sotalol in human medicine is 40-80 mg twice daily; the dose can be increased if necessary every 3 to 4 days until a maximum of 640 mg per day is reached [99].

Pharmacokinetics of sotalol in dogs are similar to those in humans. Also in dogs sotalol shows good oral F, no biotransformation and a complete and unchanged excretion in urine (Table 4) [112]. In dogs, an oral dose of 5 mg/kg bwt bid, leading to plasma concentrations of 1.1-1.6 µg/mL, resulted in β-blockade, class III activity and significant QT prolongation [105].

Table 4. Pharmacokinetic profile of sotalol in humans and in dogs after oral administration. Data are derived from [105,107,108,112]		
Parameter	**Humans**	**Dogs**
T_{max}	2.5-4 h	0.5-1.5 h
Extent of absorption	90%-100%	>90%
Bioavailability	Approximately 100%	85-90%
Plasma protein binding	0%	0%
Apparent volume of distribution	2.0 ± 0.4 L/kg	1.5-2.5 L/kg
Biotransformation	0%	<1%
Metabolites	None detected	None detected
Total body clearance	110-400 mL/min	
Renal elimination of unchanged drug	>75%	>90%
Plasma elimination half-life	15 (10-20 h)	4.8 ± 1 h
Therapeutic plasma concentration range	~1-4 µg/mL	0.8 – 1.6 µg/mL*
Disposition kinetic model	First order/2-compartment	First order/2-compartment
Dose proportionality of C_p?	Yes (linear)	Yes (linear)
Special features	Water soluble, little central nervous system penetration	
	Accumulates in patients with renal failure, not in patients with hepatic failure	
	No pharmacokinetic drug interactions	

: Therapeutic C_p range not well established; limited clinical use. T_{max}: time to reach maximal plasma concentration; C_p: plasma concentration.

Adverse Effects

The side effects of sotalol are related to either its β-adrenergic antagonism or its capability of lengthening the QT interval. Sotalol is a non-cardioselective β-blocker, blocking both β_1 and β_2 receptors. Fatigue, dizziness, dyspnea or worsening of bronchospasm are common side effects associated with β_2-blocking agents [99]. Due to its class III effects, the chances of exacerbating congestive heart failure, a serious adverse effect of most β_1-blockers, are significantly reduced with sotalol [99,108]. The most dangerous adverse effects of sotalol are related to the lengthening of cardiac repolarization. Drugs that prolong the QT interval may

aggravate preexisting arrhythmias or provoke new arrhythmias, the most notorious one being torsade de pointes, a polymorphic ventricular tachycardia. Predisposing factors such as hypokalemia, bradycardia or concomitant use of other QT interval prolonging drugs warrant close clinical, electrocardiographic and drug monitoring [99]. In human clinical trials, adverse reaction leading to discontinuation of oral sotalol included fatigue (4%), bradycardia (3%), dyspnea (3%), proarrhythmia (3%), asthenia (2%) and dizziness (2%) [108]. Special care should be taken when using sotalol in patients suffering from diabetes, renal failure, electrolyte disturbances and other drugs prolonging the QT interval. The use of sotalol is contraindicated in cases of bronchial asthma, bradycardia, uncontrolled congestive heart failure and cardiogenic shock [108].

Although sotalol is frequently used in dogs suffering from ventricular arrhythmias, side effects of the therapy are rarely reported. In dogs, proarrhythmia is a rare adverse effect of sotalol, although the risk of proarrhythmic events is increased when high dosage is used or hypokalemia is present [105,106,113].

Use in the Horse

Because of its promising pharmacokinetic profile and its demonstrated antiarrhythmic effects in humans and small animals, sotalol is being increasingly used by equine veterinarians, at different dosages and dose intervals. However, apart from 2 recent reports describing the use of sotalol in 2 single cases, no studies on its pharmacokinetics, pharmacodynamics and clinical antiarrhythmic properties have been published [114,115]. Therefore, studies determining a safe dose schedule and evaluating the clinical effects of sotalol in horses are necessary.

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SCIENTIFIC AIMS

The first objective of this dissertation was to improve the diagnosis of atrial rhythm disorders both by veterinarians and by horse owners in the field. First, specific ECG characteristics associated with atrial premature depolarizations were studied to improve ECG interpretation. We hypothesized that an atrial premature depolarization may result in QRS complex and T wave morphology alterations, making differentiation between an atrial and a ventricular origin challenging. In addition, the use of heart rate variability as a novel diagnostic tool was studied. We hypothesized that heart rate variability parameters may allow to distinguish atrial fibrillation from sinus rhythm in horses and that heart rate monitors automatically calculating these parameters can be used to detect atrial fibrillation in the field. For this first part of the dissertation the specific aims were:

- To describe the QRS complex and T wave morphology following atrial premature depolarizations in horses (Chapter 1)
- To evaluate whether certain heart rate variability parameters differ between horses in atrial fibrillation and in sinus rhythm (Chapter 2)
- To assess whether a heart rate monitor with automatic heart rate variability calculations can be used to detect atrial fibrillation in horses (Chapter 3)

The second major objective was to investigate a new pharmacological treatment for atrial arrhythmias in horses by using oral sotalol. The hypothesis was that sotalol influences the electrophysiological properties of the heart and results in antiarrhythmic effects in horses. First, the pharmacokinetic profile and absolute oral bioavailability of sotalol in horses was studied as well as its effect on the surface ECG. Subsequently, the electrophysiological effects of different doses of sotalol on the equine heart were studied. Finally, the rate control properties of sotalol are evaluated in patients with naturally occurring atrial fibrillation. The specific aims of the second part of this dissertation were:

- To determine the pharmacokinetic profile and absolute oral bioavailability of sotalol in healthy horses (Chapter 4)
- To develop a technique for intracardiac monophasic action potential recording in standing horses (Chapter 5)
- To study the effect of sotalol on the electrophysiological properties of the myocardium in healthy horses (Chapter 4 and 6)
- To assess the effect of sotalol on heart rate, QT interval and atrial fibrillatory rate in horses with naturally occurring atrial fibrillation (Chapter 7)

CHAPTER 1:

ATRIAL PREMATURE DEPOLARIZATION-INDUCED CHANGES IN QRS AND T WAVE MORPHOLOGY ON RESTING ELECTROCARDIOGRAMS IN HORSES

Summary

Background: The electrocardiographic differentiation between atrial (APDs) and ventricular (VPDs) premature depolarizations is important. P wave prematurity and normal QRS and T wave morphology generally are used as discriminating criteria for APDs.

Hypothesis/Objectives: To determine whether P, Q, R, S and T wave amplitude, PQ interval, QRS and P wave duration and P and T wave morphology differ between APDs and sinus beats. To determine the relationship between the RR coupling interval and the change in S wave amplitude between sinus beats and APDs.

Methods: Case-control study. From a modified base-apex configuration of 30 horses with APDs at rest, sinus beat and APD-associated preceding RR interval, P, PQ and QRS duration and P, R, S and T wave amplitudes were measured. Linear mixed models and logistic regression were used to determine the effect of APDs on the ECG variables studied.

Results: In comparison to sinus beats, APDs were associated with a significant ($P<0.001$) change in P amplitude (-0.03 ± 0.01 mV) and increase in S (0.20 ± 0.02 mV) and T (0.08 ± 0.03 mV) amplitude. PQ (-20.3 ± 5.2 ms) and RR (-519 ± 14 ms) interval and P duration (-21.1 ± 3.0 ms) decreased ($P<0.001$). APDs were significantly associated with a singular positive P wave (OR: 11.0, CI 6.2-20.0.0, $P<0.001$) and were more likely to have a monophasic positive T wave (OR: 9.2, CI 5.1-16.5, $P<0.001$). A smaller RR coupling interval was associated with an increased relative difference in S amplitude ($P<0.01$).

Conclusions: APDs may lead to changes in QRS and T wave morphology. Knowledge of these changes is important in order to avoid interpreting certain APDs as VPDs.

Introduction

Electrocardiography is of increasing importance in equine medicine. Correct classification of different electrocardiographic complexes is important to differentiate clinically relevant abnormalities from physiological complexes. Electrocardiographic differentiation between ventricular (VPDs) and atrial premature depolarizations (APDs) is important because the clinical relevance of both types of premature cardiac complexes is very different. During exercise, VPDs, especially when consecutive, are thought to be a risk factor for the induction of ventricular tachyarrhythmia, which can cause an abrupt decrease in blood pressure and may even lead to cardiovascular collapse. APDs can occur in normal horses but do, however, predispose to the development of atrial fibrillation [1,2]. Because management and treatment options can vary depending on the diagnosis, it is important to correctly differentiate between APDs and VPDs.

Ventricular dysrhythmias are the consequence of abnormal impulses arising somewhere in the ventricular myocardium, leading to a different ventricular conduction, and resulting in changes in QRS and T wave morphology and duration [1-3]. However, depending on the site of origin, changes in ventricular conduction and morphology can sometimes be minimal [2]. VPDs arise from the ventricular myocardium and result in AV dissociation: they are not associated with a preceding P wave and the normal sinus P wave often is not conducted, resulting in a compensatory pause [4]. Atrial dysrhythmias are caused by abnormal impulse formation in the atrial myocardium outside of the sinoatrial (SA) node. In the case of APDs, ventricular conduction is not affected and QRS complex and T wave morphology remain unchanged [1,3,5]. A premature P wave, with normal or abnormal morphology, usually is present, but can sometimes be buried in the preceding QRS or T wave and thus be difficult to visualize. If a P wave is not clearly visible, differentiation between a VPD and an APD can be challenging, especially when there are only mild changes in QRS and T wave morphology. Using multiple lead recordings can be helpful [6,7]. Each lead measures the cardiac depolarization from a different angle, so minor electrical changes sometimes can be more easily identified in 1 lead compared to the other.

The aim of our study was to report P, QRS and T wave morphology associated with APDs at rest in order to improve the interpretation of electrocardiogram recordings. Therefore, the amplitude of P, Q, R, S and T waves and the PQ interval and P and QRS duration of APDs and normal sinus beats were compared. Associations between APDs and specific T and P

wave morphologies were investigated. Finally, the effect of the RR interval on the relative change in S wave amplitude following an APD was studied.

Material and Methods

Study design and population

A case-control study was performed on 30 ECGs of 30 horses with APDs. Per horse, 10 single APDs were included, whereby for each APD the preceding sinus beat was taken as its negative control. All ECGs belonged to horses presented to the Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University for cardiac examination. All data were used with owner informed consent. Mean age and body weight of the horses was 11 ± 5 years and 569 ± 81 kg, respectively. Two stallions, 18 geldings and 10 mares were included. Breed was reflective of the hospital population, with 26 warmblood horses, 2 Arabian horses, 1 French trotter and 1 Belgian draft horse. Nineteen horses had been presented for cardiac examination after electrical cardioversion (TVEC) of atrial fibrillation (4 days [n=14] and 6 weeks [n=5] after TVEC). Two horses were diagnosed with mild to moderate mitral regurgitation, 1 horse had an aortacardiac fistula and 1 horse had suffered from intoxication with cardiac glycosides. Seven horses had APDs without any other cardiac abnormalities. Horses with VPDs were excluded from the study.

Electrocardiography

In all 30 horses, an ECG was recorded using a Televet100 recording system[a]. Four self-adhesive electrodes were placed underneath a girth[b]. The right arm electrode was positioned 15 cm right of the withers, the left leg electrode caudal to the left elbow on the thorax and the left arm electrode 10 cm above the left leg electrode, resulting in a modified base-apex configuration [6,8]. A reference electrode was placed on the left side of the withers. All electrodes were connected to the recording device, placed in the girth. The ECG recordings were analyzed offline using dedicated software[c]. Standard gain (10 mm/mV) and paper speed (25 mm/s) were increased to 20 mm/mV and 200 mm/s to allow accurate analysis. Three different leads were displayed simultaneously to improve ECG interpretation (Fig 1). In the Televet system, leads 1 and 2 are recorded, whereas lead 3 is calculated, assuming an Einthoven triangle. Measurements always were performed in lead 2 (between the right arm [-] and left leg [+] electrode). For each horse, the first 10 APDs and their preceding normal sinus beats were included. An APD was defined as an individual atrial depolarization, occurring prematurely, meaning with an RR coupling interval at least 20% (arbitrary value) shorter than the RR interval of the preceding sinus beat. Only APDs followed by a QRS complex were included in the study. The ECGs were recorded at rest and heart rate varied between 35 and 55 beats per minute. For both the sinus beats and the APDs, preceding RR interval, P, PQ and

QRS duration and P (P1 and P2 if present), Q, R, S and T (T1 and T2 if present) wave amplitudes were measured (Fig.2).

Although measurements always were performed in lead 2 of the ECG, 3 different leads were recorded to allow for correct interpretation of the electrocardiogram (Fig.1). When P waves were difficult to visualize in lead 2 of the ECG, they often were better visualized in lead 1 or lead 3, so that a correct diagnosis of APD could be made.

Figure 1: Electrocardiogram with 3 lead recording (1, 2, 3) showing an atrial premature depolarization (APD). In lead 2 the P wave of the APD is partially buried in the preceding T wave and difficult to identify. The S wave amplitude after the APD is increased and also T wave morphology has changed. Lead 1 and 3 clearly show the premature P wave. Gain: 20 mm/mV, paper speed: 100 mm/s.
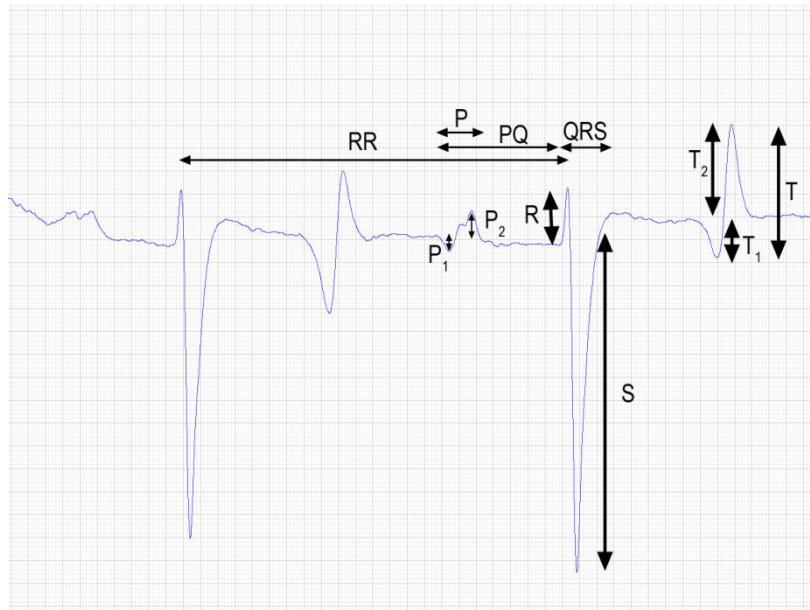
Figure 2: *Method of measurement of the different electrocardiographic variables used in the present study. For all atrial premature depolarizations and their preceding sinus beat RR and PQ interval, QRS duration and P1, P2, Q, R, S and T1 and T2 wave amplitude were measured. If a P wave was biphasic or bifid, only the largest amplitude was included in statistical analysis. For biphasic T waves, the total T wave amplitude (T), calculated as the sum of the absolute amplitudes of the positive and negative T wave (T1, T2) was used for statistical analysis.*

Data management and statistical analysis

Statistical analyses were performed using SAS 9.4[d]. In Figure 2, the different measurements applied are shown. If the P wave was bifid or biphasic, only the largest amplitude was selected. Singular, bifid or biphasic nature of the wave was recorded as a categorical variable. For the T wave, if biphasic, the sum of the positive amplitude and the absolute value of the negative amplitude were used for statistical analysis. The T wave morphology was recorded as a categorical variable with 4 categories: biphasic positive-negative, biphasic negative-positive, monophasic positive and monophasic negative.

To determine the effect of an APD on P, Q, R, S and T amplitude and P, PQ and QRS duration, a linear mixed model was constructed with horse added as a random factor to account for clustering of measurements within a horse (PROC MIXED). A maximum likelihood model with Satterthwaite approximation was used. The outcome variables were tested for normal distribution (histograms and Q-Q plots) and validity of the final models was checked by inspecting the residuals.

Logistic regression was performed to determine associations between P and T wave morphology and APDs and RR intervals. A generalized linear model (PROC GLIMMIX) was used with binomial distribution and logit link function with Wald's statistics for type 3 contrasts. Horse was added as a random factor to account for the clustering of ECG recordings within a horse. Post hoc tests were done with Bonferroni corrections for multiple comparisons. Model fit was evaluated by the Hosmer-Lemeshow goodness-of-fit test for logistic models. In all models, significance was set at P<0.05.

A mixed model approach (PROC MIXED) was applied to determine the relationship between the RR coupling interval of APDs and the change in S wave amplitude. The percentage change in S wave amplitude between a normal sinus beat and an APD was calculated as follows: S wave amplitude (sinus beat)/ S wave amplitude (APD) x 100. A maximum likelihood model with Satterthwaite approximation was used, and horse was added as a random effect. The outcome variables were tested for a normal distribution (histograms and Q-Q plots) and validity of the final models was checked by inspecting the residuals. In addition to the general analysis, in order to better understand the horse effect, the relationship between the RR coupling interval and the change in S wave amplitude was visually inspected and analyzed by simple linear regression in every individual horse. Validity of the models was checked by inspecting the residuals.

Results

Changes in ECG measurements

In total, 588 complexes (294 APDs and 294 preceding sinus beat controls) from 30 horses were collected. In 2 horses, only 6 and 8 APDs and preceding sinus beats could be measured, respectively. In all other horses (n=28) 10 APDs and their preceding sinus beats were included. For 96 of 300 APDs (32%) the ectopic P wave could not be accurately measured because it was fully or partially buried in the preceding T wave. In all horses, the majority of the QRS complexes, both from sinus beats and APDs, had an rS morphology, in 6 horses an S morphology was present in 5-35% of the complexes per recording. A Q wave was not visible in any of the horses. The S and the T amplitudes of APDs were significantly ($P<0.001$) increased as compared to sinus beats (mean difference±SD 0.20±0.02 mV and 0.08±0.03 mV, respectively) and PQ (-20.3±5.2 ms) and RR (-519±14 ms) intervals were decreased as compared to sinus beats ($P<0.001$). Furthermore, the P wave of an APD significantly differed from the P wave of a regular sinus beat (amplitude -0.03±0.01 mV, duration -21.1±3.0 ms). The R amplitude ($P=0.51$) and QRS duration ($P=0.09$) were not significantly different between APDs and sinus beats (Table 1).

Table 1. Changes in ECG morphology in 588 recordings of 30 horses with APDs						
Variable	n	sinus beat mean ± SD (range)	APD mean ± SD (range)	Estimated difference	95% CI of difference	P-value
P amplitude (mV)	492	0.25±0.08 (0.05-0.60)	0.22±0.11 (0.05-0.70)	-0.03±0.01	-0.04 - -0.02	<0.001
R amplitude (mV)	574	0.27±0.24 (0.00-1.00)	0.26±0.21 (0.00-1.00)	-0.01±0.01	-0.04 - 0.02	0.51
S amplitude (mV)	588	-2.16±0.54 (-4.20- -0.90)	-2.35±0.61 (-4.10- -1.00)	-0.20±0.02	-0.24 - -0.16	<0.001
T amplitude (mV)	588	0.97±0.05 (-2.10- 1.10)	0.90±0.05 (0.30-1.80)	0.08±0.03	0.04 - 0.11	<0.001
P duration (ms)	477	168±42 (56-272)	147±45 (60-254)	-21.1±3.0	-26.95 - 15.3	<0.001
PQ interval (ms)	495	337±74 (148-748)	321±97 (0-633)	-20.3±5.2	-30.4 - -10.2	<0.001
RR interval (ms)	588	1468±327 (566-2320)	1002±225 (522-2000)	-519±14	-546 - -492	<0.001
QRS duration (ms)	588	132±12 (98-164)	133±11 (98-162)	1.0±0.6	-2.1 - -0.2	0.09
Horse was added as a random factor and was significant in each model ($P<0.001$)						

SD: standard deviation APD: atrial premature depolarization: CI, confidence interval

The change in morphology was significantly horse-dependent for all variables, especially for the S wave amplitude (P<0.0001). In some horses, there was a large increase in the S wave amplitude in case of an APD whereas in other horses S wave amplitude hardly changed. The horses with the highest and lowest change in S wave amplitude had a mean±SD increase in S amplitude of 44±10% and 3±5%, respectively.

Changes in P and T wave morphology

To determine associations between P wave and T wave morphology and APDs, 492 P waves (40% APDs, 60% sinus beats) and 588 T waves (50% APDs, 50% sinus beats) could be used. The APDs were significantly associated with a singular positive P wave (OR: 11.0, P<0.001) and were respectively more and less likely to have single positive (OR: 9.2, P<0.001) or single negative (OR: 0.2, P<0.001) T waves (Table 2). The RR interval significantly influenced T wave morphology (P<0.05). Within APDs, a monophasic positive T wave was associated with a shorter RR interval (mean ±SD: 935±46 ms), whereas monophasic negative T waves were associated with the longest mean RR interval (1485±62 ms). Those APDs with biphasic negative-positive T waves had a mean RR interval of 1286±42 ms. In normal sinus beats, the P wave was most often bifid (79.6%), whereas 62.2% of APDs had a monophasic positive P wave. As for the T wave, in normal sinus beats it was most often biphasic negative-positive (67.3%), with a fairly small percentage monophasic positive (16.7%) or monophasic negative (15.3%) T waves. In APDs, however, almost half of the T waves changed polarity towards a monophasic positive morphology (41.5%; Table 2). APD morphology was not always consistent within the individual horse. The horse effect was significant (P<0.001) for P wave morphology, but not for T wave morphology.

Table 2. Changes in P and T wave morphology associated with APDs						
		Sinus beat	APD	OR	95% CI	P-value
P wave	Bifid	223 (79.6%)	60 (30.0%)	Ref.		
	Singular	66 (22.7%)	125 (62.2%)	11	6.1-20.0	<0.001
	Biphasic	2 (6.9%)	16 (8.0%)	4	5.7-333.0	<0.001
Total		291	201			
T wave	Biphasic: Pos-neg	2 (0.7%)	0 (0%)	Excluded from analysis		
	Biphasic: Neg-pos	198 (67.3%)	149 (50.7%)	Ref.		
	Monophasic: Pos	49 (16.7%)	122 (41.5%)	9.2	5.1-16.5	<0.001
	Monophasic: Neg	45 (15.3%)	23 (7.8%)	0.2	0.1-0.5	<0.001
Total		294	294			
Horse effect was significant for P wave morphology (*P*<0.001) not for T wave morphology						

APD, atrial premature depolarization; OR, odds ratio; CI, confidence interval

Relationship between RR coupling interval and S wave amplitude

A total of 294 observations could be used to study the relationship between the RR interval and S wave amplitude. A significant negative relationship between RR coupling interval of the APD and the relative difference in S wave amplitude between a sinus beat and an APD was detected ($P<0.01$). The random horse effect was significant ($P<0.001$), and therefore detailed observations were made in every horse. In only 13% (4/30) of the horses, there was a significant ($P<0.05$) negative relationship between the RR coupling interval and the difference in S wave amplitude, and in 1 horse a significant positive relationship ($P<0.01$) was found. In the other horses there was no significant relationship.

Discussion

Our study shows that, besides a change in P wave morphology, APDs can induce changes in QRS complex and T wave morphology. In APDs, the S and T wave amplitude increased and PQ and RR interval decreased, whereas R amplitude and QRS duration did not change. The APDs were significantly associated with singular positive P waves and were more likely to have a single positive T wave.

In the present study, only conducted APDs for which the RR interval was at least 20% shorter than the RR interval of the preceding sinus beat were included. This value is probably relatively high, but was arbitrarily chosen because currently no data are available for differentiation between APDs and sinus arrhythmia. We chose not to include horses with VPDs because in these horses one might argue whether an altered QRS morphology is a results of fusion beats. In horses with only APDs, this was very unlikely to occur.

On visual inspection, the most prominent feature of an APD compared to its preceding sinus beat was a more negative S wave and a singular, instead of bifid, P wave. Furthermore, T waves often changed polarity in the case of an APD. Another important finding in this study was that horse was a strongly significant factor for all observations. This was especially clear for the change in S wave amplitude. Among the different horses, the mean increase in S wave amplitude for an APD ranged from 3±5% to 44±10%.

Overall, a significant negative relationship between RR interval and the relative increase in S wave amplitude was found, meaning that the shorter the RR coupling interval, the larger the S wave amplitude of the APD, when compared with the preceding sinus beat. This relationship, however, was strongly horse dependent and can be influenced by differences in vagal tone, intraventricular conduction and altered QRS depolarization or repolarization [9]. The RR interval also influenced T-wave morphology. Those APDs with short RR interval were more likely to have monophasic positive T waves.

Current veterinary literature about ECG in horses usually states that, in the case of an APD, P wave morphology changes, whereas QRS and T wave morphology remains similar [1,3,10]. In our study, P wave morphology did indeed change. The APDs were more likely to have singular positive P waves, instead of the bifid P waves which were seen in most normal sinus beats. In the case of an APD, the impulse originates from another location and conducts differently over the atrial myocardium, which might lead to altered ECG deflections [1,11]. In

our study, ECG registration was performed in a modified base-apex configuration because this configuration frequently is used in equine practice, especially during exercise and 24-hour ECG recording. This modified base-apex configuration produces fewer motion artifacts but lower P waves compared with a true base-apex configuration in which the right arm electrode is placed in the jugular groove [8]. Furthermore, although in our study all ECG measurements were performed using the second lead, the P wave in our electrode configuration generally was best visualized in the first lead of the ECG (between the right arm [-] and the left arm [+] electrode). Those P waves buried in preceding T waves were therefore sometimes difficult to identify and could not be measured in 32% of the APDs. In these cases, leads 1 and 3 were very helpful to identify the APD. Inspecting multiple lead traces is therefore useful for ECG interpretation.

Although it usually is stated that in the case of APDs, ventricular conduction is not affected and QRS complex and T wave morphology do not change, our study shows that, in addition to changes in P wave morphology, APDs also can lead to changes in QRS complex and T wave morphology. The APDs resulted in PQ shortening, increased S wave amplitude and a more positive T wave. Similar changes occur during exercise because of a positive inotropic effect and an increase in sympathetic tone [12,13].

Shortly coupled APDs may lead to very premature QRS complexes. For such short RR intervals, especially when occurring after a relatively long RR interval, the conduction system or parts of it might not be fully recovered yet, resulting in intraventricular conduction block. In other species, this conduction block commonly occurs at the right bundle branch and is known as Ashman phenomenon. The conduction block results in an aberrant conduction whereby part of the myocardium is depolarized from cell to cell. The ECG typically shows QRS widening with changes in QRS and T wave morphology. Whether similar mechanisms occur in horses is not known, but QRS widening did not occur. The increase in S amplitude and the T wave change toward a positive deflection are very similar to what occurs when heart rate physiologically increases (e.g. during exercise). Indeed, in 13% of the horses we found that, for the APDs, the RR interval and the change in S wave amplitude were inversely correlated, meaning that the shorter the RR coupling interval, the larger the difference in S wave amplitude between an APD and a sinus beat. In 83% of the horses, the relationship did not reach significance but this might be related to the small number of APDs (n=10) per horse. Only in 1 horse was a positive relationship found. Also, T wave morphology was

significantly influenced by changes in RR interval with a shorter RR interval leading to a more positive T wave.

A decrease in left ventricular preload has been shown to result in smaller QRS amplitudes, as explained by the Brody theory [14,15]. Premature depolarization results in a shorter diastolic interval and consequently a lower left ventricular preload. The increase in S wave amplitude found in our study can therefore not be explained by the Brody theory. Respiration also influences ECG amplitudes because of alterations in transthoracic impedance and changes in cardiac filling [16,17]. However, this effect is not associated with APD occurrence and therefore not likely to have any effect on our results.

In our study, a modified base-apex lead was used for ECG measurements because this lead often is used in clinical situations. The other Einthoven leads also were displayed for better interpretation of the ECG. However, one should be aware that most ECG units, such as the Televet100, only record lead 1 and 2 whereas lead 3 is calculated assuming an Einthoven triangle with the heart in the middle. In horses, these criteria are not fully met which results in minor changes, especially in amplitude. Nevertheless, using multiple leads can be helpful for ECG interpretation [7].

This study has several limitations. Observers were not blinded to the electrocardiographic complexes, so they were aware of whether a cardiac cycle was a normal sinus beat or an APD. Blinding the observers would have been difficult to accomplish, because, for measuring the RR interval, the previous cycle must be included. As mentioned before, larger P waves could have been recorded from a true base-apex ECG. However, the placement of electrodes and lead recording were chosen to mimic the clinical situation. Furthermore, there was no control group of healthy horses included. A control group of horses without APDs would have allowed us to determine the natural variability in electrocardiographic measurements between successive normal sinus beats in order to compare those results with the variability between an APD and its preceding normal sinus beat. In our study, an APD was defined as an individual atrial depolarization with an RR coupling interval at least 20% shorter (arbitrary value) than the RR interval of the preceding sinus beat. We chose this relatively large difference as a criterion to minimize inclusion of early sinus beats. There is, however, a chance that some early sinus beats were included as APDs.

In conclusion, our study demonstrated that APDs can lead to important changes in ventricular morphology. Clinically, the most important observation is the fact that the S wave becomes

more negative and the T wave often changes polarity from negative or biphasic to a monophasic positive T wave. These changes, which are strongly horse dependent, may lead to incorrect diagnoses, particularly when P waves are difficult to identify on the ECG. Clinicians should be aware of a possible change in QRS complex and T wave morphology not only with VPDs but also in the case of an APD in order to avoid mistakes. The use of multiple lead recordings can be useful to diagnose certain cardiac arrhythmias.

Footnotes

a: Televet100, Engel Engineering Services GmbH, Heusenstamm, Germany.

b: Mainat Vet, Barcelona, Spain.

c: Televet100 software version 5.1.2, Engel Engineering Services GmbH, Heusenstamm, Germany.

d: SAS Institute Inc., Cary, North Carolina, USA.

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CHAPTER 2:

HEART RATE VARIABILITY PARAMETERS IN HORSES DISTINGUISH ATRIAL FIBRILLATION FROM SINUS RHYTHM BEFORE AND AFTER SUCCESSFUL ELECTRICAL CARDIOVERSION

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Summary

Background: Atrial fibrillation (AF) is the most common pathological arrhythmia in horses. After successful treatment, recurrence is common. Heart rate monitors are easily applicable in horses and some devices offer basic heart rate variability (HRV) calculations. If HRV can be used to distinguish between AF and sinus rhythm (SR), this could become a monitoring tool for horses at risk for recurrence of AF.

Objectives: The purpose of this study was to assess whether in horses AF (before cardioversion) and SR (after cardioversion) can be differentiated based upon HRV parameters.

Study Design: Cohort study with internal controls.

Methods: Six HRV parameters were determined in 20 horses, both in AF and in SR, at rest (2- and 5-minute and 1- and 4-hour recordings) and during exercise (walk and trot, 2-minute recordings). Time-domain (SDNN, RMSSD and Triangular index), frequency-domain (LF/HF ratio) and nonlinear parameters (SD1 and SD2) were used. Statistical analysis was done using paired Wilcoxon Signed Rank Tests and Receiver Operating Curves.

Results: HRV was higher during AF compared to SR. Results for the detection of AF were good (AUC 0.8-1) for most HRV parameters. RMSSD and SD1 yielded the best results (AUC 0.9-1). Sensitivity and specificity were high for all parameters at all recordings, but highest during exercise. Although AUCs improved with longer recordings, short recordings were also good (AUC 0.8-1) for the detection of AF. In horses with frequent second degree atrioventricular block, HRV at rest is increased and recordings at walk or trot are recommended.

Main limitations: Animals served as their own controls and there was no long-term follow-up to identify AF recurrence.

Conclusions: AF (before cardioversion) and SR (after cardioversion) could be distinguished with HRV. This technique has promise as a monitoring tool in horses at risk for AF development.

Introduction

Atrial fibrillation (AF) is the most common clinically important arrhythmia in horses. Reported prevalence ranges between 0.3 and 2.5% of the population, with an overrepresentation in Standardbreds and Warmblood horses [1-3]. Timely diagnosis and treatment are important and definite diagnosis is made with electrocardiography (ECG) [4]. Successful restoration of sinus rhythm (SR) by electrical or pharmacological cardioversion can often be attained, but reported recurrence rates range between 16 and 43% [5-9]. Diagnosis of early recurrence cannot always be performed by lay people and may require frequent veterinary visits.

Heart rate variability (HRV) describes and quantifies the beat-to-beat variability and the long-term variation in heart rate [10]. Under the influence of both the autonomic nervous system and the neuroendocrine system, cyclical and beat-to-beat variations in heart rate are normal in the healthy individual. Decrease in parasympathetic tone and increase in sympathetic tone result in a decrease in short-term HRV. In human medicine, HRV is often used to predict prognosis in various cardiac disease states [11]. In horses it has been used to study the autonomic regulation of the heart and it is frequently used in animal welfare studies, quantifying stress and pain [12-17]. Since arrhythmia, especially AF, leads to an increased variation in beat-to-beat intervals, HRV parameters describing short-term (beat-to-beat) variability are expected to be increased compared to horses in SR. An increase in HRV in human patients with AF has been described and HRV parameters have been used to diagnose arrhythmias [18-22]. If HRV parameters can also be used to differentiate AF from SR in horses, a heart rate monitor or smartphone app with automated HRV calculations would be an accessible diagnostic tool for both veterinarians and horse owners to monitor their horses for the presence of AF. The purpose of the current study was to determine whether HRV parameters at rest, during exercise at different paces and at different recording durations, differ between horses in AF and in SR, before and after successful transvenous electrical cardioversion (TVEC), respectively.

Materials and Methods

Experimental design and population

In this cohort study, each horse was used as its own internal control. Twenty Warmblood horses presented to the Department of Equine Internal Medicine, Faculty of Veterinary Sciences, Ghent University, for the treatment of AF using TVEC, were used [23]. Inclusion criteria were electrocardiographic confirmation of AF and successful electrical cardioversion. Horses ranged between 5 and 15 years, with a mean±SD age of 10±3 years. Two stallions, 11 geldings and 7 mares were included. Echocardiography was performed in all horses in AF. A resting ECG and a standardized exercise ECG were taken from each horse before (AF) and 5 days after (SR) TVEC.

Electrocardiography

In all 20 horses an ECG was recorded using a Televet100 recording system [a]. Four self-adhesive electrodes were placed underneath a girth [b]. The right arm electrode was positioned 15 cm right of the withers, the left leg electrode caudal to the left elbow on the thorax and the left arm electrode 10 cm above the left leg electrode resulting in a modified base-apex configuration [24,25]. A reference electrode was placed on the left side of the withers. All electrodes were connected to the recording device, placed in the girth.

For resting ECG horses were confined in a stable with access to food and water. All horses had been stabled in this environment for at least 1 day in order to minimize environmental stress factors. The standardized lunging exercise test consisted of 5 minutes of walk, followed by 10 minutes of trot, 2 minutes of walk, 4 minutes of canter, 1 minute of gallop and 10 minutes of walking. The ECG recordings were analyzed offline using dedicated software [c]. Standard gain (10 mm/mV) and paper speed (25 mm/s) were used. RR intervals were automatically calculated by the software program (RR detection Equine), peak detection was set on negative (S wave). Three different leads were displayed simultaneously to improve ECG interpretation, but S peak detection was always performed in lead 2 (between the right arm (-) and left leg (+) electrode). All ECGs were visually inspected and corrected manually if necessary. Interference, electrical interruptions or software errors in peak detection were corrected or otherwise removed from the database. All cardiac arrhythmias were documented.

Heart rate variability

RR intervals were exported into a text file and subsequently imported into commercial software for HRV analysis [d]. Each text file was blinded by giving a random number before analysis. For the resting ECG, 15 minutes after starting the ECG recording, a 2-minute, 5-minute, 1-hour and 4-hour recording were analyzed. For the exercise ECGs, a 2-minute recording at walk and trot, starting 2 minutes after the onset of the walking and trotting phase, was analyzed. Six HRV parameters were used for further analysis. Three time-domain parameters: SDNN (standard deviation of the RR intervals), RMSSD (root mean squared successive differences in RR intervals) and Triangular Index (TI) (integral of RR interval histogram divided by height of the histogram); 1 frequency-domain parameter: LF/HF ratio (the ratio of the low frequency band over the high frequency band); and 2 nonlinear parameters: SD1 and SD2 (standard deviation of the Poincaré plot perpendicular to and along the line of identity, respectively) (Fig.1) [11]. For frequency-domain analyses, frequency values for horses were set at 0.001-0.005Hz for the very low frequency band (VLF), 0.005-0.07Hz for the low frequency band (LF) and 0.07-0.5Hz for the high frequency band (HF) [10-12]. There were no filters nor artifact corrections applied.

Data analysis

Statistical analysis was performed using dedicated software [e]. Descriptive statistics, quantile-quantile plots and Shapiro-Wilk tests were used to check for normality of the data. Data were not normally distributed. Paired Wilcoxon Signed Rank Tests were used to detect significant differences between observations. Significance was set at $P < 0.05$. Receiver operating characteristic (ROC) curves were used to study the performance of a parameter as a discriminatory variable for the detection of AF. The coordinate points of the ROC curves were used to determine the most fitting cut off values for each parameter at each recording. Cut off values were chosen to maximize sensitivity whilst retaining good ($\geq$80%) specificity, where possible.

Results

Echocardiography revealed mild to moderate mitral (11/15; 3/15), aortic (5/15, 0/15), tricuspid (7/15; 3/15) and pulmonary (1/15; 0/15) valve regurgitation in 15 horses and mild dilatation of the right (3/3) and left (2/3) ventricle and the right (1/3) and left (1/3) atrium in 3 of those horses. In 5 horses, no structural or functional echocardiographic abnormalities were detected.

All HRV values were significantly different in SR compared with AF (Table 1). In 11 horses in SR at rest, occasional arrhythmias (<1% of RR intervals) such as supraventricular premature depolarizations (SVPD) (8/11), second degree atrioventricular block (AVB2) (6/11), ventricular premature depolarizations (VPD) (2/11) and ventricular tachycardia (VT) (1/11) increased HRV, but not above the cut off values for AF. In 6 horses with frequent episodes of sinus arrhythmia, HRV parameters were within the limits of the other horses in SR. Where more arrhythmias (1-10% of RR intervals, n=2) were present, HRV values increased and sometimes hampered differentiation between SR and AF at rest. In 2 horses with very frequent (12% and 18% of the RR intervals) AVB2, HRV values at rest in SR approached or even exceeded those in AF. When these horses were exercised, AVB2 disappeared and HRV values decreased to within the range of the other horses in SR (Fig.1).
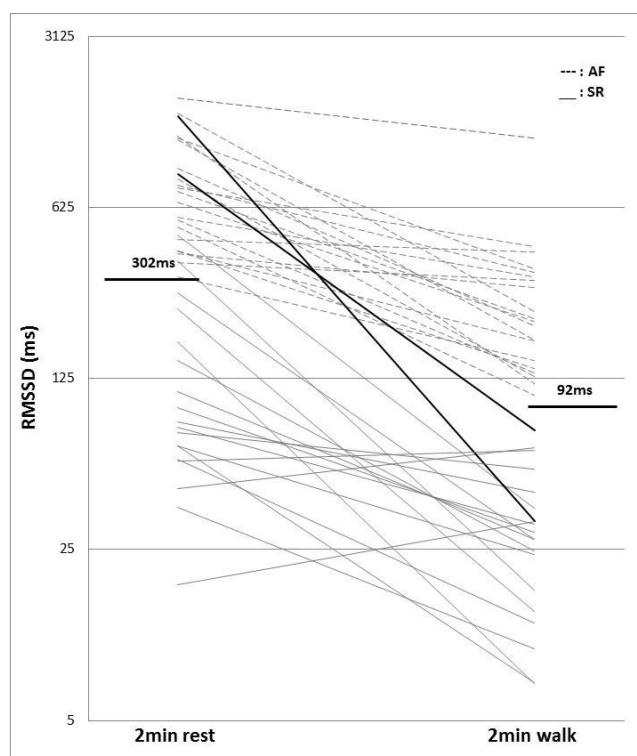
Fig 1: RMSSD of 20 Warmblood horses in atrial fibrillation(AF; dotted lines) and in sinus rhythm (SR; solid lines) during a 2 minute ECG recording at rest and walk. Cut off RMSSD values for the detection of atrial fibrillation (302ms at rest and 92ms at walk) are represented by the horizontal lines. Notice that 2 horses (black lines) with frequent second degree atrioventricular block in SR (black lines) show high RMSSD at rest, above the cut off for AF. At walk, both horses show an RMSSD below the cut off value.

Phase	Variable	SR			AF			P- value
		Median	Min	Max	Median	Min	Max	
2 min rest	SDNN (ms)	158	22	901	462	240	1111	0.001
	RMSSD (ms)	89	18	1472	689	325	1745	0.001
	TI	14	4	21	22	13	34	<0.001
	SD1 (ms)	63	13	1051	490	21	1243	0.001
	SD2 (ms)	201	22	741	440	247	475	0.002
	LF/HF ratio	0.93	0.1	24.08	0.19	0.06	1.45	0.001
5 min rest	SDNN (ms)	154	65	781	469	306	1057	0.001
	RMSSD (ms)	102	29	1215	675	375	1686	0.001
	TI	17	8	40	34	24	45	<0.001
	SD1 (ms)	75	21	862	478	266	1196	0.001
	SD2 (ms)	196	63	838	478	308	902	0.002
	LF/HF ratio	2.4	0.09	70.23	0.16	0.85	0.43	<0.001
1 hour rest	SDNN (ms)	190	127	768	55	307	1077	<0.001
	RMSSD (ms)	128	47	1057	813	292	1647	<0.001
	TI	29	18	72	58	29	73	<0.001
	SD1 (ms)	91	33	748	575	277	1165	<0.001
	SD2 (ms)	242	156	788	544	327	982	0.001
	LF/HF ratio	2.79	0.17	39.86	0.26	0.13	0.36	<0.001
4 hours rest	SDNN (ms)	231	130	765	627	279	1148	<0.001
	RMSSD (ms)	153	42	1103	886	366	1804	<0.001
	TI	44	23	80	70	46	88	0.001
	SD1 (ms)	109	30	78	627	259	1276	<0.001
	SD2 (ms)	298	181	749	626	298	1003	<0.001
	LF/HF ratio	2.41	0.2	25.85	0.25	0.19	0.37	<0.001
2 min walk	SDNN (ms)	79	24	123	186	93	935	<0.001
	RMSSD (ms)	28	7	77	216	106	1195	<0.001
	TI	12	4	19	20	16	29	<0.001
	SD1 (ms)	20	5	55	153	75	850	<0.001
	SD2 (ms)	110	33	172	199	100	1017	<0.001
	LF/HF ratio	7.54	0.39	45.82	0.4	0.08	3.62	<0.001
2 min trot	SDNN (ms)	19	7	39	70	39	112	<0.001
	RMSSD (ms)	7	3	53	84	48	130	<0.001
	TI	4	2	9	13	8	21	<0.001
	SD1 (ms)	5	2	37	59	34	92	<0.001
	SD2 (ms)	24	8	51	73	43	138	<0.001
	LF/HF ratio	5.35	0.22	24.5	0.26	0.08	0.92	<0.001

Table 1: Median and range of 6 heart rate variability parameters in 20 warmblood horses in sinus rhythm and in atrial fibrillation at rest and during exercise.

CI: conficence interval, SR: sinus rhythm, AF= atrial fibrillation, SDNN= standard deviation of the RR intervals, RMSSD = root mean squared successive differences, TI = Triangular Index, SD= standard deviation of the Poincaré plot perpendicular to (1) and along (2) its line of identity, LF= low frequencies, HF= high frequencies, ms = milliseconds

Table 2. Area under the Receiver Operating Curves (ROC) with cut off values, sensitivity and specificity for 6 heart rate variability parameters in 5 different recording phases for the detection of atrial fibrillation (AF) in 20 horses. A value above the cut off indicates AF, except for * where it indicates SR.												
Phase	Variable	AUC	95% CI		Cut off	Sens	95% CI		Spec	95% CI	
			Lower B	Upper B			Lower B	Upper B		Lower B	Upper B
2 min rest	SDNN (ms)	0.89	0.78	1	248	1	0.8	1	0.8	0.56	0.93
	RMSSD (ms)	0.9	0.79	1	302	1	0.8	1	0.8	0.56	0.93
	Triangular I	0.92	0.84	1	17	0.9	0.67	0.98	0.8	0.56	0.93
	SD1 (ms)	0.9	0.79	1	215	1	0.8	1	0.8	0.56	0.93
	SD2 (ms)	0.87	0.75	0.99	283	0.9	0.67	0.98	0.8	0.56	0.93
	LF/HF ratio*	0.83	0.7	0.96	0.41	0.95	0.73	1	0.7	0.45	0.87
5 min rest	SDNN (ms)	0.89	0.78	1	279	1	0.8	1	0.8	0.56	0.93
	RMSSD (ms)	0.93	0.83	1	367	1	0.8	1	0.9	0.67	0.98
	Triangular I	0.9	0.8	1	24	1	0.8	1	0.8	0.56	0.93
	SD1 (ms)	0.93	0.83	1	260	1	0.8	1	0.9	0.67	0.98
	SD2 (ms)	0.87	0.74	1	299	1	0.8	1	0.8	0.56	0.93
	LF/HF ratio*	0.88	0.76	1	0.38	0.95	0.73	1	0.85	0.61	0.96
1 hour rest	SDNN (ms)	0.92	0.82	1	305	1	0.8	1	0.8	0.56	0.93
	RMSSD (ms)	0.94	0.85	1	310	1	0.8	1	0.9	0.67	0.98
	Triangular I	0.88	0.76	0.99	44	0.9	0.67	0.98	0.8	0.56	0.93
	SD1 (ms)	0.94	0.85	1	219	1	0.8	1	0.9	0.67	0.98
	SD2 (ms)	0.88	0.76	0.99	430	0.85	0.61	0.96	0.85	0.61	0.96
	LF/HF ratio*	0.96	0.87	1	0.39	1	0.8	1	0.95	0.73	1
4 hour rest	SDNN (ms)	0.93	0.83	1	350	0.94	0.73	1	0.85	0.61	0.96
	RMSSD (ms)	0.94	0.85	1	318	1	0.8	1	0.9	0.67	0.98
	Triangular I	0.89	0.78	1	50	0.94	0.73	1	0.75	0.51	0.9
	SD1 (ms)	0.94	0.85	1	225	1	0.8	1	0.9	0.67	0.98
	SD2 (ms)	0.91	0.8	1	394	0.94	0.73	1	0.8	0.56	0.93
	LF/HF ratio*	0.96	0.88	1	0.59	1	0.8	1	0.9	0.67	0.98
2 min walk	SDNN (ms)	0.97	0.93	1	112	0.9	0.67	0.98	0.9	0.67	0.98
	RMSSD (ms)	1	1	1	92	1	0.8	1	1	0.8	1
	Triangular I	0.96	0.91	1	16	0.9	0.67	0.98	0.9	0.67	0.98
	SD1 (ms)	1	1	1	65	1	0.8	1	1	0.8	1
	SD2 (ms)	0.91	0.82	1	148	0.85	0.61	0.96	0.8	0.56	0.93
	LF/HF ratio*	0.96	0.9	1	1.13	0.95	0.73	1	0.9	0.67	0.98
2 min trot	SDNN (ms)	1	1	1	39	1	0.8	1	1	0.8	1
	RMSSD (ms)	1	0.98	1	46	1	0.8	1	0.95	0.73	1
	Triangular I	0.99	0.98	1	7.8	1	0.8	1	0.9	0.67	0.98
	SD1 (ms)	1	0.98	1	33	1	0.8	1	0.95	0.73	1
	SD2 (ms)	0.99	0.96	1	42	1	0.8	1	0.9	0.67	0.98
	LF/HF ratio*	0.96	0.9	1	0.94	1	0.8	1	0.9	0.67	0.98

AUC: area under the curve, CI: confidence interval,Lower B: lower bound, Upper B: upper bound, SR: sinus rhythm, AF= atrial fibrillation, SDNN= standard deviation of the RR intervals, RMSSD = root mean squared successive differences, TI = Triangular Index, SD= standard deviation of the Poincaré plot perpendicular to (1) and along (2) its line of identity, LF= low frequencies, HF= high frequencies, ms = milliseconds

Area under the ROC curves and cut off values with their sensitivity and specificity for each parameter at each recording duration at rest or during exercise, are summarized in Table 2. AUCs were high (>0.8) for all parameters at rest and very high (>0.9) during exercise. RMSSD (Fig. 2) and SD1 overall had the highest sensitivity (100%) and specificity (80-100%). From a 2-minute walk recording, RMSSD and SD1 had an AUC of 1 and 100% sensitivity and specificity.

Discussion

The current study demonstrated that AF (before TVEC) and SR (after TVEC) can be differentiated based upon HRV parameters. Cut off values with good sensitivity and specificity were determined for SDNN, RMSSD, TI, LH/HF ratio, SD1 and SD2 at rest and during walking and trotting. At rest, good results with high sensitivity and specificity were obtained, even for short recordings. Light exercise (walk and trot), however, led to the most reliable cut off values.

In this study, HRV is not used in its traditional way to study the autonomic control of the heart, but to quantify the variation in RR intervals to detect the presence of a certain arrhythmia. Ranges of HRV parameters are therefore different and not comparable with values published in previous studies using HRV as a tool to study autonomic tone. Higher variability represents more arrhythmia. In horses, due to their high parasympathetic tone, arrhythmias are common. AF, however, leads to an extremely irregular heart rhythm. The variability in heart rate was therefore expected to be higher when horses were in AF compared to SR. In human medicine several algorithms using HRV parameters are implemented in automatic devices detecting AF [18-22].

Apart from AF, other arrhythmias might also increase HRV. We chose to study horses before and after TVEC as arrhythmias, such as SVPD, were expected to occur more frequently in the post cardioversion period, making the differentiation between AF and SR challenging. The presence of occasional (<1% of the RR intervals) arrhythmias did not increase HRV above the cut off values for AF but very prevalent arrhythmias might make the differentiation between SR and AF more difficult. In this study, frequent AVB2 resulted in increased HRV values but AVB2 was abolished at walk or trot. Most HRV parameters are based on beat-to-beat variability in RR interval. In AVB2, a blocked beat leads to a major increase in RR interval, while other arrhythmias, such as premature beats or sinus arrhythmia, lead to smaller changes, with less influence on HRV. In horses with frequent AVB2 or other arrhythmias, care should thus be taken when drawing conclusions from recordings at rest and suspicious recordings should be repeated while walking or trotting the horse. If HRV remains increased during exercise, a pathological arrhythmia is likely to be present. However, more studies are necessary to assess the influence of different pathological arrhythmias on HRV.

Based on the results of a preliminary study investigating 144 different HRV parameters, data available from literature and availability of parameters on different heart rate monitors, HRV

devices and internet applications, we used 6 HRV parameters for our study [11,26]. The simplest HRV value to calculate is SDNN. This is a time-domain parameter expressing overall HRV[11]. It is easy to use and readily available on various commercial heart rate monitors. Good results were obtained for SDNN, especially during exercise. At rest, the presence of frequent AVB2 can lead to false positives. RMSSD is mainly used to represent short-term HRV (vagal influences) [10,13]. Cut off values for this parameter had a sensitivity of 100% and a specificity 80-100%, identifying all cases of AF with only a small chance of false positives. RMSSD is routinely used and available, which makes it an excellent tool for home monitoring. TI was chosen to include a geometrical time-domain parameter and represents overall HRV. It has the advantage of being less sensitive to the analytical quality of the RR interval, excludes outliers and covers many problems of artifacts [10]. Its major disadvantage is the need for a relatively large number of RR intervals to construct the histogram. In horses, recordings of at least 60 minutes are advised to ensure the correct performance of geometric methods for studying autonomic tone [10]. SD1 and SD2 are derived from nonlinear dynamics and based on the Poincaré Plot of the RR intervals. While excellent results were obtained for SD1, SD2 yielded moderate results. Since SD2 mainly represents long-term HRV, and beat-to-beat variability is averaged out, it was expected to be less influenced by AF. The Poincaré plot displays the RR intervals against their previous RR interval. Arrhythmias will thus cause a dispersion of the plot towards the upper left and lower right area of the graph, increasing SD1 in particular (Fig.2). Finally, the ratio between LF and HF components was used as a power spectral density analysis method of HRV. In contrast to the other parameters, a value lower (and not higher) than the cut off value indicates the presence of AF. For short recordings at rest moderate sensitivity and specificity was obtained, so longer recordings at rest or recordings during exercise are necessary.

Cut off values determined in this study were chosen to optimize sensitivity whilst retaining good specificity. As AF may be associated with potentially dangerous ventricular rhythms during exercise and because early diagnosis positively affects treatment outcome, our focus was to identify all horses with AF, even if this would include some false positives [4]. Furthermore, if RR identification is correct and no artifacts or electrical interference are present on the ECG, false positives will most likely arise from horses with frequent arrhythmias. If these horses remain positive during exercise (walk and trot), it is likely that a pathological arrhythmia is present and further examination (ECG) is indicated.
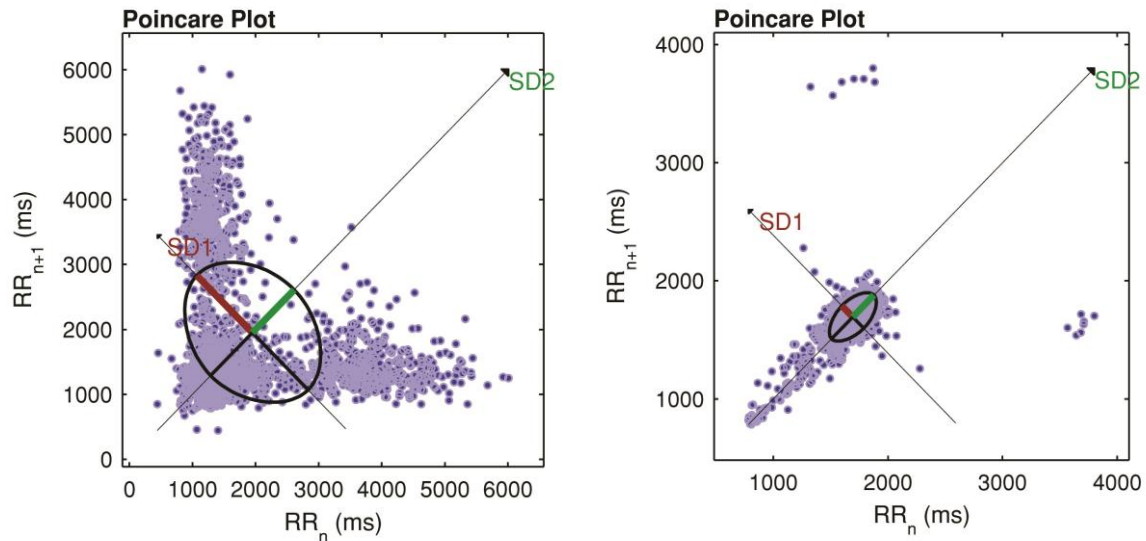
Fig 2: Poincaré plots of a horse at rest during atrial fibrillation (left) and sinus rhythm (right). RR intervals are plotted against their preceding RR interval. SD1 and SD2 are calculated as the standard deviation of the Poincaré plot perpendicular to and along the line of identity, respectively. Notice the dispersion of the values towards the upper left and lower right corner, increasing SD1 in case of atrial fibrillation. In case of sinus rhythm, notice how 7 second degree atrioventricular blocks, increasing SD1, are visible in the upper left and lower right corner of the graph.

This study demonstrated that AF can be distinguished from SR before and after TVEC using HRV. Because arrhythmias, especially SVPD, often appear more frequently in the post-cardioversion period, this differentiation was probably more challenging in the current study than if a control group of normal horses had been used. Therefore it seems highly likely that HRV monitoring can be used to monitor horses at risk for developing AF, such as horses with mitral valve regurgitation, a dilated atrium, SVPD or horses after successful AF treatment. However, an important limitation of this study is that we did not include long-term follow-up until recurrence.

Heart rate monitors can automatically generate some basic HRV calculations, mostly SDNN, RMSSD and/or SD1. The majority of equine HRV studies have used Holter recordings, but equine heart rate monitors have also been used [14,27,28]. This is an accessible tool for both veterinarians and horse owners. In the current study, we used very short recording durations (2 and 5 minutes). Our study demonstrated only slight improvements in sensitivity and specificity when using longer (1 or 4 hour) recordings. Heart rate monitors have the benefit of being affordable and easy to use, but they also have inherent limitations. Heart rate monitors

automatically detect the R-peak of the ECG. In horses, the T wave can by very pronounced and systems based on detecting R peaks by looking for sharp deflections from the baseline, can erroneously register T waves. Movement and electrical artifacts are common, especially during exercise, and may lead to erroneous R peak registrations. Most heart rate monitors are not validated for the use in horses and significant differences between results from heart rate monitors and ECG recordings have been shown [28]. Further studies are necessary to assess whether heart rate monitors can correctly detect RR intervals in horses and whether they can differentiate between horses in AF and in SR.

We did not include exercise at high speed in this study. The exercise tests in SR were performed 5 days after TVEC and only low-level exercise was performed. In addition, based upon HRV parameters, discriminating between AF and SR at high heart rates is expected to be more difficult. In human medicine, HRV after cardioversion is used to predict AF recurrence. This was beyond the scope of the current study, but warrants further attention.

Conclusions

This study demonstrated that HRV is significantly increased when horses are in AF, compared to when they are in SR. HRV could potentially be used as a tool for early detection of AF in horses judged to be at risk. ECG would then be required to confirm the diagnosis.

Manufacturers' Details

a: Televet100, Engel Engineering Services GmbH, Heusenstamm, Germany.

b: Mainat Vet, Barcelona, Spain.

c: Televet100 software version 6.0, Engel Engineering Services GmbH, Heusenstamm, Germany.

d: Kubios 2.2,University of Eastern Finland, Finland

e: IBM SPSS Statistics 24, IBM corp., United States

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CHAPTER 3:

HEART RATE VARIABILITY PARAMETER RMSSD OBTAINED FROM A HEART RATE MONITOR CAN DISTINGUISH ATRIAL FIBRILLATION FROM SINUS RHYTHM IN HORSES

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Summary

Background: Heart rate variability (HRV) parameters, and especially RMSSD (root mean squared successive differences in RR interval), can be used to diagnose atrial fibrillation (AF) in horses. Some heart rate monitors (HRM) offer basic HRV calculations.

Objectives: To assess whether RMSSD values obtained from a HRM can differentiate AF from sinus rhythm (SR) in horses. Furthermore the impact of artifact correction before HRV analysis was evaluated.

Animals: Fourteen horses before and after AF treatment.

Methods: Prospective clinical trial. Horses were equipped with two HRMs, one using plastic electrodes and another using an equine electrode belt, and an ECG. A two-minute recording at rest, walk and trot, before and after cardioversion, was obtained. RR intervals were determined automatically by the HRM and by equine ECG analysis software, and manually from the ECG trace. RMSSD was calculated by the HRM software and by dedicated HRV software, using six different artifact filters. Statistical analysis was performed using paired t-tests, one-way ANOVA and receiver operating curves.

Results: RR detection using an electrode belt was inconsistent. The HRM with plastic electrodes, which applies a low level filter, produced high AUCs (>0.9) and cut off values with high sensitivity and specificity for AF detection. Similar results were obtained for the ECG, when low level artifact filtering was applied. When no artifact correction was used during trotting, an important decrease in AUC (0.75) occurred.

Conclusion: HRMs with automatic RMSSD calculations can distinguish between AF and SR and might be useful as a monitoring tool for AF detection.

Introduction

With a prevalence of 0.3-2.5% of the population, atrial fibrillation (AF) is the most common clinically important arrhythmia in horses. Treatment, either by pharmacological or electrical cardioversion, is often successful, but recurrence rates are reported to be relatively high (16-43%) [1-5]. Most horses with AF have little or no underlying cardiac diseases and clinical signs limited to different grades of poor performance. During strenuous exercise horses with AF can reach extremely high heart rates which may lead to R-on-T phenomenon and even collapse or sudden death [6-8]. Timely diagnosis and treatment are important. After successful AF treatment, home monitoring by means of frequent auscultation or ECG is advised to check for AF recurrence. However, this cannot always be performed by lay people, requiring frequent veterinary visits or specialized equipment, which is time consuming and expensive.

Heart rate variability (HRV) describes and quantifies the beat-to-beat variability and the long-term variation in heart rate [9]. The heart rate is influenced by both the autonomic nervous system and the neuroendocrine system. Cyclical and beat-to-beat variations in heart rate are normal in the healthy individual. In horses, HRV has been used to study the autonomic regulation of the heart and in animal welfare studies, quantifying stress and pain [10-12]. Aside from its use as indicator of autonomic tone, HRV is used in human medicine to diagnose arrhythmias [13,14]. Since arrhythmia, especially AF, leads to an increased beat-to-beat interval variation, HRV parameters describing short-term variability are increased compared to sinus rhythm (SR) and algorithms using HRV parameters are implemented in devices detecting AF[15-19]. In horses, HRV has already been evaluated for the detection of AF [20]. In that study, 6 different HRV variables were calculated from RR intervals of a manually corrected ECG, both in AF and in SR. RMSSD, the root mean squared successive differences in RR interval, yielded the best results with high sensitivity and specificity to distinguish AF from SR.

Different types of heart rate monitors (HRM) are increasingly being used in horses and some of them offer basic HRV calculations, including RMSSD [11,21,22]. They might be an accessible diagnostic tool for both veterinarians and horse owners to monitor horses for AF. Most HRMs, however, have not been validated for use in horses and significant differences between their RR registration and RR intervals from ECG recordings have been shown [23]. Therefore the purpose of this study was to assess whether AF and SR can be differentiated in horses based upon RMSSD generated by a HRM and to compare this technique with manual ECG interpretation as golden standard. Furthermore the impact of artifact correction before HRV analysis was evaluated.

Materials and Methods

Study design and study population

Prospective clinical trial. Fourteen warmblood horses were presented to the Faculty of Veterinary Medicine, Ghent University for the treatment of AF. All horses were warmblood horses with a mean±SD age, bodyweight and height at the withers of 12±6 years, 544±62kg and 167±13cm. The study population consisted of 5 mares, 8 geldings and 1 stallion. All horses were included in the study with informed owner consent and cared for according to the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals. Each horse was subjected to a recording at rest and a standardized exercise test including 5 minutes of walk and 10 minutes of trot, both in AF (before cardioversion) and in SR (5 days after cardioversion).

Electrodes and heart rate detection

Four different systems of heart rate detection (electrodes + RR detection) were compared (Table 1). As golden standard, a modified base-apex ECG, as described elsewhere [20], using adhesive electrodes [a] was manually analyzed using a commercial ECG software program [b] to obtain RR intervals (ECG$_{Man}$). The same surface ECG was subsequently automatically analyzed by the commercial ECG software program [b] (ECG$_{Aut}$). Furthermore, a commercial plastic electrode set [c] and an equine heart rate electrode belt [d], each with their own heart rate sensor for RR detection [e], were attached according to the manufacturer's instructions (HRM$_{Elec}$ and HRM$_{Belt}$).

Because subjective assessment of heart rate detection suggested inconsistency, a preliminary evaluation of the different heart rate detection methods was conducted after examination of the first 8 horses. All RR interval files obtained by the different heart rate detection methods (ECG$_{Man}$, ECG$_{Aut}$, HRM$_{Elec}$ and HRM$_{Belt}$) were imported into a software program for HRV analysis [f] and time-matched 2 minute recordings at rest and during walk and trot, were analyzed. Due to inconsistent results using the electrode belt, this technique was not further used in the remaining horses and all belt data were omitted from further analysis.

Table 1. Four different methods for heart rate detection and heart rate variability calculations in 14 warmblood horses. Due to inconsistent results, heart rate detection using the equine heart rate electrode belt was not continued after the first 8 horses and all belt data were further omitted from the study.

Method	Electrodes	Heart rate detection	Heart rate variability
ECGMan	Adhesive electrodes (Skintact)	Manual RR detection (Televet 100)	Kubios HRV Analysis
ECGAut	Adhesive electrodes (Skintact)	Automatic RR detection (Televet 100)	Kubios HRV Analysis
HRMElec	Plastic heart rate electrodes (Polar)	Heart rate sensor Equine H7 (Polar)	Polar Flow software
HRMBelt	Equine heart rate electrode belt (Polar)	Heart rate sensor Equine H7 (Polar)	/

Heart rate variability and artifact correction methods

From all 14 horses, RR intervals from both ECG_{Man} and ECG_{Aut} were imported in Kubios Software [f] for HRV analysis, while RR data from HRM_{Elec} were imported into its own commercial software program [g] (Table 1). Subsequently, a time-matched 2-minute recording at rest, walk and trot, both in AF and SR, was selected for calculation of RMSSD. Each recording was obtained 2 minutes after a change in gait, in order to exclude movement artifacts and stress reactions from the gait change.

Furthermore, for the ECG_{Aut} method, using the Kubios HRV software program [f], 5 different levels of artifact correction were applied. The artifact correction algorithm of this program compares every RR interval value against the average RR interval of the 2-minute recording. The average is obtained by median filtering of the RR interval time series, and is therefore not affected by single outliers in RR interval time series. RR intervals that differ from the local average more than a specified threshold value, are identified as artifacts and corrected by replacing the corrupted RR intervals by interpolated RR values. The threshold values used in this study were 0.45s (very low artifact correction), 0.35s (low), 0.25s (moderate), 0.15s (high) and 0.05s (very high).

Statistical analysis

Statistical analysis was performed using dedicated software [h]. Descriptive statistics, quantile-quantile plots and Shapiro-Wilk tests were used to check for normality of data.

To evaluate the RR detection methods, mean differences in RMSSD and its standard deviations (SD) between the evaluated method and the golden standard were calculated and student paired t-tests were used to assess significant differences between the evaluated method and the golden standard. Significance was set at $P < 0.05$.

One-way ANOVA was used to detect significant differences in RMSSD between horses in AF and SR at different paces using the different detection methods and artifact correction levels. Receiver operating characteristic (ROC) curves were used to study the performance of each method and each artifact correction level as a discriminatory variable for the detection of AF. The coordinate points of the ROC curves were used to determine the most fitting cut off values for each parameter at each recording. Cut off values were chosen to maximize sensitivity whilst retaining good ($\geq 80\%$) specificity, where possible.

Results

Electrodes and heart rate detection

Since RMSSD was calculated using the same software program for each RR detection method, differences in RMSSD were caused by differences in RR intervals and therefore by differences in RR detection. A graphical comparison between golden standard RMSSD from manually analyzed ECG and the 4 different methods for heart rate detection in the first 8 horses is presented in Fig.1. RMSSD values using HRM_{Belt} were significantly different (P<0.05) from the golden standard method (ECG_{Man}) at all paces, both in AF and in SR, except for the recording at rest in SR (P=0.2). Mean differences between the ECG_{Man} and ECG_{Aut}, HRM_{Elec} and HRM_{Belt} were ranging, for the different paces, between -38ms and -41ms, -22ms and -43ms and between +7ms and -20ms, respectively. SD of the mean differences between RMSSD obtained from ECG_{Man} and HRM_{Belt} were large (range 171 – 241 ms), indicating inconsistent heart rate detection with the belt. Therefore, the use of the belt was not continued and all belt data were omitted from the study.

Heart rate detection using the other methods was not significantly different from the golden standard (P>0.05) and SD of the mean differences in RMSSD for ECG_{Aut} and HRM_{Elec} were small (range 38-42 ms) and medium (range 79-118 ms), respectively.
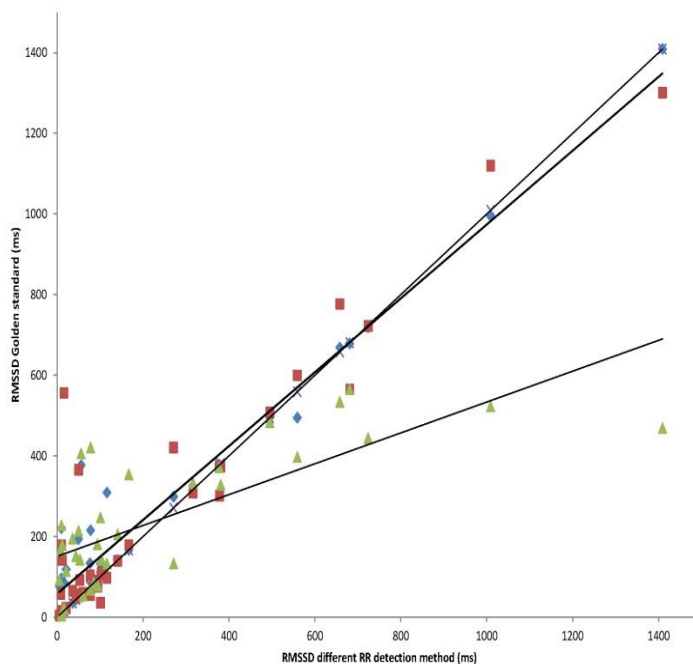
Fig. 1. Graphical comparison of RMSSD obtained by 4 different heart rate detection methods in 8 horses in atrial fibrillation.

ECG_{Man}= *a manually analyzed ECG (Televet 100) with adhesive electrodes which served as golden standard. ECG_{Aut}= an automatically analyzed ECG (Televet 100) with adhesive electrodes. HRM_{Elec}= heart rate monitor (Polar Equine H7) using two plastic electrodes. HRM_{Belt}= a heart rate monitor (Polar Equine H7) using an equine heart rate electrode belt. Kubios HRV software was used to calculate RMSSD values.*

Distinguishing AF from SR and effect of artifact correction level

Mean values for RMSSD are displayed in Table 2. RMSSD values were significantly different (P<0.001) between horses in SR and horses in AF, except when a very high level of artifact correction was used and at trot if no artifact correction was applied.

AUCs, cut off values and their sensitivity and specificity for the use of RMSSD as a discriminatory variable for the detection of atrial fibrillation are displayed in Table 3. ECG_{Aut}, without artifact correction, led to reliable results at rest and at walk (AUC 0.96), but there was an important decrease in AUCs during trotting exercise (AUC: 0.75). Low level artifact correction during trotting exercise, led to a clear improvement in AUC (=1) and cut off values with very high sensitivity and specificity. High levels of artifact correction resulted in an important drop in AUCs, especially at rest. Using the Polar Flow software[g] in combination with a plastic electrode set[c] (HRM_{Elec}), led to reliable cut off values, especially during exercise.

| Pace | Method | Artifact correction | RMSSD SR | | RMSSD AF | | P-value |
			Mean	SD	Mean	SD	
Table 2. Mean ± SD RMSSD values of 14 horses before (in atrial fibrillation (AF)) and after cardioversion (in sinus rhythm (SR)).							
Rest	ECGMan	none	107.21	86.99	725.71	339.3	< 0.001*
	ECGAut	none	164.29	150.84	725.79	340.37	< 0.001*
	ECGAut	Kubios very low	82.86	44.37	232.71	31.32	< 0.001*
	ECGAut	Kubios low	80.07	42.42	195.5	18.62	< 0.001*
	ECGAut	Kubios moderate	71.36	36.46	147.79	18.17	< 0.001*
	ECGAut	Kubios high	57.71	22.52	80.07	14.47	0.006*
	ECGAut	Kubios very high	31.5	10.18	32.07	21.72	0.93
	HRMElec	Polar Flow	86.14	38.66	216.93	76.35	< 0.001*
Walk	ECGMan	None	55.29	45.49	252.71	106.47	< 0.001*
	ECGAut	None	90.5	58.61	262.86	96.33	< 0.001*
	ECGAut	Kubios very low	42.36	26.68	182.79	31.18	< 0.001*
	ECGAut	Kubios low	38.29	24.87	164.79	30.12	< 0.001*
	ECGAut	Kubios moderate	35.64	19.96	134.07	18.58	< 0.001*
	ECGAut	Kubios high	30.36	15.09	82.29	10.12	< 0.001*
	ECGAut	Kubios very high	22.14	9.64	31.5	10.9	0.29
	HRMElec	Polar Flow	37.21	22.22	115.71	30.34	< 0.001*
Trot	ECGMan	None	11	4.17	92.07	25.81	< 0.001*
	ECGAut	None	84.69	66.76	142.71	91.01	0.08
	ECGAut	Kubios very low	23.92	16.88	97	32.13	< 0.001*
	ECGAut	Kubios low	20.23	13.64	84.14	21.9	< 0.001*
	ECGAut	Kubios moderate	16.46	8.45	75.14	20.44	< 0.001*
	ECGAut	Kubios high	14.62	6.29	56.29	13.99	< 0.001*
	ECGAut	Kubios very high	11.77	6.48	23.57	9.69	0.02*
	HRMElec	Polar Flow	8.31	3.79	42.27	22	< 0.001*

*Two-minute recordings at rest, walk and trot were analyzed using 4 different methods of RR detection and HRV analysis. The manually analyzed ECG (ECG$_{Man}$) with HRV analysis using Kubios HRV Software was considered the golden standard method. HRM$_{Elec}$= Polar heart rate monitor (Equine H7) with plastic electrodes and Polar Flow Software. ECG$_{Aut}$= ECG with automatic RR interval detection (Televet 100) and Kubios HRV software with 5 different artifact correction levels. The artifact correction algorithm identifies every RR interval that differs from the average RR interval of the 2-minute recording more than a specified threshold value as artifacts and replaces the corrupted RR interval by interpolated RR values. The threshold values used in this study were 0.45s (very low artifact correction), 0.35s (low), 0.25s (moderate), 0.15s (high) and 0.05s (very high). * Indicates significant differences.*

					sensitivity		specificity	
Pace	Method	Artifact correction	AUC	cut off		95% CI		95% CI
Rest	ECGMan	none	0.99	215	1	0.77-1	1	0.77-1
	ECGAut	none	0.96	383	0.93	0.66-1	0.93	0.66-1
	ECGAut	Kubios very low	1	188	1	0.77-1	1	0.77-1
	ECGAut	Kubios low	0.99	131	1	0.66-1	0.93	0.66-1
	ECGAut	Kubios moderate	0.96	111	1	0.77-1	0.93	0.66-1
	ECGAut	Kubios high	0.8	65	0.93	0.66-1	0.69	0.42-0.92
	ECGAut	Kubios very high	0.38	24	0.5	0.23-0.77	0.38	0.13-0.65
	HRMElec	Polar Flow	0.92	136	0.86	0.57-0.98	0.86	0.57-0.98
Walk	ECGMan	None	0.97	121	0.93	0.66-1	0.93	0.66-1
	ECGAut	None	0.96	164	0.93	0.66-1	0.86	0.57-0.98
	ECGAut	Kubios very low	1	119	1	0.77-1	1	0.77-1
	ECGAut	Kubios low	1	111	1	0.77-1	1	0.77-1
	ECGAut	Kubios moderate	1	92	1	0.77-1	1	0.77-1
	ECGAut	Kubios high	0.99	57	1	0.77-1	0.93	0.66-1
	ECGAut	Kubios very high	0.74	22	0.78	0.49-0.95	0.57	0.29-0.82
	HRMElec	Polar Flow	0.97	70	0.93	0.66-1	0.93	0.66-1
Trot	ECGMan	None	1	36	1	0.77-1	1	0.77-1
	ECGAut	None	0.75	90	0.86	0.57-0.98	0.72	0.42-0.91
	ECGAut	Kubios very low	1	51	1	0.77-1	0.93	0.66-1
	ECGAut	Kubios low	1	42	1	0.77-1	0.93	0.66-1
	ECGAut	Kubios moderate	1	40	1	0.77-1	1	0.77-1
	ECGAut	Kubios high	1	33	1	0.77-1	1	0.77-1
	ECGAut	Kubios very high	0.93	15	0.93	0.66-1	0.93	0.66-1
	HRMElec	Polar Flow	1	21	1	0.77-1	1	0.77-1

Table 3. Area under the curve (AUC), cut off values and their sensitivity and specificity and 95% confidence intervals (95% CI) for the use of RMSSD as a discriminatory variable for the detection of atrial fibrillation in 14 warmblood horses.

Two-minute recordings at rest, walk and trot were analyzed using 4 different methods of RR detection and HRV analysis. The manually analyzed ECG (ECG$_{Man}$) with HRV analysis using Kubios HRV Software was considered the golden standard method. HRM$_{Elec}$= Polar heart rate monitor (Equine H7) with plastic electrodes and Polar Flow Software. ECG$_{Aut}$= ECG with automatic RR interval detection (Televet 100) and Kubios HRV software with 5 different artifact correction levels. The artifact correction algorithm identifies every RR interval that differs from the average RR interval of the 2-minute recording more than a specified threshold value as artifacts and replaces the corrupted RR interval by interpolated RR values. The threshold values used in this study were 0.45s (very low artifact correction), 0.35s (low), 0.25s (moderate), 0.15s (high) and 0.05s (very high).

Discussion

The ultimate goal of this study was to find an easily applicable tool for AF detection. In this study, AF could be differentiated from SR based upon RMSSD using a commercially available HRM. Investigation of different artifact filtering levels learned that high levels of artifact correction decreased test sensitivity and specificity, while low levels of artifact correction significantly improved sensitivity and specificity, especially during trotting exercise.

In this study RMSSD was chosen as the HRV parameter to detect AF. RMSSD is a time-domain HRV parameter and especially useful in assessing short-term HRV [9,24]. In a previous study, using 6 different HRV parameters for AF detection in horses, RMSSD yielded the best results with cut off values with high sensitivity and specificity, identifying all cases of AF with only a small chance of false positives [20]. Furthermore, RMSSD is a well-known and frequently used HRV parameter, available on a variety of HRMs, HRV software programs and mobile applications, which makes it a suitable tool for home monitoring. However, if RMSSD calculations are not available, SDNN (the standard deviation of the RR intervals) and SD1 (nonlinear parameter, derived from Point Caré plot), other frequently used HRV parameters and inter-related with RMSSD, might also be useful to detect AF [20].

Several methods to record heart rate were used in this study. Manual analysis of an ECG trace using adhesive electrodes [a] and calculation of RMSSD by specialized software [f] was used as golden standard. However, this requires specialized equipment, expertise for ECG interpretation and data handling (exporting, importing), which is time consuming and expensive. Automatic analysis of the ECG trace by dedicated software [b] reduces analysis time, but also introduces error. HRMs are increasingly being used by both veterinarians and horse owners, because they are relatively cheap and easy to use. They automatically detect heart rate and some devices also generate HRV calculations, including RMSSD. Previous studies, however, have demonstrated significant differences between HRMs and ECG recordings and many HRMs have not been thoroughly validated in horses [23]. In our study 2 different electrode systems for heart rate monitoring, were evaluated [12,21-23]. Heart rate detection using a commercially available equine heart rate electrode belt [d] was not reliable in our study, probably due to frequent movement artifacts, loss of contact and T wave detection, especially during exercise. Heart rate detection using a plastic electrode set [c] was moderately accurate.

For the data obtained by the HRM, we used Polar Flow commercial software [g] for HRV analysis. There are, however, numerous other, often cheaper, software programs and mobile phone applications available automatically importing RR data from HRMs via Bluetooth, displaying them in real time and immediately generating some basic HRV calculations. These applications might be useful candidates for home monitoring of horses at risk for AF recurrence but need to be validated.

Different HRV software programs might apply a different artifact correction method. The second goal of this study was to assess the effect of artifact correction levels on RMSSD as a discriminatory parameter for the AF detection. Artifact filters omit outliers, i.e. RR intervals that strongly differ from other RR intervals, and/ or replace them by interpolated values. Although this filtering helps to delete artifacts, it will also blunt the large beat-to-beat variation which is typical for AF. Low level artifact correction resulted in improved sensitivity and specificity approaching 100% at all paces. Furthermore, in horses in SR at rest, second degree atrioventricular block (AVB) was a common finding with a large impact on HRV. Low level artifact correction omitted AVB from the analysis, leading to better differentiation with horses in AF. The Polar Flow software[g] also uses an algorithm for artifact correction, which cannot be adapted. Unfortunately the authors were not able to obtain the details of this algorithm, but comparison with Kubios artifact correction[f] indicated that the Polar correction algorithm is of a low to moderate level. Other HRV software or applications often allow manual adaptation of the applied artifact correction. Care should be taken when using unknown algorithms, filters or artifact correction.

In human medicine, artifact correction algorithms are also used in AF detection devices[16,17,25]. Reported sensitivity and specificity of these human devices range from 86.6-100% and 84.3-99.9%, respectively [15,18,19,25].

Cut off values determined in this study were chosen to optimize sensitivity whilst maintaining a good specificity. Because early diagnosis positively affects outcome and AF may be associated with dangerous ventricular tachyarrhythmias, our focus was to identify all horses with AF, even if this would include some false positives. False positives, however, will most likely arise from either incorrect RR registration, electrical interference and movement artifacts, or from horses with frequent arrhythmias other than AF. If these horses remain positive at consecutive HRM sessions and during walk and trot, further electrocardiographic examination is necessary.

Despite the relatively small study population, differences in HRV between SR and AF were large, leading to reliable cut off values. Also, power calculations based upon RMSSD differences between horses in AF and in SR from a previous study, indicated sufficient power to detect significant differences with 5-7 horses[20]. An important limitation of the study was its self-control design. We used horses before and after cardioversion because arrhythmias, especially atrial premature depolarizations, often appear more frequently in the post-cardioversion period. This probably made differentiation more challenging than if a control group of normal horses had been used. Since our goal was to diagnose AF recurrence after successful treatment, comparing AF to SR in the immediate post-cardioversion period, when the chances of relapse are the highest, most closely resembled the clinical situation in which this technique would be used.

We conclude that in horses, AF can be differentiated from SR using RMSSD values obtained from automatically analysed ECGs but also from an equine HRM. One should be aware that heart rate detection technique as well as level of artifact correction are important.

Footnotes:

a Skintact, Leonhard Lang GmbH, Innsbruck, Austria

b Televet 100 software version 5.1.2, Engel Engineering Services GmbH, Heusenstamm, Germany

c Polar equine heart rate electrode set, Polar Electro Benelux, Dendermonde, Belgium

d Polar equine heart rate electrode belt, Polar Electro Benelux, Dendermonde, Belgium

e Polar heart rate sensor H7, Polar Electro Benelux, Dendermonde, Belgium

f Kubios Heart Rate Variability Analysis Software, Varsitie 22, 70150 Kuopio, Finland

g Polar Flow Software, Polar Electro Benelux, Dendermonde, Belgium

h SPSS 24, IBM Analytics, Brussels, Belgium

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CHAPTER 4:

PHARMACOKINETICS OF INTRAVENOUSLY AND ORALLY ADMINISTRATED SOTALOL HYDROCHLORIDE IN HORSES AND EFFECTS ON SURFACE ELECTROCARDIOGRAM AND LEFT VENTRICULAR SYSTOLIC FUNCTION.

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Summary

Arrhythmias are common in horses. Some, such as frequent atrial or ventricular premature beats, may require long-term antiarrhythmic therapy. In humans and small animals, sotalol is often used for chronic oral antiarrhythmic therapy. Sotalol prolongs repolarization and the effective refractory period in all cardiac tissues. No information on sotalol pharmacokinetics or pharmacodynamics in horses is available and the aim of this study was to evaluate the pharmacokinetics of intravenously (IV) and orally (PO) administered sotalol and the effects on surface electrocardiogram and left ventricular systolic function. Six healthy horses were given 1 mg sotalol/kg bodyweight either IV or PO. Blood samples to determine plasma sotalol concentrations were taken before and at several time points after drug administration. Electrocardiography and echocardiography were performed at different time points before and after IV sotalol administration.

Mean peak plasma concentrations after IV and PO administration of sotalol were 1624 ng/mL and 317 ng/mL, respectively. The oral bioavailability was intermediate (48%) with maximal absorption after 0.94 h, a moderate distribution and a mean elimination half-life of 15.24 h. After IV administration, there was a significant increase in QT interval, but no significant changes in other electrocardiographic and echocardiographic parameters. Transient transpiration was observed after IV administration, but no adverse effects were noted after a single oral dose of 1 mg/kg in any of the horses. It was concluded that sotalol has an intermediate oral bioavailability in the horse and might be useful in the treatment of equine arrhythmias.

Introduction

In horses, supraventricular and ventricular arrhythmias can be associated with a wide range of cardiac and non-cardiac diseases. Frequent ventricular premature beats carry a risk of ventricular tachyarrhythmia and even ventricular fibrillation and sometimes requires long-term, antiarrhythmic therapy [1]. A high number of atrial premature beats may require treatment as atrial premature beats increase the risk of atrial fibrillation developing [1,2]. In the first few days to weeks after cardioversion of atrial fibrillation, reverse remodeling takes place. During this remodeling phase, suppression of atrial premature beats may be important in reducing recurrence rates [3-5].

In human medicine, long-term oral antiarrhythmic treatment is often prescribed to reduce the chances to relapse into atrial fibrillation [6,7]. Sotalol, a potent, non-cardioselective β-adrenergic blocking agent with class III antiarrhythmic action, has been shown to have an efficacy equivalent to propafenone and quinidine in preventing atrial fibrillation recurrence, but is significantly better tolerated by human patients [8-10]. In dogs, sotalol is used to prevent recurrence of atrial flutter and atrial fibrillation [11,12]. Unlike many other antiarrhythmic drugs, sotalol has a good oral bioavailability (F) (90%) in humans, no major interaction with other drugs, no metabolism and is solely cleared through the urine [13]. The most important side effects of sotalol are related to its β-blocking actions and the risk of torsade de pointes (a form of polymorphic ventricular tachycardia). Most antiarrhythmic drugs currently used in horses have a low F, are difficult for long-term administration or are very expensive. The present study investigates the pharmacokinetics of sotalol in horses and its effect on the surface electrocardiogram (ECG) and left ventricular systolic function.

Materials and methods

Study design

Six healthy Standardbred horses, with a mean ± standard deviation (SD) age, bodyweight (BW) and height at the withers of 17±3 years, 527±46 kg and 156±23 cm, respectively, belonging to the teaching herd of the Faculty of Veterinary Medicine, Ghent University, were used. The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine at Ghent University (case number EC 2012/149, date of approval 23 November 2012).

The horses received sotalol at an intravenous (IV) and oral (PO) dose of 1 mg/kg BW in a two-way cross-over design, with a wash-out period of 40 days. For the IV study, horses received 1 mg/kg sotalol (Sotalol Carino, Carinopharm) as a constant rate infusion over a period of 10 min. For the PO administration, after being withheld from food for 12 h, horses received 1 mg/kg of crushed sotalol tablets (Sotalol Sandoz) in 2 L of tap water by means of nasogastric intubation.

Blood was withdrawn in heparinized polyethylene tubes just before and at 5, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 480, 600 and 720 min after administration, and every 12 h after that, until 72 h after administration. Blood samples were immediately centrifuged for 10 min at 4000 g and plasma was frozen at -18 °C until drug assay. Clinical signs, heart rate (HR) and respiratory rates were recorded just before each blood sampling. The horses receiving IV sotalol were under continuous ECG surveillance until 12 h after drug administration. Echocardiography was performed before, and at 60 and 180 min after sotalol administration.

Plasma analysis

Quantitation of sotalol in the plasma samples was performed using a liquid chromatographic (LC) tandem mass spectrometric (MS/MS) method, developed and validated in house. To 250 µL of plasma, 25 µL of the internal standard (IS) working solution (1 µg/mL, atenolol) were added and the sample was vortex mixed for 15 s. Subsequently, the sample clean-up consisted of a protein precipitation step using 750 µL of acetonitrile. After a vortex mixing (15 s) and centrifugation step (10 min, 7800 g), the supernatant was evaporated to dryness using a nitrogen stream (40 °C). The dry residue was re-dissolved in 250 µL ultra-performance liquid chromatography (UPLC)-grade water by vortex mixing (15 s) and filtered using a syringe

filter (Millex-GN, Merck). The filtrate was transferred to an autosampler vial and a 5 μL aliquot was injected onto the LC-MS/MS system.

The LC system consisted of an UPLC Acquity Binary Solvent Manager and Sample Manager with temperature controlled tray and column oven (Waters). Chromatographic separation was achieved on an Acquity UPLC BEH C18 column in combination with an Acquity BEH C18 Vanguard pre-column (Waters). The mobile phases used consisted of 20 mM ammonium formate in UPLC-grade water (A) and UPLC-grade acetonitrile (B). A gradient elution was performed (0-2 min: 97% A, 2-5 min: linear gradient to 20% A, 5-6 min: 20% A, 6-6.5 min: linear gradient to 97% A, 6.5-10 min: 97% A) at a flow-rate of 0.3 mL/min. The LC column effluent was interfaced to a Quattro Premier XE triple quadrupole mass spectrometer with an electrospray ionisation source operating in the positive mode (Waters). Instrument parameters were optimized for both analytes (sotalol and IS) and the following multiple reaction monitoring transitions were selected: sotalol: mass to charge ratio (m/z) = 273.01 > 255.01 (quantifier ion) and 212.96 (qualifier ion); IS: m/z = 267.06 > 144.96.

The method was validated in house by a set of parameters that were in compliance with the recommendations as defined by the European Community [1], international guidelines [2] and in literature [14]. The following parameters were evaluated: linearity (2-2000 ng/mL), within-run and between-run accuracy and precision, limit of quantification (LOQ, 2 ng/mL), limit of detection (LOD 0.26 ng/mL), specificity and carry-over. The validation protocol and the acceptance criteria used were previously described by De Baere et al. [15].

Pharmacokinetic analysis

Two-compartmental pharmacokinetic analysis was performed with dedicated software (WinNonlin 6.3 Pharsight). The most important pharmacokinetic parameters were calculated: maximal plasma concentration (Cmax), plasma concentration at time 0 (C_0), time to maximal plasma concentration (T_{max}), area under the plasma concentration-time curve from time 0 to infinite (AUC_0-inf), absorption rate constant (k_a), absorption half-life ($T1/2_a$), distribution rate

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constant ($k_{el\alpha}$), distribution half-life ($T1/2_{el\alpha}$), elimination rate constant ($k_{el\beta}$), elimination half-life ($T1/2_{el\beta}$), clearance (Cl), volume of distribution in the central (V_c) and peripheral (V_p) compartment. The F was calculated for each horse according to the formula:

$$F (\%) = AUC_0\text{-inf PO} / AUC_0\text{-inf IV} \times 100$$

where Cl, V_c and V_p after oral administration were calculated by multiplying the output generated by the modelling software, namely Cl/F, V_c/F and V_p/F, with the F for each individual horse.

Based on the data derived from the single PO administration study, plasma concentrations were predicted for multiple dosing of sotalol: 1, 2 and 3 mg/kg twice daily for 4 days. Maximal and minimal plasma concentrations at steady state (Cp_{ss} max and Cp_{ss} min) were derived from the dosing interval between 72 h and 84 h. The average plasma concentration at steady state (Cp_{ss} av) was calculated as follows:

$$Cp_{ss} \text{ av} = AUC72 \text{ h-t} / \tau$$

with t the next time point of administration, 84 h, and τ the dose interval, 12 h.

Electrocardiography

An ECG was recorded using a Televet100 recording system (Engel Engineering Services). A modified base-apex configuration as described by Verheyen et al. [16] was used. The ECG recordings were analysed offline (Televet100 software version 5.0, Engel Engineering Services) by a blinded observer. The duration of the QRS complex and the P wave, and the RR, PQ and QT intervals were measured for 20 cycles before and at 15, 30, 60, 120, 180 and 360 min after sotalol administration. All values were also corrected using the Fridericia correction (corrected interval = interval/$RR^{(1/3)}$).

Echocardiography

All horses were examined with a HR < 45 beats/min, using an ultrasound unit (GE Vivid 7 Dimension, GE Healthcare) with phased-array transducer (3S, GE Healthcare). A base-apex ECG was recorded simultaneously. All examinations were recorded digitally and analysed offline (EchoPAC software version BT12, GE Healthcare) by a blinded observer. Echocardiographic recordings and measurements were obtained as described elsewhere [17, 18]. In brief, systolic function was assessed by calculating fractional shortening from left ventricular internal diameter at end-diastole (LVIDd) and end-systole (LVIDs) (FS =

((LVIDd–LVIDs)*100)/LVIDd) on a right parasternal short axis view M-mode at the chordal level. From a right parasternal left ventricular outflow tract view M-mode of the aortic valve, left ventricular pre-ejection period (LVPEP) was measured from the onset of the QRS complex to the opening of the aortic valve and left ventricular ejection time (LVET) from the opening to closure of the aortic valve. Pre-ejection period to ejection time ratio (LVPEP/LVET) was calculated to reduce the influence of HR on systolic time intervals. For each cycle the instantaneous HR was recorded. Three consecutive cycles were measured to calculate a mean value at each time point (0, 60 and 180 min) for each horse.

Statistical analysis.

Statistical analysis was performed using dedicated software (SPSS 21 for Windows, IBM). Pharmacokinetic parameters are reported as mean values ± SD. Linear mixed models with post hoc Dunnett's comparison with baseline values were used to assess the relationship between ECG measurements and between echocardiographic measurements at different time points before and after the administration of sotalol. Differences were considered statistically significant when $P < 0.05$.
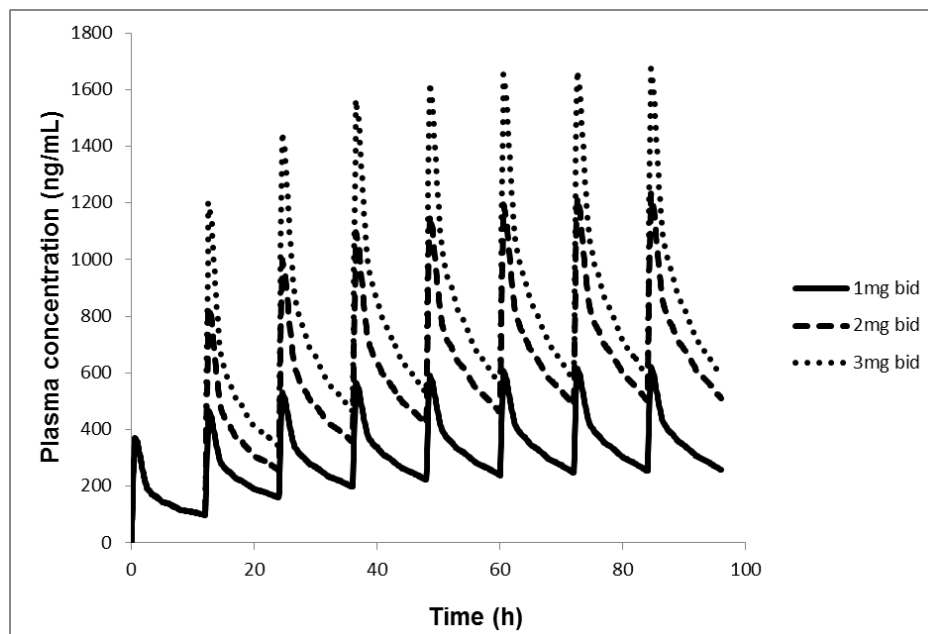
Results

Pharmacokinetics

Pharmacokinetic variables and plasma concentrations of sotalol after IV and PO administration are summarized in Table 1 and Fig. 1, respectively. In Fig. 2, the predicted steady state concentrations at a dose of 1, 2 and 3 mg/kg PO twice daily in fasted horses are shown. Table 2 summarizes the predicted AUC, Cp_{ss} av, Cp_{ss} max and Cp_{ss} min.

Table 1. Main pharmacokinetic parameters of sotalol hydrochloride after oral (PO) and intravenous (IV) administration (1 mg/kg BW) to fasted horses (n = 6). Values are presented as mean ± standard deviation.		
Parameter	**PO**	**IV**
C_{max} (PO) or C_0 (IV) (ng/mL)	316.53 ± 56.59	1624.39 ± 297.37
T_{max} (h)	0.94 ± 0.31	/
$AUC_{0\text{-}inf}$ (h.µg/mL)	3.38 ± 0.88	7.18 ± 1.10
k_a (h^{-1})	2.21 ± 1.46	/
$T_{1/2a}$ (h)	0.50 ± 0.23	/
Cl (L/h/kg)	0.15 ± 0.02	0.15 ± 0.03
$k_{el\ \alpha}$ (h^{-1})	1.10 ± 0.15	2.37 ± 0.39
$k_{el\ \beta}$ (h^{-1})	0.05 ± 0.01	0.08 ± 0.01
$T_{1/2el\ \alpha}$ (h)	0.66 ± 0.11	0.31 ± 0.05
$T_{1/2el\ \beta}$ (h)	15.24 ± 1.76	8.66 ± 0.70
Vc (L/kg)	0.74 ± 0.11	0.66 ± 0.15
Vp (L/kg)	1.89 ± 0.14	1.02 ± 0.01
F (%)	47.84 ± 12.43	/

C_{max}, maximal plasma concentration; C_0, plasma concentration at time 0; T_{max}, time to maximal plasma concentration; $AUC_{0\text{-}inf}$, area under the plasma concentration-time curve from time 0 to infinite; k_a, absorption rate constant; $T_{1/2a}$, absorption half-life; Cl, clearance; $k_{el\ \alpha}$, distribution rate constant; $k_{el\ \beta}$, elimination rate constant; $T_{1/2el\ \alpha}$, distribution half-life; $T_{1/2el\ \beta}$, elimination half-life; Vc, volume of distribution in the central compartment; Vp, volume of distribution in the peripheral compartment; F, absolute oral bioavailability.
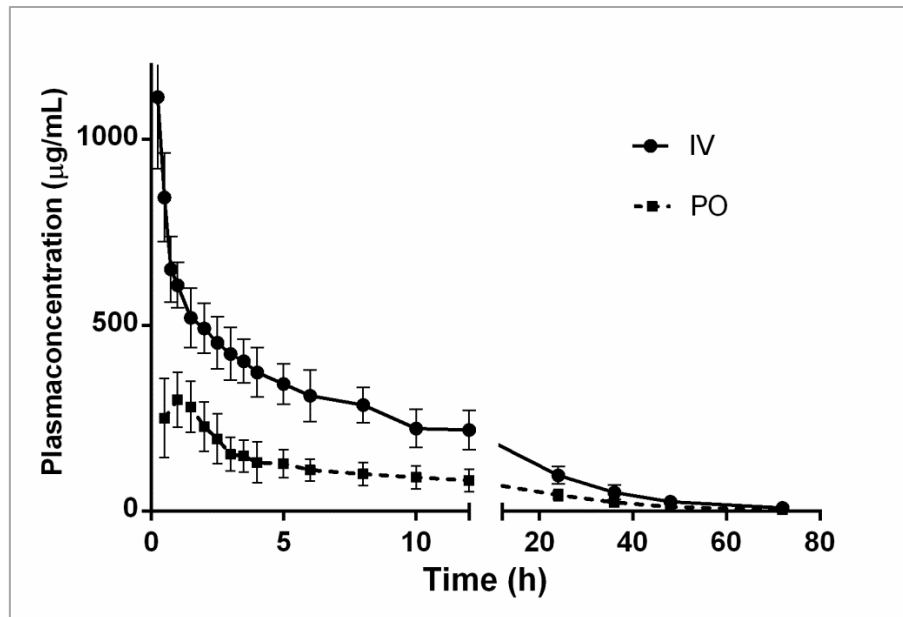
Fig. 1: Mean plasma concentrations ± standard deviations of sotalol after a single intravenous and orally administered dose (1 mg/kg bodyweight) in six healthy Standardbred horses.

Fig. 2: Predicted steady state plasma sotalol concentrations based on the study results when an oral dose of 1, 2 and 3 mg/kg bodyweight sotalol is administered twice daily to a fasted horse.

	1 mg/kg bid	2 mg/kg bid	3 mg/kg bid
Table 2. Predicted area under the plasma concentration-time curve (AUC) for one dosing interval at steady state (72-84 h) and average, maximum and minimum plasma concentration of sotalol at steady state after oral administration of 1, 2 or 3mg sotalol/kg bodyweight two (bid) times a day to fasted horses ($n = 6$).			
$AUC_{72-80/84 h}$ (h.ng/mL)	3212.52 ± 859.96	6392.48 ± 1695.80	9673.13 ± 3284.22
$Cp_{ss\ av}$ (ng/mL)	267.71 ± 71.66	532.71 ± 141.32	806.09 ± 273.69
$Cp_{ss\ max}$ (ng/mL)	498.11 ± 96.10	992.75 ± 189.72	1498.09 ± 344.00
$Cp_{ss\ min}$ (ng/mL)	178.09 ± 56.60	349.06 ± 115.02	548.88 ± 250.84

AUC: area under the curve, $Cp_{ss\ av}$: sotalol plasma concentration at steady state; $Cp_{ss\ max}$: maximal sotalol plasmaconcentration, $Cp_{ss\ min}$: minimal sotalol plasma concentration.

Electrocardiography

Electrocardiographic variables at baseline and at different time points after IV STL administration are summarized in Table 3. Compared to baseline, mean QT interval was significantly increased at 15 (mean increase $\pm$ SD: 35 ± 27 ms, 6.0%, P = 0.026), 30 (37 ± 24 ms, 6.2%, P = 0.018) and 60 (35 ± 25 ms, 5.6%, P = 0.039) min after STL administration. No significant difference was found for the corrected QT interval (QTc), although QTc at 15 min after STL administration was increased by 37 ± 18 ms (7.8%, P = 0.051). There were no significant differences in P wave and QRS duration or in RR and PQ interval, between baseline and any time point after IV STL administration, neither with nor without correction.

Echocardiography

Echocardiographic variables at baseline and at 60 and 180 min after IV STL administration are summarized in Table 3. There were no significant differences in FS, LVPEP, LVET and LVPEP/LVET between baseline and different time points after IV STL administration.

	Variable	baseline		15min		30min		60min		180min		360min		Unit
Electrocardiography	P	163	17	157	14	159	16	158	20	168	15	164	16	ms
	PQ	359	52	361	47	367	48	371	56	385	54	381	50	ms
	QRS	141	9	136	11	136	7	136	11	141	10	141	9	ms
	QT	593*†ᵞ	28	628*	23	630†	24	626ᵞ	34	616	59	619	37	ms
	RR	1984	26	188	14	1885	14	1927	27	2019	22	2146	23	ms
	Pc	130	14	127	16	129	13	126	18	133	11	127	12	ms
	PQc	286	40	292	38	297	35	298	37	305	45	296	41	ms
	QRSc	113	7	111	8	110	6	110	10	111	7	109	8	ms
	QTc	473	29	509	22	511	23	505	31	490	54	482	38	ms
Echocardio	FS	36	8	NA	NA	NA	NA	37	5	37	5	NA	NA	%
	LVPEP	13	2	NA	NA	NA	NA	13	2	14	3	NA	NA	ms
	LVET	47	3	NA	NA	NA	NA	48	1	49	3	NA	NA	ms
	LVPEP/LVET	27	5	NA	NA	NA	NA	26	4	29	7	NA	NA	

Table 3. Summary of electro- and echocardiographic data (mean±SD) of 6 horses recieving 1mg/kg BW sotalol hydrochloride intravenously.

*C=corrected interval using the Fridericia correction; FS: fractional shortening; LVPEP= left ventricular pre-ejection period; LVET= left ventricular ejection time; NA= not available. *, † and ᵞ indicate significant differences: there is a significant difference between values with the same superscript. *: p= 0.026, †: p= 0.018, ᵞ: p=0.039.*

Tolerability

After IV administration sweating (n = 3) and mild colic signs (n = 1) were observed. Oral administration of 1 mg/kg sotalol did not result in any adverse effects.

Discussion

In contrast to most available antiarrhythmic drugs, sotalol has a simple pharmacokinetic profile in humans. In our study in horses, the decline in sotalol plasma concentrations after IV or PO administration follows a two compartment pharmacokinetic model, as is the case in humans and small animals. In the present study, the absorption rate of sotalol in horses, was slower compared to dogs ($T1/2a$ = 11-17 min) and similar to that in humans (Tmax = 1-4 h). Also F in horses was lower than in humans and dogs (F = 75-90%) [19-21].

For many drugs the absorption after oral administration is lower in horses than in other monogastric species. This can be attributed to the herbivorous diet of horses which causes them to maintain some fermentative bacteria in the stomach flora, possibly interfering with drug absorption. Quinidine, administered by nasogastric tube, has a similar F (48±21%) compared to sotalol [22]. Phenytoin has a medium F of 35±8% in the horse and amiodarone (F = 6-33%) and propranolol (F = 1-32%) are poorly available [23-25]. In the present study, horses were fasted before receiving oral sotalol. Further work is needed to better understand the influence of feeding on F of sotalol. Animal studies indicate that sotalol distributes into a number of tissues including the liver, heart and kidney [26,27]. In our study, after PO administration, tissue distribution of sotalol was moderate and slightly lower than in humans (Vd = 1.2-2.4 L/kg) [21]. The elimination half-life and clearance of sotalol in horses were similar to those published in humans ($T1/2el\beta$ = 10-20 h, Cl = 0.15 L/h/kg), but slower than those described in small animals ($T1/2el\beta$ = 4 h) [13].

The pharmacological effects of sotalol are strongly related to its plasma concentrations. β-blockade in humans occurs at concentrations between 0.8 and 1 µg/mL, while class-III activity starts from 1.2 µg/mL [21,28]. Therapeutic concentrations used in human medicine lie therefore between 0.8 and 4 µg/mL, depending on the clinical indication [13]. In small animals, an oral dose of 5 mg/kg twice daily, leading to plasma concentrations of 1.1 -1.6 µg/mL, results in β-blockade, class-III activity and QT prolongation [29]. As displayed in Fig. 2 and Table 2, 2 and 3 mg/kg twice daily in fasted horses resulted in a predicted average plasma concentration approximating the therapeutic concentration used in human medicine. However, further studies are necessary to establish the efficacy and safety of these dosing protocols in horses.

The class-III action potential prolonging effect of sotalol is important for its antiarrhythmic action. On the surface ECG, this can be seen as lengthening of the QT interval. Excessive QT

prolongation, however, has been associated with torsade de pointes, a life-threatening ventricular tachyarrhythmia. In human medicine, the dose-effect relationship between oral sotalol and QT prolongation has been widely studied [30-32]. In our study in horses, a single IV dose of 1 mg/kg caused a small QT prolongation. After correction of the QT interval for HR, results were close to significant with an increase in QTc interval of 37±18 ms. In this study, the Fridericia formula was used to diminish the influence of HR on QT interval. However, there is no general consensus on which formula to use and all are subject to possible under- and overcorrection. It is therefore possible that a different correction formula would have been more appropriate.

Several studies on IV dosed sotalol in humans and in dogs revealed negative chronotropic and limited negative inotropic effects, because of the β-blocking action counterbalancing the class-III activity of the drug [33,34]. Similar as in human studies, in our study FS, LVEP, LVET and LVEP/LVET were unchanged [35-37].

Sotalol toxicity is associated with proarrhythmia. Generally adverse effects of sotalol administration occur after overdosing or in association with electrolyte disturbances or renal failure, but on rare occasions sotalol can induce ventricular arrhythmia even at therapeutic dosages [38,39]. The risk of initiating torsade de pointes can be attributed to its propensity to lengthen the QT interval, rather than its β-blocking action. Avoiding hypokalaemia, excessive QT prolongation and bradycardia is important to prevent ventricular tachycardia [13,39]. Underlying renal disease and the use of diuretics may require dose adjustments [28,40]. In human medicine, if high concentrations are required, effects on QT interval are monitored with prolongations of more than 20% being an indication to reduce the dose [41]. In our study, a small prolongation in QTc interval (7.8%) was noted after 1 mg/kg sotalol IV. Adverse effects noted after IV administration of sotalol in our study were local sweating and mild colic. Also in human medicine, abdominal discomfort and sweating have been reported as side effects of sotalol and other beta-blocking agents.

The main limitation of our study is its low power. Because of the small study population, conclusions should be drawn carefully. A larger study population would better allow the identification of small changes in electrocardiographic and echocardiographic measurements. The inclusion of a control group would have enabled us to eliminate environmental influences. Urine was not collected during the entire study period in order to clarify the eliminations process. Furthermore, a single dose of sotalol was administered, so theoretical

models had to be used to draw conclusions about steady state concentrations with different dosing protocols. These models are subject to variability and are less reliable than clinical studies on animals. Also, sotalol was given to fasted horses in this study. More studies are needed to correctly asses the influence of feeding on the F.

Conclusions

Sotalol seems promising because of its oral bioavailability and its ease of administration. The IV administration of 1 mg/kg resulted in relatively high plasma concentrations. There was a small QT prolongation, with no significant changes in other electrocardiographic measurements, or in echocardiographic assessment of left ventricular function. Further studies are needed to assess the effect of feeding on plasma concentrations and to study the effects of higher plasma concentrations on electrocardiographic and echocardiographic parameters, in order to determine a safe therapeutic oral dosing protocol.

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CHAPTER 5:

DAY-TO-DAY VARIABILITY OF RIGHT ATRIAL AND VENTRICULAR MONOPHASIC ACTION POTENTIAL AND REFRACTORY PERIOD MEASUREMENTS IN THE STANDING NON-SEDATED HORSE

Summary

Reasons for performing the study: In human medicine, monophasic action potential (MAP) analysis and determination of local refractory periods by contact electrode technique gives highly accurate information about cardiac electrophysiological properties. It is used to investigate dysrhythmias and the impact of drugs on the myocardium. Precise measurement of total MAP duration is difficult, therefore the MAP duration is usually determined at a repolarization level of 90% (APD90).

Objectives: To determine the feasibility, amplitude and APD90 of right cardiac MAP recordings and to measure local refractory period in standing non-sedated healthy horses by using a contact electrode.

Study design: prospective study.

Methods: In 6 healthy Warmblood horses, on two different days, an 8F quadripolar contact catheter was passed through a jugular introducer sheath and placed under ultrasound guidance at the level of the tuberculum intervenosum or right atrial free wall (RA), and in the right ventricular apex (RV) to record the MAP. The MAP amplitude and APD90 were measured at a resting heart rate of 30-42 BPM and at pacing cycle lengths (PCL) of 1000 and 600ms. The effective refractory period (ERP) was determined at PCL of 1000 and 600ms.

Results: The overall mean (±SD) APD90(rest), APD90(1000) and APD90(600) were 263±39ms, 262±41ms, 236±47ms for the RA and 472±26ms, 412±34ms, 331±45ms for the RV. The mean ERP1000 and ERP600 were 273±24ms and 256±22ms for the RA and 386±40ms and 293±30ms for the RV. The day-to-day variability for the amplitude, APD90 and ERP measurements were high, low to moderate and low, respectively.

Conclusions: RA and RV MAP duration and ERP can be obtained by a contact electrode in standing non-sedated horses with a low to moderate day-to-day variability.

Introduction

Electrocardiography (ECG) is the most commonly used technique for recording electrical activity of the myocardium. However, it does not provide precise information regarding the dispersion of repolarization or after-depolarizations since an ECG represents the summation of electrical activity of many myocardial cells from a relatively large region of the heart [1]. The local electrical activity of the in situ beating heart can be assessed by monophasic action potentials (MAP). This waveform can be obtained by slight pressure of a contact electrode on the heart muscle and is a result of membrane potential discharges (depolarization) and slow recharges (repolarization). MAP is useful for investigation of the electrophysiology of different arrhythmias and the impact of drugs on the heart [2-4]. In humans and animals, MAP recordings by contact electrode can give highly accurate information about local atrial or ventricular activation time and the entire duration of myocardial repolarization [2,5-10]. The underlying principles concerning MAP recordings are described elsewhere [1,11,12].

This paper describes the technique and the day-to-day variability of right cardiac MAP recording and refractory period measurement in the standing non-sedated horse.

Materials and methods

The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine and the Faculty of Bioscience Engineering at Ghent University (case number EC 2015/85, date of approval 9th of September, 2015).

The study population consisted of 6 healthy warmblood horses (3 geldings, 3 mares) aged 10±5 years, with a body weight of 531±70 kg and a height at the withers of 160±6 cm. Cardiovascular or other diseases were excluded based on a physical examination, a complete blood exam, an echocardiographic examination, a 30-minute resting ECG and a 20-minute standardized exercise ECG. All horses underwent the procedure on 2 different days with at least 2 weeks interval. In each horse an 8.5F introducer sheath (Introflex)[a] was placed using the Seldinger technique in the lower third of the jugular vein. An 8F quadripolar contact catheter with separated recording and pacing electrodes (EasyMap MAP)[b] [7-8] was introduced via the introducer sheath. The catheter was placed under ultrasonograpic guidance at the level of the tuberculum intervenosum (n=9) or the right atrial free wall (RA) (n=3) and in the right ventricular apex (RV) (n=12). The catheter tip was pressed softly against the endocardium. MAPs were recorded after placement of the catheter in a position that provided continuous recordings with a stable amplitude of >2 mV in the RA and >3mV in the RV, a stable baseline, a sharp positive phase and an upward convex plateau phase [11]. MAP signal and a surface ECG (base-apex) were recorded simultaneously using a multichannel recorder (PowerLab 8/35)[c] with amplifier (Bio Amp and Quad Bridge Amp)[c] and digitized on a computer. Measurements were performed off-line using the cardiac action potential peak analysis module of a specialized semi-automatic data analysis software program (LabChart 8)[c]. The minimum peak height detection was set at 1.5 mV and an automatic resting membrane potential detection was used. The MAP signals were amplified using a low-pass filter with settings between 20-50 Hz because it reduces noise which results in a smoother signal. Each MAP curve was checked separately and only MAPs where the automatic resting membrane potential was automatically set at the level of true baseline were used. In every horse, 15 cardiac cycles were analysed per chamber and per day. The APD90 was automatically measured by the software as the duration between the upstroke and 90% repolarization. The amplitude was measured as the height from baseline to the crest of the plateau phase. First, measurements were made in sinus rhythm at rest at a cardiac rate between 30-42 BPM. Subsequently, to have a more stable rhythm and to follow the impact of heart frequency on action potential characteristics, the pacing electrodes of the MAP catheter were connected to a pacemaker programmer[d] and pacing was performed at a pacing cycle

length 1000 and 600ms to record MAPs at each pacing rate. Finally, atrial and ventricular effective refractory periods (ERP) were determined as previously described [13,14]. In brief, after pacing for two minutes with a fixed pacing interval (S1-S1), an extra stimulus (S2), at two times threshold amplitude, was introduced with a coupling interval (S1-S2) below the expected refractory period. The coupling interval was then increased in steps of 8 ms until capture of the extra stimulus occurred (the atrial or ventricular S2 was followed by a P wave or QRS complex on the surface ECG, respectively). The longest S1-S2 interval without capture was taken as the effective refractory period. ERP was always determined five times to obtain a mean value. After the procedure, all catheters were removed under ultrasound guidance.

Data analysis

Statistical analyses were performed using dedicated software[e]. Mean and standard deviation (SD) were calculated from pooled measurements of all examinations for each horse. Measurement variability was obtained by comparing the results of the measurements from day 1 and day 2 in a one-way repeated measures analysis of variance with the horse as the unit of repeated measure. The numerical values for the reported coefficients of variation (CV) were calculated by dividing the square root of the mean square error (MSE) by the grand mean, multiplied by 100. A value of $P<0.05$ was considered significant.

Results

Right atrial (Fig.1A) and right ventricular (Fig.1B) MAP recordings of sufficient quality could be obtained and analysed in non-sedated standing horses. However, horse movements occasionally resulted in loss of contact requiring MAP catheter repositioning. The total recording time per horse and per day ranged between 35-165 minutes. Catheter positioning was occasionally associated with self-limiting atrial or ventricular depolarizations which terminated after slight catheter tip movements. During atrial ERP measurements, a short episode of self-limiting atrial tachycardia was occasionally found and one horse showed a 20-minute paroxysm of atrial fibrillation. Ventricular ERP measurements were never associated with tachyarrhythmias. No complications were observed after the procedure. Typical right atrial and ventricular MAP recordings are shown in Figure 1. The results of the two different days and the overall mean±SD are shown in Table 1. The day-to-day variabilities for the amplitude, APD90 and ERP measurements were high, low to moderate and low, respectively (Fig.2 and 3). A difference of up to 100ms was observed during VERP1000 measurement in one horse.

Table 1: Mean ±SD and coefficient of variation (CV) of monophasic action potential amplitude and duration at 90% repolarization (APD90) and effective refractory periods (ERP) measured at rest and at a pacing cycle length of 1000 and 600ms in the right atrium and right ventricle in 6 horses.							
Parameter	RIGHT ATRIUM				RIGHT VENTRICLE		
	mean±SD				mean±SD		
	Day 1	Day 2	Overall	CV %	Day 1	Day 2	Overall	CV %
amplitude (rest)(mV)	6.9±1.9	4.4±1.9	5.7±2.3	35.6	8.7±2.6	8.1±4.7	8.4±3.6	42.7
amplitude(1000)(mV)	6.0±2.0	4.0±1.0	5.0±2.0	44	9.0±4.0	8.0±5.0	9.0±4.0	58.1
amplitude(600)(mV)	6.0±1.0	6.0±2.0	6.0±2.0	37.3	9.0±3.0	8.0±5.0	9.0±4.0	64.2
APD90(rest)(ms)	282±41	244±29	263±39	9.1	477±31	467±21	472±26	6.7
APD90(1000)(ms)	281±47	243±23	262±41	12.3	418±28	406±41	412±34	10
APD90(600)(ms)	242±17	231±67	236±47	21.6	324±36	338±56	331±45	8.8
ERP1000 (ms)	269±18	277±31	273±24	7.9	401±34	370±43	386±40	11.8
ERP600 (ms)	251±14	261±28	256±22	6.5	295±38	291±23	293±30	9.6
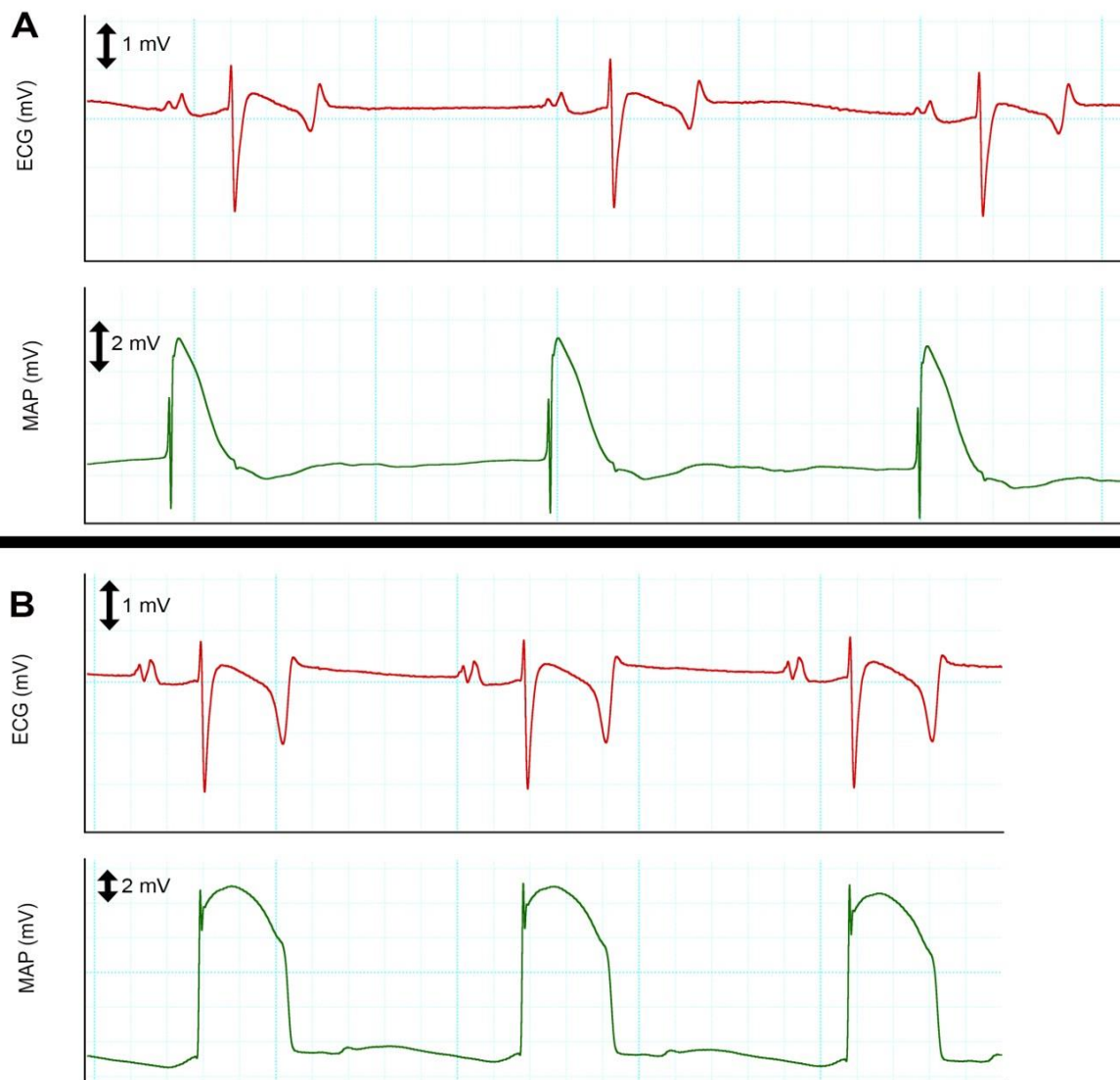
Figure 1: Electrocardiogram (red) and monophasic action potential recording (green) from the right atrium (A) and ventricle (B) in a standing, non-sedated horse.
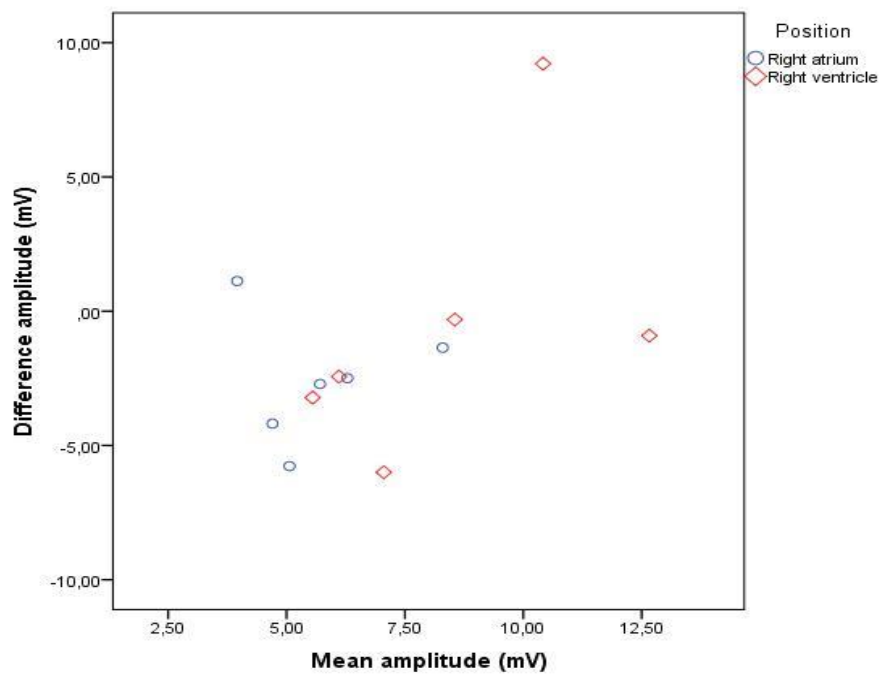
Figure 2: Day-to-day variability of the amplitude of the monophasic action potential in the right atrium and right ventricle of 6 warmblood horses.
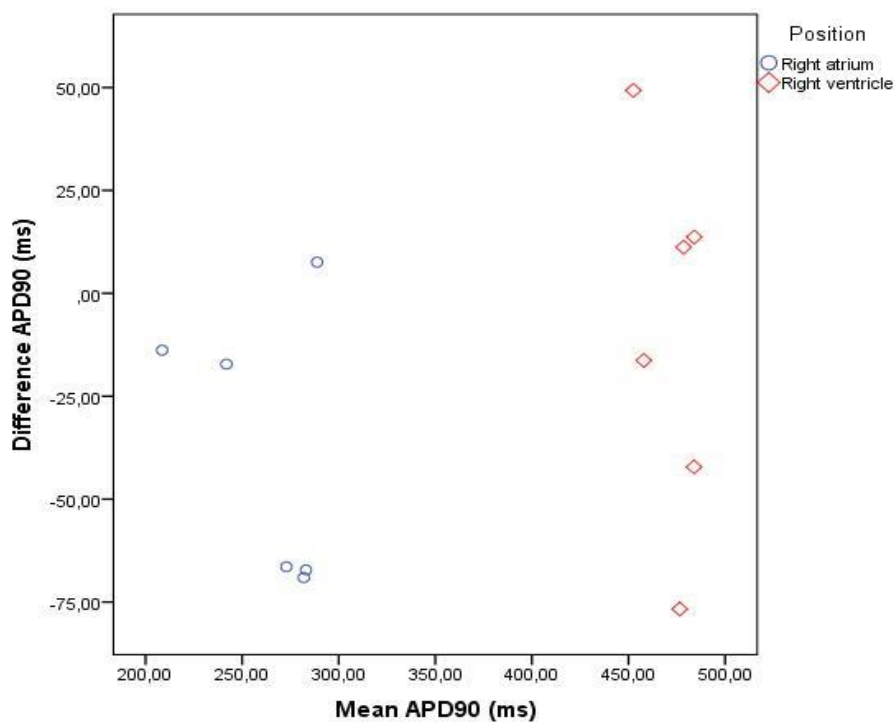

Figure 3: Day-to-day variability of the monophasic action potential duration at 90% repolarization in the right atrium and right ventricle of 6 warmblood horses.

Discussion

The present paper describes the technique to determine right atrial and right ventricular repolarization and refractoriness in the standing non-sedated horse. In humans, dogs, cats and pigs, MAP recordings play an important role in the investigation of myocardial electrophysiology under physiological, pathological and pharmacological conditions and have a low risk [3,15-19]. However, complications such as sustained dysrhythmias, movement artefacts or pericardial effusion have been reported [20]. In our study, complications were limited to a few short episodes of ectopic rhythms and one short episode of atrial fibrillation during ERP measurement. The ERP values in our study were comparable with previously reported results [14]. The coefficient of variation of the ERP measurements ranged between 7 and 12% but in one horse the difference in VERP1000 measurement on 2 different days was >100ms. As observed in awake dogs, neck and body movements of the horse occasionally resulted in dislocation of the catheter and loss of tip pressure, increasing recording time and measurement variability [20]. Due to a more fixed catheter position in the apex, dislodgement of the catheter tip was less frequently observed in ventricular MAP recordings. Probably, some spatial repolarization differences also contributed to the measurement variability [11,14]. MAP duration and amplitude can be variable due to angle differences of the catheter tip relative to the myocardium, the catheter location and varying endocardial contact pressures in the beating heart [11,20]. Prolonged good quality MAP recordings from the same endocardial site (up to 1 hour), as described for awake dogs, could not be achieved in our horses [20]. Mild sedation or the use of another type of catheter could have facilitated the procedure in our study. In humans and sedated dogs, the MAP amplitude of the atrium and ventricle ranges between 5 and 50mV due to a variability in contact pressure but also due to tissue type and species [11,18,21]. The difference in amplitude between the atrium and the ventricle can partly be explained by differences in thickness of the endocardial tissue beneath the tip of the catheter[18]. The reason for the species- and tissue-related difference in MAP amplitude is still not clear [20]. Intracellular recordings have amplitudes of approximately 120 mV regardless of species or tissue . The MAP amplitude in our horses ranged between 2 and 10 mV for the atrium and between 3 and 16 mV for the ventricle, which could be related to endocardial properties. The large size of the equine heart seems to be a large disadvantage and has probably also contributed to a less stable catheter position. The most frequently used filters during MAP measurements are the low-pass filter and the high-pass filter. The guidelines of the program we used, recommends the use of a low-pass filter since it lets low frequencies pass and stops high frequencies. This results in a reduction of noise and leads to a smoother

MAP signal. The use of a high pass filter allows high frequencies to pass and removes any steady component or slow fluctuations from the signal. This setting is used to stabilize baseline. In this study we have chosen a low pass filter to have smoother MAP signals. In addition, each MAP signal was checked to evaluate if the automatic resting membrane potential was really automatically set at the level of baseline.

Despite ultrasonographic guidance, altered catheter location, changes in contact pressure by the beating heart and horse movements contributed to the variability. Further research is necessary to identify whether repeating the measurements in a higher number of horses, harder pressure on the catheter tip, measurement under general anaesthesia or using another type of catheter (for example with a spring-steel stylet that is inserted into the myocardium) results in a lower variability in horses [11]. Also the influence of spatial differences within right atrium and right ventricle, and between the left and right heart should be investigated.

The present study describes the feasibility and measurement variability of MAP recording and refractory period measurement with a quadripolar contact catheter in the standing non-sedated horse. Further research is necessary to evaluate whether this MAP recording technique can be used to investigate equine dysrhythmias and the impact of drugs on the myocardium.

Manufacturers' addresses

[a] Edwards Lifesciences, Dilbeek, Belgium

[b] Medfact, Lörrach, Germany

[c] ADInstruments, Oxford, UK

[d] Medtronic, Jette, Belgium

[e] SPSS Statistics 22.0, SPSS Inc, Chicago, IL

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CHAPTER 6:

PHARMACOKINETICS AND ELECTROPHYSIOLOGICAL EFFECTS OF SOTALOL AT DIFFERENT MULTIPLE DOSE SCHEDULES IN HORSES

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Summary

Background: Arrhythmias in horses may require long-term antiarrhythmic therapy. Unfortunately, oral antiarrhythmic drugs for use in horses are currently scarce. In human patients and small animals, sotalol, a β-blocker with class III antiarrhythmic properties, is often used for long-term treatment.

Objectives: To determine the pharmacokinetics of sotalol at multiple oral dosages in unfasted horses, as well as the effects on electro- and echocardiographic measurements, right atrial and ventricular monophasic action potential (MAP) and effective refractory period (ERP).

Study design: Prospective, placebo controlled, double-blinded experimental study.

Materials and Methods: 6 healthy, unfasted warmblood horses were given either 0, 2, 3 or 4 mg/kg body weight sotalol orally (PO) twice daily (bid) for 9 days in a randomised cross-over design with a wash-out period of 4 weeks. Echocardiography and surface electrocardiography were performed and plasma concentrations of sotalol and right atrial and right ventricular MAPs and ERPs were determined at steady state conditions. Statistical analysis was performed using a repeated measures univariate analysis with post hoc Bonferroni corrections.

Results: Calculated mean steady state plasma concentrations determined by non-linear mixed effect modeling were 287 (range 234-339), 409 (359-458) and 543 (439-646) ng/mL for 2, 3 and 4 mg/kg sotalol PO bid, respectively. Sotalol significantly increased the QT interval and ERPs, but, despite increasing plasma concentrations, higher dosages did not result in a progressive increase in QT interval or ERPs. Echocardiographic and other electrocardiographic measurements did not change significantly. MAP durations at 90% repolarization were not significantly different during sotalol treatment. Besides transient local sweating, no side effects were noted.

Main limitations: Small study population and variable location of MAP catheter.

Conclusions: Sotalol at a dose of 2, 3 and 4 mg/kg body weight PO bid increases the QT interval and ERP in horses and might be a useful drug for long-term antiarrhythmic therapy in horses.

Introduction

Arrhythmias, both physiological and pathological, are relatively common in horses. While physiological arrhythmias are mostly caused by high vagal tone, pathological arrhythmias can be associated with a wide range of both cardiac and non-cardiac diseases. Aside from treating the underlying disease, long-term antiarrhythmic therapy may be indicated. So far, a limited number of oral antiarrhythmic drugs are available for long-term use in horses because of low oral bioavailability, important side effects, high costs and a lack of knowledge and data supporting their use [1-3]. In human medicine, sotalol, a potent β-blocking agent with additional class III antiarrhythmic properties, is frequently used for long-term oral antiarrhythmic therapy. Sotalol blocks both β_1 and β_2 receptors and has potassium channel blocking activity. By blocking outward potassium channels, sotalol inhibits the delayed rectifier potassium current, thereby slowing the repolarization phase of the cardiac tissues. Because of its dual action, it is often preferred over other β-blockers as treatment for ventricular tachycardia, symptomatic atrial fibrillation or atrial flutter, or to reduce the chances to relapse into atrial fibrillation [4,5]. In dogs, sotalol has also been used experimentally to prevent recurrence of atrial flutter and atrial fibrillation [4,6,7]. Due to its class III antiarrhythmic effects, sotalol lengthens the QT interval, monophasic action potentials (MAPs) and effective refractory periods (ERPs) in human and small animal cardiac tissues. Adverse reactions to sotalol are almost entirely accounted for by events related to β-blocker activity and QT prolongation, specifically torsade de pointes. The pharmacokinetic properties of a single dose of 1 mg/kg body weight (BW) of sotalol have been studied in horses, demonstrating a moderate absolute oral bioavailability of 48% in the fasted horse [8]. The purpose of the current study was to determine the pharmacokinetics and electrophysiological effects of 2, 3 and 4 mg/kg BW sotalol administered orally (PO), twice daily (bid) for 9 days in unfasted horses.

Materials and Methods

Study design

Six healthy warmblood horses, 3 mares and 3 geldings, with a mean ± standard deviation (SD) age, BW and height at the withers of 11±5 years, 532±70 kg and 160±6 cm, were used. All horses belonged to the teaching herd of the Faculty of Veterinary Medicine. The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (case number EC 2015/85).

In a randomized cross-over design, the horses received 0, 2, 3 and 4 mg/kg BW sotalol orally twice daily for 9 days, with a dose interval τ of 12 h. A washout period of 4 weeks was included before starting a new study period. Sotalol was administered as 80mg tablets (Sotalol Sandoz)[a] given uncrushed in 100 g of concentrates (Fibermix)[b]. Tablets could be divided in half to approximate correct dosage, leading to a dose (range 1000-2000mg) accuracy of 20mg (=1-2%). Horses receiving 0 mg/kg BW were given a sham tablet. Horses were housed in straw-bedded boxes and given 4-5 kg of hay, twice daily, 30 min after the medication, for *ad libitum* consumption during the day. Each morning during the study period, body temperature, heart rate (HR) and respiratory rates were recorded. On day 8 of each study period, non-invasive blood pressure (NIBP) measurements, echocardiography and electrocardiography were performed between 2 and 3 hours (h) after administration of medication and blood samples were taken for determination of sotalol plasma concentrations. More specifically, blood was withdrawn in heparinized polyethylene tubes just before (0 h) and at 1, 1.5, 2, 3 and 4 h after sotalol administration in the morning. On day 9 of each study period, ERPs and MAPs were recorded between 2 and 3 h after administration of medication and again blood samples were taken for determination of sotalol plasma concentrations at the same time points as on day 8. Observers conducting the clinical examinations, echocardiography, electrocardiography, NIBP, ERP and MAP recordings and data analysis were blinded to the dose of sotalol used.

Sotalol plasma analysis

Blood samples were immediately centrifuged for 10 minutes at 4000*g* and plasma was frozen at −18 °C until analysis. Sotalol plasma concentrations were measured using a validated liquid chromatography-tandem mass spectrometry method, as described before [8]. Observers performing the analysis were blinded to the dose of sotalol used.

Pharmacokinetic analysis

Plasma concentration–time data were analyzed for each dose with a nonlinear mixed-effects modeling approach using first-order conditional estimation with extended least squares (FOCE-ELS) as an estimation method in Phoenix NLME[®c]. The structural pharmacokinetic model was a one-compartmental with first order absorption and elimination.

$$dAa/dt = -Ka \cdot Aa$$
$$dA1/dt = Ka \cdot Aa - Cl \cdot C$$

Where dAa/dt and $dA1/dt$ are the decrease of amount of sotalol in the intestinal tract and in plasma, respectively, Aa and A1 are the amount of drug in the intestinal tract and in the plasma, respectively, ka is the absorption rate constant, Cl is the total body clearance and C is the plasma concentration.

Interindividual variability was expressed using an exponential error model according to the equation:

$$P_i = \theta_P \cdot e^{\eta_{Pi}}$$

where P_i is the parameter value in the ith patient, θ_P is the typical value of the parameter in the population, and η_{Pi} is a random variable in the ith patient with a mean of 0 and a variance of ω^2. Interindividual variability is reported as ω. Residual variability (ε), with a mean of zero and a variance of σ^2, was evaluated within the best structural pharmacokinetic model. The best residual error model was multiplicative:

$$C_{obs} = C_{pred} \cdot (1+\varepsilon)$$

where C_{obs} is the observed concentration for the individual and C_{pred} is the model predicted concentration plus the error value (ε).

Structural and error model selection was guided by visual inspection of goodness-of-fit plots (observed vs. predicted plasma concentrations, weighted residuals vs. predicted concentrations, and weighted residuals vs. time), -2 log likelihood (-2LL), Akaike information criterion (AIC) and Bayesian information criterion (BIC) as well as precision of the parameter estimates. The models were chosen based on the smaller values of -2LL, AIC and BIC, better precision of estimates, and superior goodness-of-fit plots.

BW was evaluated as covariate (continuous variable). A stepwise forward-backward process was used to evaluate whether inclusion of the covariates significantly improved the model fit

using a -2LL test. A decrease in -2LL with a p-value <0.01 was considered significant for addition and p<0.001 for exclusion of the covariate.

Electrocardiography

A 30-minute ECG was performed on the stabled horses in undisturbed rest on day 8 of the study between 2 and 3 hours after sotalol administration using a Televet100 recording system[d]. A modified base-apex configuration as described by Verheyen et al. (lead II) was used [9]. The ECG recordings were analyzed offline using dedicated software (Televet100 software version 6.0)[d]. QRS complex and P wave duration, and RR, PQ and QT intervals were measured for 10 non-consecutive cycles at least 10 minutes after the start of the recording. All measurements were performed on cycles with RR intervals between 1450 (42 beats/minute) and 1650ms (37 beats/minute) to minimize influence of heart rate.

Echocardiography

Echocardiography was performed using an ultrasound unit (GE Vivid 7 Dimension)[e] with phased-array transducer (3S)[e]. A modified base-apex ECG was recorded simultaneously. All examinations were recorded digitally and analyzed offline by a blinded observer (EchoPAC software version BT12)[e]. Fractional shortening, left ventricular pre-ejection period, left ventricular pre-ejection time and pre-ejection period to ejection time ratio were measured as described elsewhere at a heart rate (HR) between 32 and 40 beats/min [8,10]. For each horse, three consecutive cycles were measured to calculate a mean value for each study period.

Blood pressure

NIBP was measured using a non-invasive, oscillometric blood pressure monitor (Cardell 9401)[f] with an average cuff width-to-tail circumference ratio according to the manufacturers guidelines [11]. For each horse, 10 consecutive blood pressure measurements (mean arterial pressure) were determined to calculate a mean value for each study period.

Effective refractory period and monophasic action potential recordings

Under ultrasound guidance, a contact electrode MAP catheter[g] was inserted through the right jugular vein into the right atrium and right ventricle. ERP measurements were performed at twice baseline pacing[h] threshold and of 0.5 ms pulse duration [12]. ERP was measured by delivering 10 basic stimuli (S1) with a fixed pacing interval (S1-S1) followed by an extra stimulus (S2) with a coupling interval below the expected refractory period. The S1-S2 coupling interval was then increased with 8 ms increments until capture of the extra stimulus occurred. ERP was defined as the longest S1-S2 interval failing to elicit a new action

potential[12-14]. Five consecutive ERP measurements were recorded at 1000 (63 BPM) and 600 ms (100 BPM) basic pacing cycle length. Measurements were performed with the catheter in the right atrium (AERP) at the level of the tuberculum intervenosum or at the free wall, and in the right ventricle (VERP) at the level of the apex. Subsequently, right atrial and ventricular MAPs were recorded (Powerlab)[i] at 1200 (50 BPM) and 600 ms (100 BPM) basic pacing cycle length as described by De Clercq et al [15]. In each horse, 20 action potential durations at 90% repolarization (APD90) were measured off-line semi-automatically (Labchart 8)[i] by a blinded observer to obtain a mean value for each study period.

Statistical analyses

Descriptive statistics (SPSS Statistics 24)[j], visual inspection, the Kolmogorov-Smirnov and Shapiro-Wilk tests were used to explore the normality of the data. Univariate analysis with horse as the unit of repeated measures and post hoc Bonferroni correction for multiple comparisons was performed to determine significant differences between different sotalol dosages. Significance was set at $P<0.05$.

Results

Clinical parameters and adverse effects

The sotalol tablets were well ingested by all horses. No changes in clinical parameters (heart rate, respiratory rate and rectal temperature) were observed during the study period (results not shown), but clinical parameters were only assessed once daily (8 am). Local sweating in the region of the head, neck and chest, was sometimes present, independent of the dose or plasma concentration of sotalol, at 1 to 4 hours after sotalol dosing. No other adverse effects were noted.

Pharmacokinetics

The main population pharmacokinetic (PK) parameters following either 0, 2, 3 or 4 mg/kg BW sotalol PO twice daily for 9 days are presented in Table 1 and Figure 1. Calculated mean steady state plasma concentrations (Cp_{ss}) increased proportionally with the dose and were 287 (range 234-339), 409 (359-458) and 543 (439-646) ng/mL for 2, 3 and 4 mg/kg BW sotalol PO bid, respectively. Individual peak plasma concentrations were variable between and within horses, ranging from 188-711 ng/mL, 448-1016 ng/mL and 542-2324 ng/mL following 2, 3 and 4 mg/kg BW, respectively. BW was not included in the final model since it did not significantly improve the model fit. Visual evaluation of the population model can be found in Figure 2. Assuming that the PK remains linear over the range, an average Cp_{ss} of 1118 ng/mL (95% CI: 403-1834 ng/mL) was predicted following a 8 mg/kg BW dose twice daily.

Table 1. Population pharmacokinetic parameters of sotalol from 6 warmblood horses receiving either of 2, 3 and 4mg/kg BW PO bid for 9 days					
Dose (mg/kg)	θ	tvθ	95% CI	CV (%)	ω
2	Vd/F (L/kg)	8.95	3.83 - 14.06	28.63	0.407
	Cl/F (L/h.kg)	0.58	0.48 – 0.69	9.1	0.095
	K_a (h^{-1})	1.21	0.08 – 2.34	46.91	< 0.001
	$T_{1/2a}$ (h)	0.57	0.04 – 1.11	46.91	/
	T_{max} (h)	2.56	1.01-4.11	30.37	/
	$AUC_{0-\tau}$ (ng.h/mL)	3438.74	2813.65 – 4063.83	9.1	/
	Cpss (ng/mL)	286.56	234.47 – 338.65	9.1	/
	K_e (h^{-1})	0.07	0.03 - 0.10	24.53	/
	$T_{1/2el}$ (h)	10.66	5.44 – 15.89	24.53	/
	Res. Error	0.23	0.14 – 0.33	20.07	/
3	Vd/F (L/kg)	9.92	5.87 – 13.97	20.43	0.235
	Cl/F (L/h.kg)	0.61	0.54 – 0.69	6.08	0.028
	K_a (h^{-1})	2.61	0.26 – 4.97	45.12	<0.001
	$T_{1/2a}$ (h)	0.27	0.03 – 0.50	45.12	/
	T_{max} (h)	1.47	0.50 – 2.43	32.91	/
	$AUC_{0-\tau}$ (ng.h/mL)	4903.36	4308.05 – 5498.67	6.08	/
	Cpss (ng/mL)	408.61	359.00 – 458.22	6.08	/
	K_e (h^{-1})	0.06	0.04 – 0.09	21.16	/
	$T_{1/2el}$ (h)	11.24	6.49 – 15.99	21.16	/
	Res. Error	0.23	0.17 – 0.29	13.46	/
4	Vd/F (L/kg)	9.54	59.56 – 13.12	18.8	0.469
	Cl/F (L/h.kg)	0.61	0.50 – 0.73	9.54	0.117
	K_a (h^{-1})	1.37	0.04 – 2.70	48.64	<0.001
	$T_{1/2a}$ (h)	0.51	0.01 – 1.00	48.64	/
	T_{max} (h)	2.34	0.78 – 3.91	33.37	/
	$AUC_{0-\tau}$ (ng.h/mL)	6514.37	5272.62 – 7756.12	9.54	/
	Cpss (ng/mL)	542.86	439.39 – 646.34	9.54	/
	K_e (h^{-1})	0.06	0.04 – 0.08	15.52	/
	$T_{1/2el}$ (h)	10.77	7.43 – 14.11	15.52	/
	Res. Error	0.22	0.17 – 0.26	10.77	/

Ɵ: fixed effect parameter; tvθ: population typical value of the fixed effect parameter; 95% CI: 95% confidence interval of the population estimate; CV%: coefficient of variation; ω: variance of the interindividual variability; Vd/F: volume of distribution uncorrected for oral bioavailability; Cl/F: total body clearance uncorrected for oral bioavailability; Ka: absorption rate constant; T1/2a: absorption half-life; Tmax: time to maximal plasma concentration; AUC0-τ: area under the plasma concentration-time curve between two dosing intervals at steady state, with τ the dose interval of 12 h; Cpss: plasma concentration at steady state; Ke: elimination rate constant; T1/2el: elimination half-life; Res. Error: residual error.
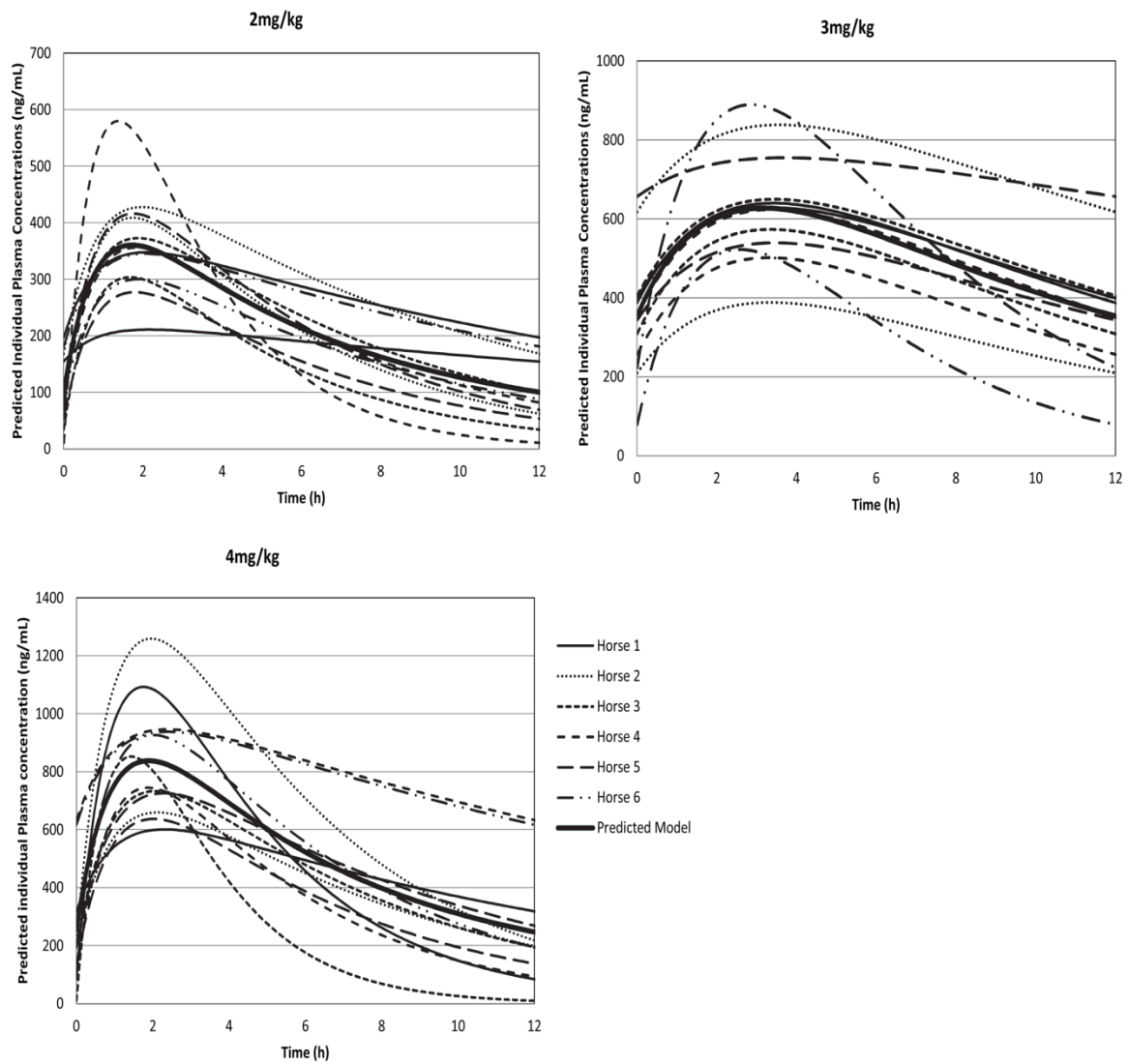
Fig. 1: Modelled individual plasma concentration-time curves for 2, 3 and 4 mg/kg BW of sotalol administered PO bid to 6 warmblood horses. Each horse was sampled on day 8 and 9 of each study period
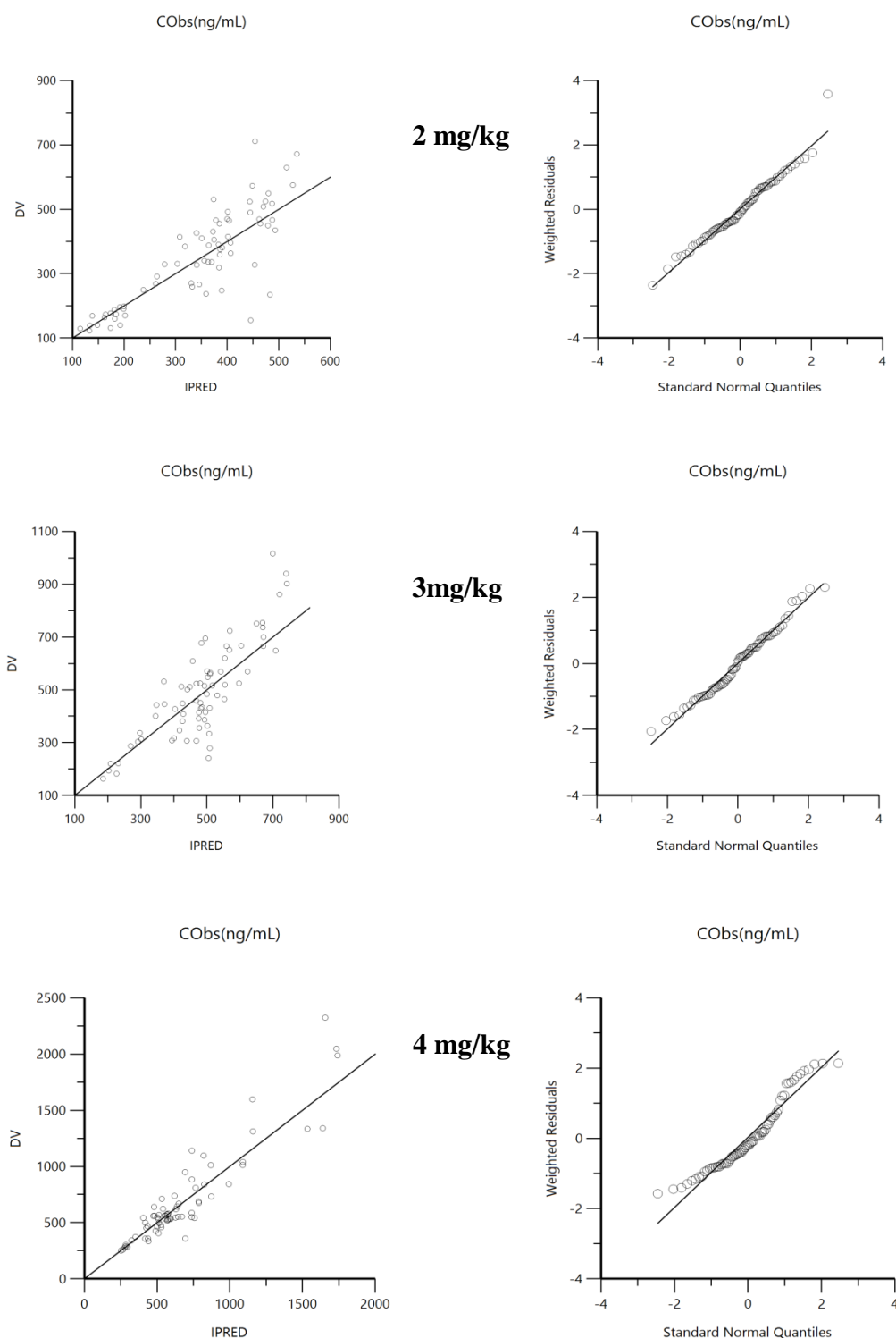
Fig. 2: Visual evaluation of the population model of sotalol after 2, 3 and 4 mg/kg body weight dosing to horses: scatter plot of the population dependent variable (DV), namely observed plasma concentration (Cobs) versus the individually predicted plasma concentration values (IPRED) (a) and QQ plot of the conditionally weighted residuals of Cobs (b).

Electrocardiography

Mean ± SD of the different electrocardiographic measurements are given in Table 2. Compared to baseline, sotalol significantly lengthened the QT interval with 49±5 (9%, P=0.001), 55±11 (10%, P<0.001) and 49±11 ms (9%, P=0.001) at 2, 3 or 4 mg/kg BW sotalol PO bid, respectively. P wave and QRS duration and PQ and RR interval did not change significantly (Table 2).

Echocardiography and NIBP

Mean ± SD of the different echocardiographic measurements and NIBP are given in Table 2. Sotalol treatment did not result in significant changes compared to baseline.

Effective refractory period

Mean ± SD of the AERPs and VERPs are given in Table 2. Stimulation threshold at 0.5 ms pulse duration was <1.5 volt for all measurements. In the right atrium, there was an increase in mean AERP1000 of 41±9 (15%), 64±8 (23%) and 65±6 ms (23%) and in mean AERP600 of 38±4 (15%), 34±8 (13%) and 53±5 ms (20%) in horses on 2, 3 or 4 mg/kg BW sotalol bid, respectively. For the right ventricle there was an increase in mean VERP1000 of 64±11 (17%,), 88±16 (24%) and 78±12 ms (21%) and in mean VERP600 of 17±4 (6%,), 43±6 (15%) and 19±4 ms (7%), respectively. Despite a consistent increase in all ERPs, these increases were not always statistically significant (Table 2).

Monophasic action potential

Mean ± SD of APD90 are given in Table 2. Despite changes in mean APD90 in both the atrium (+59 ms ±8 (23%), +22±6 ms (9%) and +31±6 ms (12%) at 1200 ms pacing cycle length and +65 ms±2 (27%), +61±6 ms (26%) and +98±13 ms (48%) at 600 ms pacing cycle length) and the ventricle (+53±8 ms (18%), +20±6 ms (4%) and +42±11 ms (9%) at 1200 ms pacing cycle length and -6±11 ms (-2%), -24±5 ms (-6%) and +8±9 ms (2%) at 600 ms pacing cycle length) in horses on 2, 3 and 4 mg/kg BW sotalol PO bid, only the 48% increase in atrial APD90 on 4 mg/kg BW sotalol was statistically significant.

Table 2: Mean±SD of electrocardiographic, echocardiographic and cardiac electrophysiological parameters in 6 warmblood horses receiving either 0, 2, 3 or 4 mg/kg BW sotalol PO bid for 9 days.				
	Dose sotalol (mg/kg bwt, PO, bid)			
Parameter	0	2	3	4
Mean arterial NIBP (mmHg)	81±2	80±2	80±2	79±1
RR (ms)	1532±15	1555±12	1553±8	1564±15
P (ms)	141±8	141±8	162±25	143±7
PQ (ms)	338±13	323±15	334±8	330±12
QRS (ms)	130±4	134±3	138±5	134±2
QT (ms)	533±12[a]	582±11[b]	588±12[b]	582±10[b]
LVIDd (cm)	11.0±0.2	11.0±0.4	11.0±0.4	11.0±0.2
FS (%)	38±2	38±2	38±1	41±2
PEP (ms)	91±5	97±6	89±3	91±7
ET (ms)	464±13	497±8	462±20	480±5
PEP/ET	0.20±0.01	0.20±0.01	0.19±0.01	0.19±0.02
AERP (1000ms) (ms)	277±5[a]	318±6	341±5[b]	342±8[b]
AERP (600ms) (ms)	261±5[a]	299±6	295±6	314±5[b]
VERP (1000ms) (ms)	370±7[a]	434±8[b]	458±4[b]	448±3[b]
VERP (600ms) (ms)	291±4[a]	308±6	334±4[b]	310±4
Atrial APD90 (1200ms) (ms)	254±16	313±27	276±34	285±20
Atrial APD90 (600ms) (ms)	237±29[a]	302±26	298±21	335±13[b]
Ventricular APD90 (1200ms) (ms)	451±160	504±15	471±14	493±23
Ventricular APD90 (600ms) (ms)	337±23	331±16	313±18	345±13

NIBP: noninvasive blood pressure; LVIDd: left ventricular internal diameter during diastole; FS: fractional shortening; PEP: pre ejection period; ET: ejection time; AERP1000 and AERP600: atrial effective refractory period at 1000 and 600ms basic pacing cycle length; VERP1000 and VERP600: ventricular effective refractory period at 1000 and 600ms basic pacing cycle length; APD90: action potential duration at 90% depolarization

Discussion

This study demonstrated that the pharmacokinetics of sotalol remained linear as the oral dose was increased from 2 to 3 and then 4 mg/kg BW resulting in steady state plasma concentrations of 287, 409 and 543 ng/mL, respectively. Sotalol significantly increased the QT intervals and ERPs, but, despite increasing plasma concentrations, higher dosages did not result in a progressive increase in QT intervals or ERPs. Echocardiographic measurements, other electrocardiographic measurements and MAP durations at 90% repolarization were not significantly different with sotalol treatment.

Little is known about pharmacokinetics and pharmacodynamics of sotalol in horses. For our study we chose to limit the number of blood samples drawn from each horse for ethical concerns as well as practical feasibility. Furthermore, the use of non-linear mixed effects modelling allows data analysis of sparse sampling protocols. We have previously described the PK of sotalol after single oral or intravenous administration in the horse, using a full sampling protocol for each subject [8]. It was demonstrated that there was no double absorption peak nor enterohepatic recirculation. The sampling strategy was based on these data, namely to obtain peak values.

In a previous study in fasted horses, the absolute oral bioavailability of sotalol was moderate (48%) and steady state plasma concentrations of 533±141 and 806±273 ng/mL for 2 and 3 mg/kg BW sotalol PO bid were predicted [8]. Average steady state concentrations and the AUC between dosing intervals in the present study in unfasted horses were about 50% lower than the values predicted by the previous study. Furthermore, plasma concentrations were more variable, not only between horses, but also within horses on the 2 different sampling days. These differences illustrate the effect of feeding on the bioavailability of sotalol. The horses in this study had free access to straw and hay during the day, so gastrointestinal filling might have interfered with drug absorption. Furthermore, sotalol tablets were given in a small amount of grain as opposed to crushing and administration by a nasogastric tube in the previous study. Together with our limited blood sampling point, this makes interpretation of our peak and steady state concentrations difficult. . Based on the PK results of a previous study, measurements were performed at day 8 and 9, at which point steady-state concentrations should have been reached [8]. However, feeding of hay resulted in a more variable absorption and therefore probably a less stable steady state concentration. This protocol was chosen in an attempt to mimic the actual clinical setting in which this drug

would be used. Time to reach maximal plasma concentrations ranged from 1.47 to 2.56 h, compared to 1 h in fasted horses [8], indicating that also the rate of absorption is decreased when administering the drug with food.

Cp_{ss} in most horses in our study were below the therapeutic plasma concentration range of 1000-3000 ng/mL aimed for in human medicine [16]. However, concentrations were variable and in 1 and 3 horses peak concentrations above 1000 ng/mL were measured at a dose of 3 and 4 mg/kg, respectively. Significant β-blockade in humans occurs at concentrations as low as 800 ng/mL, while class-III activity starts from 1200 ng/mL [17]. In dogs, an oral dose of 5 mg/kg bwt bid, leading to Cp_{ss} of 1100–1600 ng/mL, results in β-blockade, class-III activity and significant QT prolongation [3]. If, in analogy with humans and dogs, a Cp_{ss} above 1000 ng/mL would be aimed for in horses, 8 mg/kg sotalol bid would be necessary based on the predictions using the population PK model. However, because of the variability in plasma concentrations, using such a high dose would, in some horses, lead to even higher plasma concentrations and possibly adverse effects. Furthermore, in the current study in horses, 2 mg/kg BW PO bid, with steady state plasma concentrations of 400-700 ng/mL, already resulted in class-III activity with a significant QT prolongation of about 9%.

The class-III action potential prolonging effect of sotalol is important for its antiarrhythmic action. Aside from its β-adrenergic effects, sotalol is a potent competitive inhibitor of the rapid component of the delayed rectifier potassium current, I_{kr} [18]. Blockade of I_{kr} by sotalol results in APD and ERP lengthening in both the atria and ventricles. In the ventricles, this results in QT prolongation, which is visible on the surface ECG. Excessive QT prolongation, however, increases the risk of ventricular proarrhythmia and the development of torsade de pointes, a life-threatening rhythm. In the present study in horses, a significant lengthening of the QT interval following sotalol administration was demonstrated. An oral dose of 2, 3 and 4 mg/kg BW sotalol bid for 8 days resulted in an increase in QT interval of 9, 10 and 9%, respectively. In human medicine, an increase in QT interval of 15-20% is considered dangerous and an indication to reduce the sotalol dose. Moreover, a cumulative daily dosage of 320 mg, results in a sharp increase in the risk of proarrhythmia [16,18]. Although the QT lengthening in the horses in the present study remained well below 20%, care should be taken, especially when sotalol is administered to horses with hypokalaemia, bradycardia or renal disease. Furthermore, sotalol dose reduction may be required when other QT prolonging drugs are administered or when horses are submitted to anesthesia [19-21].

Another consequence of sotalol's class-III action, is the lengthening of ERPs and MAP durations in cardiac tissues. This has been demonstrated in humans, dogs and other mammals, but has not been studied in horses before [22-24]. In this study in horses there was a significant increase in AERP (13-23%) and VERP (6-24%) when horses received sotalol. MAP durations also increased but did not reach significance, probably due to the moderate day-to-day variability in MAP measurements caused by variability in electrode position and electrode contact [15]. In our study, the MAP catheter was usually placed at the level of the tuberculum intervenosum in the right atrium or in the right ventricular apex under ultrasonographic guidance. In some horses, right atrial free wall MAPs were acquired when tuberculum intervenosum recordings failed. As displayed in Table 2, there was a tendency for sotalol to increase MAP, but variations were large. Probably the day-to-day variability in this study was too large and the expected difference in APD90 (10-20%) too small, leading to insufficient study power to obtain significant results in this small population of 6 horses.

A significant increase in QT interval and ERP was observed following sotalol administration but no clear dose-response correlation was observed. In human medicine, the concentration–effect relationship between oral sotalol and QT duration showed a linear relationship [18,25]. Why an increase in dosage did not further prolong QT interval or ERP in horses, is unclear. It might be hypothesized that, in horses, an oral dose of 2 mg/kg bwt could already lead to the maximal response achievable (Emax) and that increasing the dose did not lead to increased antiarrhythmic properties and might not or only have limited additional advantage over the dose of 2 mg/kg. However, further research is needed to confirm or refute this hypothesis. Furthermore, differences in distribution, density and regulation of the I_{kr} channels have been demonstrated, not only between species, but also within one species [26]. These differences in channels may also partly explain the variability in electrophysiological effects between our horses. Electrocardiographic, echocardiographic and electrophysiological measurements were only performed once, approximately 2-3 hours after dosing. We chose to limit the number of procedures for ethical concerns as well as practical feasibility and cost limitations. The single time point for collection coincided with the theoretical peak plasma concentrations in order to assess maximal effects. Since only small effects were found, it is likely that the effects would have been smaller after several hours, when plasma concentrations decrease.

Aside from class III effects, sotalol also has β-blocking effects. In this study, no bradycardia or other β-blocking effects were observed. This was in accordance with results from our previous study [8]. However, RR intervals in this study were measured as the mean of 10

intervals and echocardiographic variables concerning cardiac contractility were only measured once. Sweating was a common side effect in both this and our previous study on sotalol in horses. In human medicine, diaphoresis is a known side effect of sotalol and several other β-blockers, but the mechanism remains to be elucidated.

Sotalol has been empirically used in horses, at different dosages and dose intervals, but no studies on its clinical antiarrhythmic properties have been published before. Electrophysiological studies on other antiarrhythmic drugs in horses are scarce. Quinidine lengthened ERP and QT intervals in healthy horses [27]. Flecainide caused a QT prolongation without significant effects on ERP, but proved to be associated with life-threatening arrhythmias and even sudden death [14,28-30]. In human medicine and dogs, sotalol is used both to treat ventricular and supraventricular arrhythmias and as a prophylactic drug to prevent recurrence of AF at an oral dose of 1-6 mg/kg bwt bid and 2 mg/kg bwt bid, respectively [4,22,31,32]. The present study demonstrated that sotalol has promising antiarrhythmic effects in horses and that, in the 6 horses included in this study, it appeared safe up to a dose of 4 mg/kg bid for 9 days. However, further studies assessing its pro-arrhythmic potential in large groups of horses are necessary in order to confirm its safety for long-term use in the horse.

We only included healthy animals in our study. However, if sotalol is to be used to suppress arrhythmias or to prevent AF recurrence, its effects might be different compared to healthy animals. AF induces electrical remodeling leading to AF stabilization, both in humans and in horses [14,33]. This remodeling might alter the electrophysiological and anti-fibrillatory effects of class III antiarrhythmic drugs [34]. A strong reduction in class III effects of sotalol was shown in goats and in humans after a period of AF and can be attributed to a reduced effect on atrial refractoriness [34].

This study has several limitations. The limited blood sampling and single time point measurements were mentioned before and a consequence of ethical, practical and cost limitations. Another limitation was the feeding of hay. Food intake and timing of food intake were not continuously monitored, so important differences in gastrointestinal filling were likely present in the individual horses. However, this protocol was chosen to mimic the clinical circumstances in which sotalol would be used in horses. Furthermore our study population was small, making it difficult for small differences to become statistically significant and the variability in MAP catheter location and angle between electrode and myocardium hampered MAP measurements. Finally, continuous ECG recording throughout

the entire study period in order to monitor for torsade de pointes, was not available and therefore conclusions regarding the safety of sotalol should be drawn carefully. We did, however, record the ECG on day 8 and 9 for 2-3 hours during the electrophysiological measurements, at 2 hours after sotalol administration. No torsade de pointes or ventricular ectopic activity was recorded in our small study population.

In conclusion, this study demonstrated that sotalol at a dose of 2, 3 and 4 mg/kg bBW bid in unfasted horses does lead to class-III antiarrhythmic actions. It might therefore be a useful drug for long-term oral treatment or prevention of tachyarrhythmias in horses.

Manufacturer's details

[a] Sandoz, Vilvoorde, Belgium

[b] Lannoo, Nevele, Belgium

[c] Certara, Cary, NC, USA

[d] Engel Engineering Services, Heusenstamm, Germany

[e] GE Healthcare, Diegem, Belgium

[f] CAS Medical Systems, Branford, CT, USA

[g] EP technologies, Inc. Mountain view, California, USA

[h] Medtronic, Jette, Belgium

[i] ADInstruments, Sidney, Australia

[j] IBM analytics, Antwerp, Belgium

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CHAPTER 7:

EFFECT OF SOTALOL HYDROCHLORIDE ON HEART RATE, QT INTERVAL AND ATRIAL FIBRILLATION CYCLE LENGTH IN HORSES WITH ATRIAL FIBRILLATION

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Summary

Background: Based on its pharmacokinetic profile and electrophysiological effects in healthy horses, sotalol could potentially be used as a long-term oral antiarrhythmic drug in horses.

Objectives: To evaluate the effect of sotalol on the heart rate, QT interval and atrial fibrillatory rate in horses with naturally-occurring chronic atrial fibrillation (AF).

Animals: 28 horses refer ed for transvenous electrical cardioversion (TVEC) of AF were treated with 2 mg/kg sotalol bid PO for three days as part of the standard treatment protocol, while 13 horses underwent the same protocol without sotalol administration.

Methods: Retrospective study. Before and after sotalol or placebo treatment, the heart rate was measured at rest and during a standardized exercise test. The QT interval and the atrial fibrillation cycle length (AFCL) using tissue Doppler velocity imaging were measured at rest.

Results: In the control group, no significant differences were found between the two examinations. In the sotalol group, the heart rate at rest and during exercise was significantly lower after sotalol treatment, while the QT interval and the AFCL measured by tissue Doppler increased significantly. Compared to the control group, horses in the sotalol group showed a significantly lower resting heart rate, lower heart rate during trot and increased QT interval after treatment. Cardioversion to sinus rhythm was achieved by a TVEC procedure in 25/28 horses in the sotalol group and all horses in the control group, but the median number of shocks required and median energy at cardioversion were significantly lower in the sotalol group.

Conclusions and clinical importance: Sotalol shows potential as an oral antiarrhythmic drug for horses with atrial fibrillation, as it decreases the atrial fibrillatory rate and heart rate.

Introduction

Atrial fibrillation (AF) is a relatively common condition in horses, which often presents as so-called 'lone AF' without underlying structural cardiac disease. Pharmacological or transvenous electrical cardioversion (TVEC) to sinus rhythm is usually recommended in equine athletes as AF limits performance in horses performing rigorous work. Even at lower levels of exercise intensity, horses may exhibit extremely high heart rates and collapse or rarely sudden cardiac death has been described [1]. The success rate for both pharmacological cardioversion and TVEC is very high, however, AF recurrence after treatment is a frequent event with an overall rate of recurrence up to 43% at 1 year after cardioversion [2-5]. The pathophysiology of AF initiation and perpetuation in horses has not yet been completely elucidated. Factors that might be associated with AF recurrence include structural changes to the atrial myocardium, atrial size and stretch, short atrial effective refractory period (AERP) and supraventricular ectopic foci [2,6].

Long-term antiarrhythmic treatment could be used to prevent AF recurrence after cardioversion through different mechanisms, such as increasing AERP, decreasing supraventricular ectopy and reducing vulnerability to AF initiation. In addition, antiarrhythmic treatment might also be useful to decrease the heart rate during exertion in horses in AF. This rate control therapy could possibly reduce the risk of collapse and sudden cardiac death. However, long-term oral antiarrhythmic therapy is scarce in horses. Sotalol is a class III antiarrhythmic drug with nonselective β-blocking activity which can be safely administered in horses, with an intermediate oral bioavailability [7]. In human medicine, sotalol is not commonly used for cardioversion of AF, but it is frequently used as an effective drug for prevention of recurrence after cardioversion [8,9]. The electrophysiological effects of sotalol include an increase in QT interval, a decrease in heart rate and prolongation of the atrioventricular nodal refractory period and PQ interval. In veterinary medicine, sotalol is the most commonly used long-term treatment for hemodynamically significant ventricular arrhythmias in dogs and cats [10]. The pharmacokinetics and electrophysiological effects of sotalol hydrochloride administered orally for 9 days have been investigated in healthy horses, demonstrating a prolongation of QT interval, AERP and ventricular refractory period at a dosage of 2 mg/kg bodyweight sotalol twice daily [11]. However, the use of sotalol has not been described in horses with cardiac arrhythmias, except in two recent reports describing single cases [12,13].

The objectives of this study were to assess the effect of sotalol on the heart rate, QT interval and atrial fibrillatory rate in horses with atrial fibrillation. We hypothesized that sotalol administration would result in a decreased heart rate at rest and during exercise, a prolonged QT interval and a decreased atrial fibrillatory rate.

Material and Methods

Study set-up

The study population consisted of 41 horses admitted for treatment of atrial fibrillation to the Department of Large Animal Internal Medicine at the Faculty of Veterinary Medicine, Ghent University. All horses were examined by an ECG at rest of at least two hours duration, an ECG during a lunging exercise test and echocardiography to assess the atrial fibrillatory rate. After these initial measurements at admission, 28 horses were treated orally with 2 mg/kg sotalol hydrochloride bid for three days before cardioversion. Echocardiography was repeated just before TVEC, after 6 doses of sotalol hydrochloride had been administered. The ECG at rest was repeated after at least 5 doses of sotalol, and the exercise test after at least 4 doses of sotalol. Measurements during sotalol treatment were always performed approximately 2-3 hours after sotalol administration. To exclude a placebo effect, the 13 remaining horses underwent the same protocol without sotalol administration. TVEC was performed according to the procedures described previously [4,14]. Shocks were administered under general anesthesia using stepwise increases of the delivered energy (150, 200, 250, 300, and 360 J) until cardioversion. If cardioversion was not acquired after the 360 J shock, the catheters were repositioned and more high energy shocks were administered.

Measurements

The heart rate at rest was measured from the resting ECG[a] as an average over a 30 minute period. The mean QT interval was measured at rest as an average from 10 RR intervals with a heart rate of 35 to 45 BPM. The lunging exercise test consisted of five minutes of walk, ten minutes of trot, four minutes of canter and one minute of gallop. The test was terminated early if the horse showed an excessively high heart rate or frequent QRS complexes with an R-on-T morphology. The heart rate at walk, trot and canter was measured over 2 minutes, starting at least 1 minute after the gait transition. The heart rate during gallop was measured over the entire gallop phase.

The atrial fibrillatory rate was assessed from tissue Doppler velocity curves of the atrial myocardial walls, as described previously [15,16]. In brief, tissue Doppler images were acquired from the left atrial free wall in a right parasternal four chamber view, from the right atrial dorsal wall at the level of the tuberculum intervenosum in a right parasternal view and from the left atrial free wall in a left parasternal long axis view. The probe frequency was 1.7/3.4 MHz, the image width was 30°, image depth ranged from 22 to 30 cm and the velocity scale

was +16/-16 cm/s[b]. This resulted in a frame rate of > 180 fps. From each view, 10 loops with a long RR interval (≥2 s) were stored. Tissue Dopper velocity curves were acquired off-line by positioning a 5x5 mm sample volume in the atrial myocardial wall[c]. The atrial fibrillation cycle length (AFCL) was measured from consecutive loops with a quality score ≥ 3, until at least 30 AFCL were measured from each view. These measurements were then averaged to obtain mean AFCL from each view. All measurements were performed with the observer blinded to the identity of the horse and treatment status.

Statistics

Statistical analysis was performed using dedicated software[d]. The number of horses that were administered sotalol was determined using a power calculation based on a paired comparison with a hypothesized increase of AFCL of 5 ms and a standard deviation within the population of 20 ms. Based on this calculation, a sample size of 28 horses could detect a significant difference with a two sided α of 5% and β of 20%. Normality of the data was checked using visual inspection, the Kolmogorov-Smirnov test and Shapiro-Wilk test. Heart rate, QT interval and AFCL were compared between each group (sotalol administration or control) and timing (pre-treatment or post-treatment) using a univariate analysis of variance with horse as the unit of repeated measure, with post-hoc Bonferroni correction for multiple comparisons. Height, body weight, age and cardioversion data of the sotalol group were compared using an independent t-test for normally distributed variables or the Mann-Whitney test for non-normally distributed data. The level of significance was defined as $P<0.05$.

Results

The sotalol group consisted of 23 Warmbloods, 3 trotters, 1 Paint and 1 Appaloosa, with a mean (± standard deviation SD) height of 169 ± 7 cm, weighing 565 ± 65 kg, aged 9 ± 3 years. The control group consisted of 12 Warmbloods and 1 trotter, with a mean (± SD) height of 172 ± 6 cm, weighing 605 ± 60 kg, aged 10 ± 3 years. Height, body weight and age were not significantly different between groups (P ≥ 0.05). During exercise, 4/13 (30.8%) horses in the control group showed QRS complexes with an R-on-T morphology during the first test compared to 3/13 horses (23.1%) during the second test (Figure 1). In the sotalol group, 5/21 (23.8%) horses showed QRS complexes with an R-on-T morphology both pre- and post-treatment. R-on-T morphology was not consistently present in the same horses during both exercise tests. In total, 7 horses in the sotalol group and 5 horses in the control group showed R-on-T morphology during either the first or the second test. Cardioversion to sinus rhythm was achieved by the TVEC procedure in 25/28 horses in the sotalol group and all horses in the control group. One of the horses that did not convert underwent a successful second TVEC procedure with amiodarone premedication two weeks later. Two horses in the sotalol group showed early recurrence of AF immediately after the procedure, during anesthesia or recovery. Both horses could be converted to stable sinus rhythm on the same day. The median number of shocks required for cardioversion to sinus rhythm was 1 (range 1-4) in the sotalol group, compared to 3 (1-8) in the control group (P=0.016). The median energy of the shock at cardioversion was 150J (150-300J) in the sotalol group, and 250J (150-360J) in the control group (P=0.023).

Figure 1: High heart rate and QRS complexes with an R-on-T morphology in one horse in the sotalol group

The resting heart rate, QT interval, heart rates during exercise and AFCL pre- and post-treatment are summarized in Table 1 for the sotalol group and the control group. Within the sotalol group, the resting heart rate and the heart rates during exercise decreased post-treatment compared to pre-treatment (Figure 2), while the QT interval and the AFCL (Figure 3) measured in the right atrium and the left atrial free wall from the left parasternal view increased. In the control group, no significant differences were found between the two examinations. Compared to the control group, horses in the sotalol group showed a lower resting heart rate, lower heart rate during trot and increased QT interval post-treatment.

Table 1: Resting heart rate, QT interval, heart rates during exercise and AFCL pre- and post-treatment for the sotalol group (n=28) and the control group (n=13)											
	Sotalol_pre			Sotalol_post			Control_pre			Control_post		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
HR rest	50a	10	28	41b	6	28	49a	7	13	49a	7	13
QT rest	513a	41	28	560b	52	28	521a	23	13	521a	40	13
HR walk	97a	30	21	77b	20	21	80b	17	13	79b	17	13
HR trot	152a	37	21	114b	24	21	140a	35	13	140a	25	13
HR canter	204a	48	21	165b	34	21	190a	30	12	183a,b	32	12
HR gallop	246a	29	18	215b	31	18	228a,b	30	11	213b	37	11
AFCL4CH	138	13	28	144	16	28	145	12	11	138	18	11
AFCLRA	171a	16	28	181b	13	28	179a,b	13	13	180b	16	13
AFCLLLA	151a	13	28	161b	16	28	158a,b	11	13	160b	13	13

HR_rest, heart rate at rest; QT_rest, QT interval at rest; HR_walk, heart rate during walk; HR_trot, heart rate during trot; HR_canter, heart rate during canter; HR_gallop, heart rate during gallop; AFCL4CH, atrial fibrillation cycle length measured in the left atrial free wall from the four chamber view; AFCLRA, atrial fibrillation cycle length from the right atrial dorsal wall at the level of the tuberculum intervenosum in a right parasternal view; AFCLLLA, atrial fibrillation cycle length measured in the left atrial free wall from the left parasternal long-axis view. Significant differences between groups are indicated by different superscripts (P<0.05).
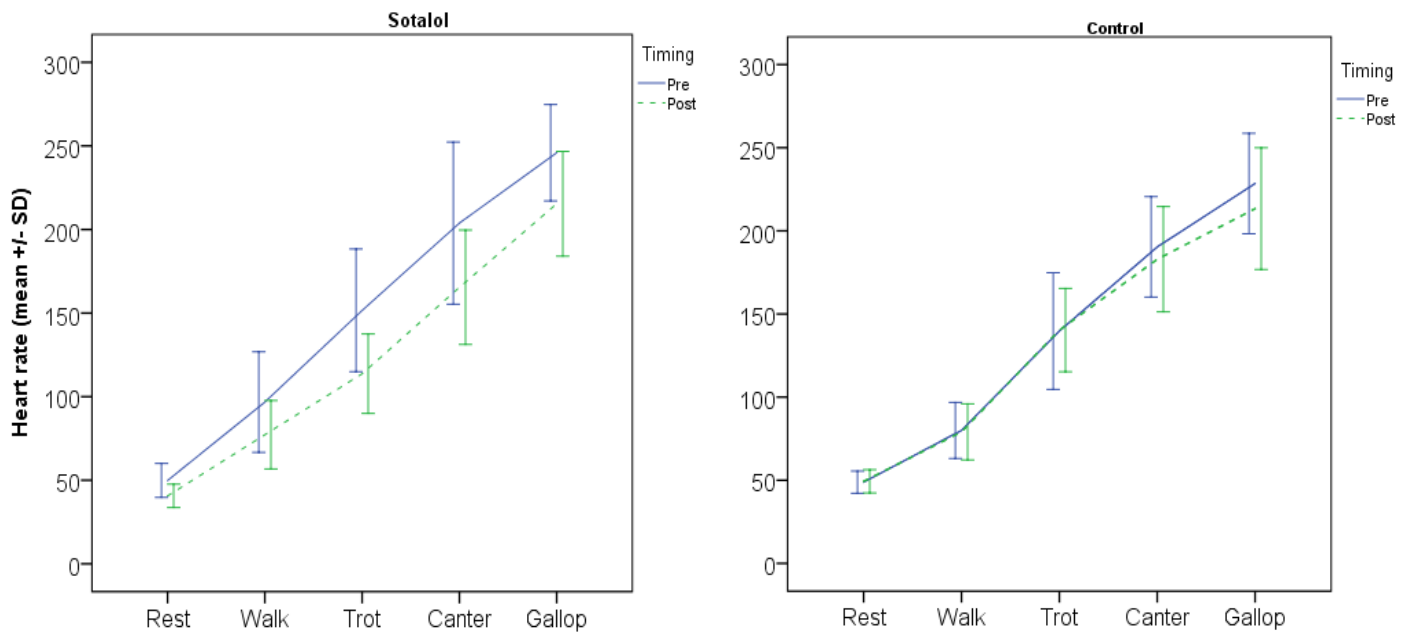
Figure 2: Mean and standard deviation (SD) of the heart rate at rest and during the exercise test in the sotalol group (left) and the control group (right).

Figure 3: Tissue Doppler velocity curve of the left atrial free wall from a left parasternal long axis view with moderate quality (quality score = 3, the lowest quality still included in the study). The biphasic atrial velocity pattern can be distinguished but there is still presence of artifacts, indicated by green arrows. The blue line demonstrates one atrial fibrillation cycle length (AFCL) measurement.

Discussion

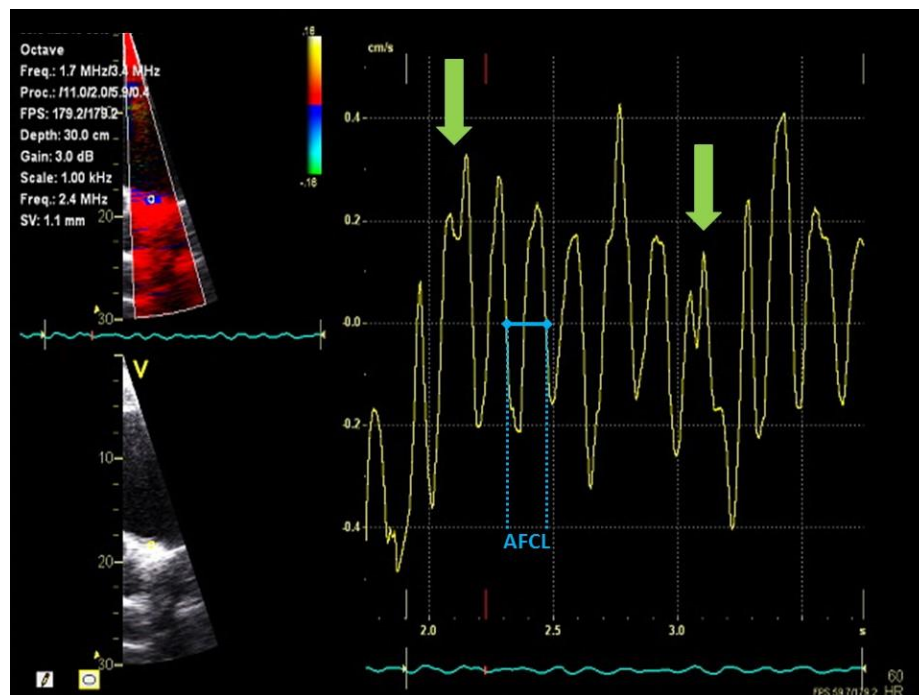This manuscript describes the effects of sotalol administered orally to horses with atrial fibrillation. Sotalol treatment resulted in a decrease in heart rate at rest and during exercise, a prolongation of the QT interval and an increase in atrial fibrillation cycle length. Significantly less energy was required for electrical cardioversion in the sotalol group, although cardioversion could not be achieved in 3/28 horses, of which 1 horses converted during a second TVEC procedure.

The increased AFCL after sotalol treatment indicates slowing of the atrial activation rate. During atrial fibrillation, the AFCL is directly related to the AERP, although the AFCL is somewhat longer than the AERP due to the presence of a small excitable gap [17]. The prolongation of AFCL after sotalol administration can be attributed to an increase of the AERP or to widening of the temporal excitable gap. A previous study in goats described a 24% increase of the AFCL during intravenous sotalol infusion at 0.2 mg/kg/minute [18]. This mainly resulted from widening of the temporal excitable gap, while the AERP remained stable. Sotalol administration in our study resulted in a modest increase of AFCL by approximately 6%. In comparison, intravenous administration of the class III antiarrhythmic drug amiodarone caused the AFCL to increase up to 100% in horses with naturally occurring chronic AF [19]. In another study, the class I drug flecainide administered intravenously to horses resulted in an AFCL increase of approximately 50% [20]. This is in line with the experimental study in goats, where administration of flecainide caused an AFCL increase of 48% [18]. This was attributed to widening of the temporal excitable gap and could be explained by a reduction in the average number of fibrillation waves through the atrium. Widening of the excitable gap results in reduced fragmentation of wavelets and fusion of waves, which causes a reduction of the number of wavelets in the atria and thus an increased chance of cardioversion [20]. Compared to amiodarone or flecainide, the effect of 2 mg/kg oral sotalol bid on AFCL was limited and cardioversion of AF using sotalol therapy alone seems unlikely in horses.

The limited effects of sotalol on AFCL can be explained by several factors. First, the sotalol plasma concentrations were probably below the therapeutic range described in human medicine. Plasma concentrations were not measured in our study, but the steady state plasma concentration was 287 ng/mL in horses receiving 2 mg/kg body weight sotalol orally twice daily for 9 days, while fed hay ad libitum during the day [7]. This is lower than the therapeutic

plasma concentrations of 1000-3000 ng/mL described in human medicine [21]. However, the significant QT prolongation indicates class III activity despite the low plasma concentrations. The limited effects on the AFCL can also be explained by the reverse use dependence of the effect of sotalol on atrial refractoriness, which results in reduced class III effects at more rapid atrial rates [22]. Reverse use dependence of sotalol probably limits the efficacy of sotalol against naturally occurring chronic atrial fibrillation with short AFCL, compared to use dependent drugs such as propafenone. Finally, the effect of sotalol might also be affected by electrical remodeling of the atrial myocardium. Reduced electrophysiological actions of class III antiarrhythmic agents have been described in a goat model of chronic AF, and this was explained by decreased contribution of the IKr potassium current to atrial repolarization in remodeled myocardium [23].

Sotalol administration resulted in a significantly lower heart rate at rest and during exercise. A 12-25% reduction in heart rate was obtained post-treatment. Atrial fibrillatory rate decreased by 6% but this was unlikely to contribute to a decrease in heart rate. Rather, the decreased heart rate was caused by the β-blocking activity of sotalol. As a competitive β-blocking agent, sotalol administration results in lower intracellular Ca2+ concentrations in the myocardial cells, causing a negative chronotropic effect by decreasing atrioventricular conduction [24]. Sotalol could be a possible candidate drug for rate control therapy in horses with AF in which cardioversion is not possible or declined by the owner. Horses with AF often show no performance limitations during low-level exercise but are deemed at increased risk to ride if the average maximal heart rate during exercise exceeds 220 BPM, as high heart rates during exercise have been associated with collapse [25]. Long-term rate control therapy could possibly reduce the heart rate during exercise in these horses. Based on our study, it is unclear whether sotalol therapy could permanently reduce the exercising heart rate in horses with AF and whether riding a horse on sotalol is safe. First, the presence of QRS complexes with an R-on-T morphology was not reduced after sotalol treatment, while this may be associated with increased risk of ventricular fibrillation [26]. Second, sotalol is not recommended for rate control therapy in human medicine because of the risk of torsade de pointes and sudden cardiac death associated with the QT prolongation [27]. The QT prolongation in our study was limited to 9%, while in human medicine an increase of 15-20% is considered dangerous. Excessive QT prolongation might occur in horses when sotalol therapy is combined with other drugs, as has been described with general anesthesia [28]. Finally, the β-blocking activity of sotalol might result in a negative inotropic effect and might affect blood pressure during

exercise. However, the negative inotropic effect is counteracted by the class III activity and no alterations of blood pressure have been described at rest in horses receiving sotalol [7].

In human medicine, sotalol is used to maintain sinus rhythm after cardioversion of AF, although proarrhythmogenic effects and a small increase of all-cause mortality have been associated with its use [29]. Our study did not allow to evaluate whether sotalol reduces the risk of AF recurrence, as this would require a long-term follow-up study in which horses are randomly administered sotalol hydrochloride or placebo. The effect of sotalol on the presence of atrial premature depolarizations during a 24-hour ECG recording after cardioversion was also not assessed. The day-to-day variability of the number of arrhythmias on Holter ECGs in horses is unknown but probably relatively high, as has been described in dogs [30]. Therefore, a large group of horses would be needed to find a significant difference in the number of atrial premature depolarizations in treated versus untreated horses.

The main limitation of this study is that sotalol administration was not performed in a double blinded study design with random assignment of horses to either the treatment group or the control group. Instead, all horses admitted for AF treatment in the timespan of 1 year were administered sotalol before TVEC. The control group consisted of horses consecutively admitted for AF treatment in a different calendar year, during which no sotalol was administered prior to treatment but the same study protocol was followed. Therefore, no difference between the two groups was expected.

In conclusion, sotalol administration resulted in a decrease of the atrial activation rate and heart rate at rest and during exercise in horses with atrial fibrillation. Further research is needed to investigate the potential of sotalol as a rate control drug in ridden horses or to prevent AF recurrence after cardioversion.

Manufacturer's Details

a Televet 100, Engel Engineering Services GmbH, Heusenstamm, Germany
b Vivid 7, GE Healthcare, Diegem, Belgium
c Echopac software version 11.2, GE Healthcare, Diegem, Belgium
d SPSS Statistics version 24, IBM, Armonk, NY

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GENERAL DISCUSSION

The purpose of this PhD research was to improve both the diagnosis and the outcome of atrial arrhythmias in horses. By better defining ECG characteristics of APDs, we highlighted some of the common mistakes and contradicted some general assumptions regarding ECG interpretation, in order to reduce erroneous diagnoses. Furthermore we introduced HRV as a novel diagnostic approach to AF. We have demonstrated that HRV parameters can distinguish AF from SR and that HRMs automatically calculating HRV can be used to monitor for AF in the field. In the future this should lead to the development of an easy-to-use, equine-specific home monitoring tool for AF.

In order to improve the outcome of supraventricular arrhythmias we focused on the evaluation of a new oral antiarrhythmic drug in horses. We studied the pharmacokinetics and –dynamics of sotalol and have shown a moderate oral bioavailability in horses. Furthermore we have demonstrated both its β-blocking activity and its capacity to lengthen the cardiac repolarization phase. Therefore, we can assume that antiarrhythmic activity is obtained in the horse. Finally, we have shown that sotalol also exerts these effects in horses with AF, possibly facilitating their treatment and preventing early AF recurrence.

This chapter discusses the challenges that were faced during and the overarching results of our studies, draws conclusions and implements them in future objectives.

The challenges of correctly diagnosing APDs

Electrocardiography is of increasing importance in equine cardiology and is becoming more widely used, not only by experienced cardiologists but also by veterinarians in the field. Therefore, clear guidelines on equine ECG interpretation are necessary. Correct differentiation between APDs and other atrial and ventricular arrhythmias is important because of their different clinical relevance.

Challenge 1: Optimal Electrode Configurations and Multiple Leads

An important factor for correctly diagnosing arrhythmias is the use of different electrode positions and multiple lead recordings. The configuration we used in our studies is a vertical modification of a classic base-apex configuration using 3 recording electrodes and one reference electrode. Our system, as most ECG recorders, recorded 2 leads (I and II) and calculated the third lead (III) [1,2]. The advantage of this configuration is that all electrodes can be placed underneath a girth or a saddle. This makes dislodging during exercise less likely and improves skin contact, reducing the number of artifacts [2]. In addition, this specific positioning of the electrodes is less subject to movement artifacts. This configuration leads to large QRS complexes and optimal registration of the electrical activity of the ventricle. The mean electrical axis of the atrium, however, is not optimally aligned, leading to smaller P waves compared to a classic base-apex lead [2].

Many studies and many veterinarians only use one single base-apex lead for ECG recording and interpretation. Although this is usually sufficient to record rate and rhythm, recording additional leads may be very useful in specific cases. Each lead measures the cardiac electrical activity from a different angle, so subtle, local changes in depolarization may be invisible in one lead while obvious in another. Therefore, the use of multiple lead recordings is advised, especially when arrhythmias are expected [3]. During exercise, however, these additional leads may show a lot of movement artifacts. In the configuration we used in our studies, lead I (right arm – left arm) showed significantly larger P wave amplitudes compared with lead II or III and an amplitude comparable to that of a classic base-apex configuration [4]. In this first lead, the positive electrode is placed 10-15 cm behind and above the left olecranon, while the negative electrode is positioned 15cm right of the withers. As such, this lead configuration is better aligned with the electrical axis of the atrium. This configuration has previously been shown to produce larger P waves in a study evaluating different electrode configurations [4]. The fact that the best P wave visualization is obtained in a non-classical lead, underlines the importance of using multiple leads for the correct interpretation of challenging

ECGs. However, the optimal electrode positions to perform multiple lead or even 12-lead recording in horses is not known yet.

Challenge 2: Sinus Arrhythmia and Wandering Pacemaker

An important problem in ECG interpretation is the correct differentiation between sinus arrhythmia and an APD. During sinus arrhythmia, a high vagal tone leads to an irregular discharge of the sinus node and an irregular RR interval on the ECG. Although sinus arrhythmia often occurs as a progressive increase and decrease in RR interval over successive beats, there might be confusion with APDs, especially when multiple successive APDs are present. There is currently no universal agreement on electrocardiographic differentiation between APDs and sinus arrhythmia. Specific studies on the ECG criteria of sinus arrhythmia are lacking, but, while from a clinical point of view P wave morphology changes seem likely, it is often stated that sinus arrhythmia does not lead to changes in ECG morphology. In our study in Chapter 1, APDs were significantly associated with singular positive P waves and larger S wave deflections and were more likely to have a single positive T wave. These findings may help in distinguishing APDs from sinus arrhythmia.

Wandering atrial pacemaker is another ECG finding which may lead to confusion with APDs. Wandering pacemaker occurs when the natural cardiac pacemaker location shifts between the sinoatrial node and various locations along the internodal tracks, as a result of high vagal tone and slow heart rate. This shifting of the pacemaker is identifiable on ECG by morphological changes in the P wave, a subtle change in PQ interval and an irregular PP and RR interval, while the QRS and T wave are not altered. Also in dogs with high vagal tone, wandering pacemaker is a common finding. Since, in horses, wandering atrial pacemaker is an ill-defined arrhythmia and its ECG characteristics are largely unknown, care should be taken not to confuse this arrhythmia with others. In addition, it is still under debate whether or not internodal tracts exist in horses.

Challenge 3: Atrial or Ventricular Origin?

A second, clinically important error is an incorrect differentiation between APD and VPD. In most cases, the difference between atrial and ventricular premature depolarizations is clear, but when P waves are difficult to identify or only small changes in the QRS complex are present, errors may occur. In contrast to what has been described in current ECG guidelines, we have shown that APDs, especially short-coupled APDs, may lead to changes in QRS and T wave morphology (Chapter 1). The increase in S wave amplitude and the change in T wave

polarity and morphology may lead to confusion, especially when the P wave is buried in the preceding T wave. Furthermore, when a VPD arises close to the atrioventricular node and His bundle (junctional VPD), the ventricular depolarization may only be slightly altered with only very subtle changes in QRS and T wave morphology, making differentiation between sinus, atrial and ventricular origin difficult and sometimes almost impossible, especially when there is a preceding or unidentifiable P wave. Guidelines for ECG interpretation should acknowledge these pitfalls in order to prevent mistakes.

Recent techniques as a solution?

Recently, new techniques improving the visualization of the depolarization path in the equine heart have been explored.

An alternative new 12-lead ECG for horses has been proposed [5]. Six precordial, unipolar leads and 3 bipolar leads were used (Table 1). This ECG configuration aims for improved evaluation of the cardiac depolarization. With this modified 12-lead ECG, the largest P wave was obtained with a unipolar precordial electrode on the left side of the thorax in the 6st intercostal space at the level of the shoulder joint [5]. In lead V2, a significant correlation between the P wave duration and left atrial size was found. A significant correlation between the QRS amplitude in lead V1 and V2 and the QRS duration in all leads and left ventricular mass was also found [5]. These results suggest that this modified 12-lead ECG should be explored further to assess diagnosis of atrial or ventricular hypertrophy, as well as arrhythmia detection in horses.

Table 1: Electrode placement for an alternative 12-lead ECG configuration in horses. Adapted from Hesselkilde *et al*., 2016 [5]	
LA	On the top of the left scapula spine
RA	On the top of the right scapula spine
LF	A little left to the midline and just caudal to the xiphoid process
V1	Between the dorsal part of the two descending superficial pectoral muscles
V2	On the left side, on the ventral part of the triceps muscle
V3	On the left side of the thorax, in the 6th intercostal space, at the level of the shoulder joint
V4	On the left side of the thorax, in the 6th intercostal space, at the level of the elbow joint
V5	On the right side of the thorax, in the 6th intercostal space, at the level of the elbow joint
V6	On the right side, on the ventral part of the triceps muscle

From human medicine, we know that the ultimate tool to unravel intracardiac impulse formation and conduction is intracardiac mapping. Results from multiple precisely positioned multi-electrode intra-cardiac catheters allow to derive the conduction pattern. Modern techniques, however, use sophisticated software algorithms and a multi-electrode exploring catheter to obtain more accurate results in a shorter time-frame. Recently, our research group performed the first successful 3D electroanatomical intracardiac mappings in adult horses [6]. An automatic, high resolution mapping system was successfully used to identify not only the electrical activation patterns from the right and left heart, but also 3D cardiac geometry in 2 adult warmblood horses (Fig.1) [6]. In human medicine this technique has revolutionized the evaluation of specific arrhythmias, the identification of diseased myocardium and ectopic foci origin. Subsequently, these techniques have allowed to develop new treatments based upon ablation of these areas. As we showed that intracardiac mapping is feasible in horses, this technique could become a promising and powerful tool for electrophysiological research, for diagnosis in clinical patients and for development of new treatment strategies such as ablation in horses.
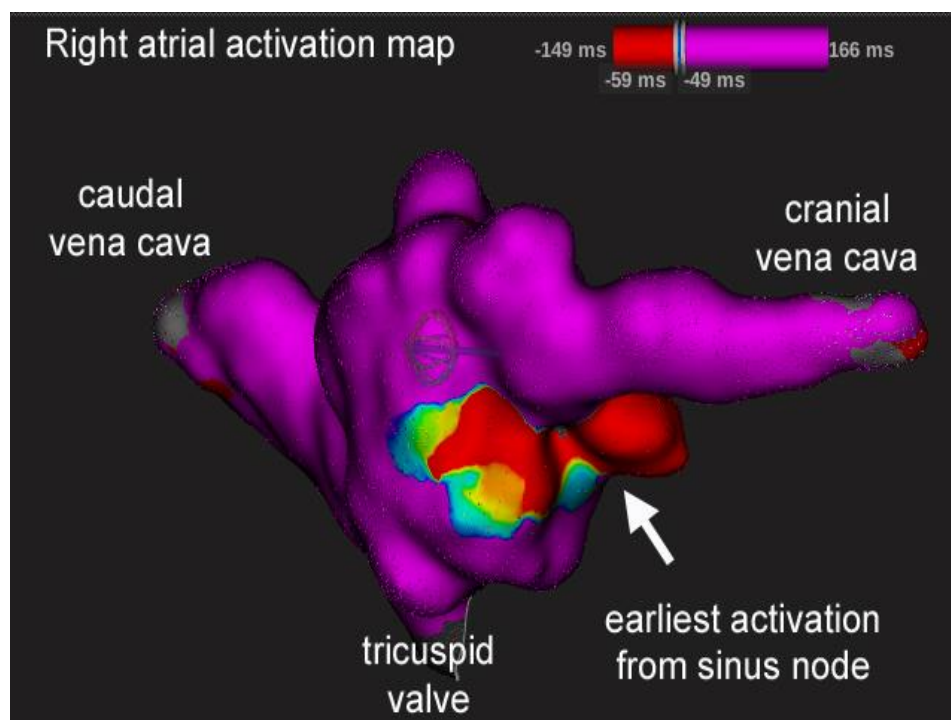
Figure 1: 3D electroanatomical intracardiac mapping of the right atrium in an adult warmblood horse [6].

Conclusions

We conclude that multiple lead recordings are useful for a correct diagnosis of atrial dysrhythmias and that care should be taken to choose an electrode configuration with optimal visualization of the P wave. Further research regarding optimal electrode placement is needed, but placement of one or more electrodes in the region of the atria seems useful to pick up optimal atrial electrical activity. Furthermore, one should be aware that sinus arrhythmia and wandering pacemaker might be challenging arrhythmias to differentiate from APDs and even differentiation between near junctional VPDs and APDs can sometimes be difficult. Guidelines for ECG interpretation acknowledging these pitfalls might improve diagnosis. Furthermore, the development of new techniques for a better evaluation of the depolarization pattern in the heart, such as 12-lead ECG or 3D electroanatomical intracardiac mapping may become important tools for diagnosing difficult or complex arrhythmias.

Is AF Really That Important?

An important part of this dissertation focused on the diagnosis and treatment of AF. AF is a relatively common condition and the most important clinically significant arrhythmia in horses with a prevalence ranging from 0.3 to 2.5% depending on the population [7,8]. Warmblood horses, which constitute the largest part of our population, are probably predisposed due to their large heart size. At our equine clinic, at least 40-50 sport horses are treated for AF each year. In racehorses, several studies have identified cardiac arrhythmias as the third most important cause of poor performance, behind musculoskeletal injury and respiratory problems [9,10].

The clinical importance of AF and whether or not to treat the arrhythmia are still subject for debate amongst equine cardiologists. The clinical consequences of AF for horses with lone AF or mild cardiac abnormalities are sometimes difficult to estimate. AF usually causes poor performance when high level exercise is expected, but when used for less intense athletic work, some horses are able to perform to expectations. Therefore, also the intended level of activity of the horse should be taken into account. Horses that remain permanently at rest do not need to be cardioverted. Treatment decision is based on two factors, namely the intended exercise level of the horse but also the underlying cardiac health. Horses with severe underlying cardiac disease and congestive heart failure are not candidates for cardioversion and treatment should be directed towards the underlying condition. Significant left atrial dilatation reduces the likelihood of successful cardioversion and increases the chances of recurrence after treatment [11]. Left ventricular dilatation may be an indication for underlying myocardial damage in horses with AF and decreases the chances of return to previous level of performance after AF treatment [12]. If no or limited underlying cardiac disease is present, treating AF may be warranted.

During exercise AF may lead to extremely high heart rates and occasionally collapse or rarely even sudden death have been reported. Collapse during exercise may not only lead to injury of the horse but is also dangerous for riders and bystanders. Several studies have reported collapse in horses with AF [13,14] and during our studies, several horses with lone AF collapsed during training at home or during canter exercise on the lunge. In some of these horses no signs of ventricular arrhythmia were observed on the ECG immediately before the collapse but heart rates were very high, often exceeding 300 BPM, probably causing an important decrease in cardiac output and hypotension, potentially leading to collapse. For this reason cardioversion is currently recommended when the average maximal heart rate during exercise

is greater than 220 BPM, at an intensity at, or slightly exceeding the horse's normal activities[11]. In human medicine, rate control is an important part in the treatment strategy of AF. Under specific circumstances, horses that are not or cannot be converted might also benefit from rate control therapy, but little is known so far. Rate control therapy aims to reduce the heart rate at rest and during exercise and might reduce the risk of exercise-related collapse in some horses. Our study on sotalol and AF (Chapter 7) showed a decrease in heart rate both at rest and during exercise when horses were under sotalol treatment. Therefore sotalol might be a possible candidate drug for rate control therapy in horses with AF in which cardioversion is not possible or not preferred by the owner. However, further research into the potential risks of using sotalol, especially in exercising horses, is first needed. Indeed, in human medicine, sotalol is currently no longer advised for rate control therapy because of the risk of torsade de pointes and sudden cardiac death associated with QT prolongation [15]. Such a mechanism might increase the risk for collapse or even sudden death in exercising horses when treated with sotalol.

Abnormal ventricular activation during exercise and/or sympathetic stimulation (stress, pain) has been reported in AF horses. Especially short coupling intervals or R-on-T phenomenon are thought to increase the risk for ventricular tachyarrhythmia [2,11,16]. R-on-T phenomenon is the superposition of a QRS complex on the T wave of the preceding beat whereby an irregular pattern is present and the isoelectric line is lost (Fig.2). This rhythm has been associated with an increased risk for sudden death in human patients [17]. During the sotalol and AF study (Chapter 7), R-on-T phenomenon was observed in 30% (11/37) of the horses and 24% (12/50) of the exercise ECGs during canter. This is in line with a previous publication regarding abnormal ventricular depolarizations in horses with AF [16]. In 3 horses, the exercise test was terminated because of repeated R-on-T episodes. In our study, sotalol treatment did lead to a significant reduction in heart rate but not in number of R-on-T episodes.

Figure 2: R-on-T phenomenon (arrow) during slow canter in a horse with AF. Heart rate during the R-on-T episode was 312 BPM.

Sudden death associated with AF and exercise-induced ventricular tachyarrhythmias, has been reported [2,11]. Therefore, treatment of AF is always recommended when concurrent ventricular arrhythmias are observed [11].

Finally, pronounced ventricular dyssynchrony during stress was observed on ultrasound in our clinic population, in the absence of R-on-T phenomenon. This dyssynchrony is probably the result of aberrant intraventricular conduction (e.g. bundle branch block) and might contribute to hypotension during stress or exercise. It is important to notice that this abnormality is very difficult to diagnose and might go unnoticed as it does not always result in R-on-T on the ECG.

It can be concluded that AF is an important and relatively common condition in horses. Since AF leads to performance problems and dangerous ventricular tachyarrhythmia at high intensity exercise and timely diagnosis increases treatment success, a rapid diagnosis and treatment are important.

Do HRV and HRMs Show Real Promise as a Diagnostic Test for AF?

The purpose of this PhD thesis was to improve diagnosis of AF by introducing a user-friendly, easy-to-interpret, diagnostic test. HRV was chosen based on examples and techniques from human medicine and the reasoning that in horses, because of their slow heart rate, differences in HRV should be larger and AF detection therefore even easier compared to human patients. These assumptions were confirmed in the studies reported in Chapter 2 and 3.

Challenge 1: Correct Heart Rate Detection

Large differences in HRV parameters between horses in AF and horses in SR were observed, provided that heart rate detection was correct. Therefore the most important factor in developing a reliable diagnostic test based on HRV is assuring correct heart rate detection. This is challenging, since all marketed devices, both ECG recorders and HRMs, are prone to identical types of errors. First, only a few RR detection software programs or algorithms are adapted to the horse's unique ECG and overcome the problem of the large T wave sensing. To make RR detection even more difficult; the most reliable cut off values for differentiation between AF and SR in our studies were obtained during walk and trot. However, during exercise the QRS deflection enlarges but the T wave enlarges even more and horses in AF rapidly reach high heart rates, even at low level exercise. This may hamper the differentiation between QRS complex and T wave or artifacts, even for an experienced observer and especially when the trace quality is suboptimal. Therefore RR detection programs specifically adapted to the horse's ECG should be designed.

A few of the possible electrode – heart rate sensor – software combinations were studied in Chapter 3 of this dissertation. Heart rate detection using a commercially available electrode belt was unreliable in our study. This belt contains two built-in electrodes and was positioned according to the manufacturer's guidelines, with one electrode a the lower left thorax, behind the olecranon and the second one halfway the left thorax. It is possible that the elasticity of the belt allowed the electrodes to move with the horse movements. However, even in the resting horse, RR detection with the belt was unreliable. According to the manufacturer, skin contact could be improved by clipping the horses or moisturizing the electrodes. In our study horses were not clipped but electrode gel was used, which should have resulted in sufficient contact between the electrodes and the skin. Electrode contact is an important issue irrespective of the recorder itself.

Apart from the systems evaluated in our study, there are various other heart rate detection systems available, most of them designed for use in humans. An ideal electrode system ensures good skin contact and stable positioning. Using adhesive electrodes, electrode gel, contact glue or ensuring stable positioning under a girth or saddle, is advised. In addition, the heart rate sensor should use an RR detection algorithm especially designed for analyzing equine ECGs. Finally, if data are not stored locally, the telemetric signal should be sufficiently strong to send data over a long distance so that recordings during exercise or work are possible without signal interruptions.

Challenge 2: Ideal Artifact Correction

Another important consideration when using HRV is the implementation of artifact correction methods. Artifact correction generally omits outliers that differ to some, variable extent from the other RR intervals, or replaces them by interpolated values. Devices used in human medicine to diagnose AF based on HRV use a very low level of artifact filtering of the RR intervals before HRV calculations are made. However movement artifacts in humans are less important compared to horses and arrhythmias such as 2nd degree AVB (AVB2) are uncommon. AVB2 is very common in horses due to their high vagal tone. Since the RR interval is doubled compared to the other RR intervals, an AVB leads to a large increase in HRV, which may hamper differentiation between AF and SR, especially when short-term recordings are used [18]. Ideal artifact correction in horses should omit all movement artifacts and AVB2s, but still allow the natural variability in heart rate caused by AF. Based on the results of our study (Chapter 3) we can conclude that low level artifact correction should be used. The low level artifact correction algorithm removed most AVBs and markedly improved the diagnostic capacity of the test. The use of PP intervals instead of RR intervals could be useful to circumvent the problem of AVBs [18], but automatic P wave detection is very difficult in horses. Another approach for the removal of AVBs is to increase sympathic and decrease vagal tone by exercising or stressing the horse, which we achieved by walking or trotting the horse. There are many heart rate sensors and software programs available, each with their own artifact correction algorithm. Sometimes the level of artifact correction can be manually adjusted, but often unknown algorithms are used. The artifact correction level should be taken into account where possible.

The difference in artifact correction methods raises questions about other studies using HRV in horses. Especially when HRV is used to evaluate psychophysiological parameters such as stress or anxiety, which induce very subtle changes in HRV, care should be taken when using

artifact correction algorithms. Most of the published HRV studies do not address the level of artifact correction and often systems with automatic, unadjustable and unknown correction algorithms are used. Considerations concerning the artifact correction method should be taken into account when interpreting the results of such studies.

Challenge 3: Optimal Cut Off Values

For the studies concerning HRV in Chapter 2 and 3 we aimed at choosing cut off values with maximal sensitivity whilst maintaining a good specificity. Since AF is a potentially dangerous condition in some horses and rapid detection improves outcome, we wanted to avoid false negative tests. As a consequence, some false positives may arise. In human medicine, a worldwide discussion concerning the obligatory ECG screening of athletes, is ongoing and led to the publication of an international consensus statement on cardiovascular care by the NCAA, the National Collegiate Athletic Association, to prevent sudden death in athletes [19]. These guidelines ceased recommending heart tests for all college students, due to the high costs of standard screening combined with a small amount of false positives, with important economical and personal consequences. In horses, similar concerns may arise when standard screening of healthy horses for AF would be recommended. In our study in Chapter 5 a mean sensitivity and specificity of 1 and 0.93 was obtained when using RMSSD after low level filtering of RR intervals. This means that, if the total riding horse population in Flanders (130 000 horses registered) would be screened for AF, this would result in around 8000 false positive test results if every horse would be screened only once. Whether the detection of the 'diseased' animals justifies the costs made for the screenings and the extra costs for the ECGs of the false positive horses, would be an interesting discussion but is beyond the scope of this dissertation. Heart rate monitors are, however, increasingly being used during training for a wide range of equine disciplines. In these cases including the registration of heart rate variability, which is already available on some of these devices, could be easy and cheap.

However, when the risk of AF development is increased, such as in horses with a dilated atrium, frequent APDs or moderate to severe mitral valve regurgitation, a regular screening could be very useful. Particularly in horses that have already been treated for AF, regular screening in order to detect recurrence is mandatory. Chances of AF recurrence after treatment are reported to be between 35 and 45%, meaning that for 100 cardioverted horses, monitoring for AF recurrence using HRV would result in 35-45 true positive and only 4-5 false positives. If this screening would be feasible with a cheap, home monitoring system, cost for regular follow-up of these patients would be greatly reduced.

Furthermore, false positive heart rate variability test results are caused either by artifacts or electrical interference or by the presence of other arrhythmias, requiring further investigation. Using low level filtering may help decrease the number of false positives. Therefore, when low level filtering is used, choosing cut off values with maximal sensitivity whilst maintaining an acceptable level of specificity, is advised.

The cut off values obtained in our studies had moderate to high sensitivity and specificity, depending on which level of filtering was used. However, some horses may have a surprisingly regular heart rate in AF, while others may have an irregular heart rate in SR, due to frequent sinus arrhythmia or premature depolarizations. It is therefore important to use these cut off values not as rigid values, but rather as a rough guide, or, even better, determine individual-specific normal values for each horse in SR. In addition, especially in case of doubt, one should evaluate the combination of the resting, walking and trotting values and not the individual data of one gait, in order to fine tune the diagnosis.
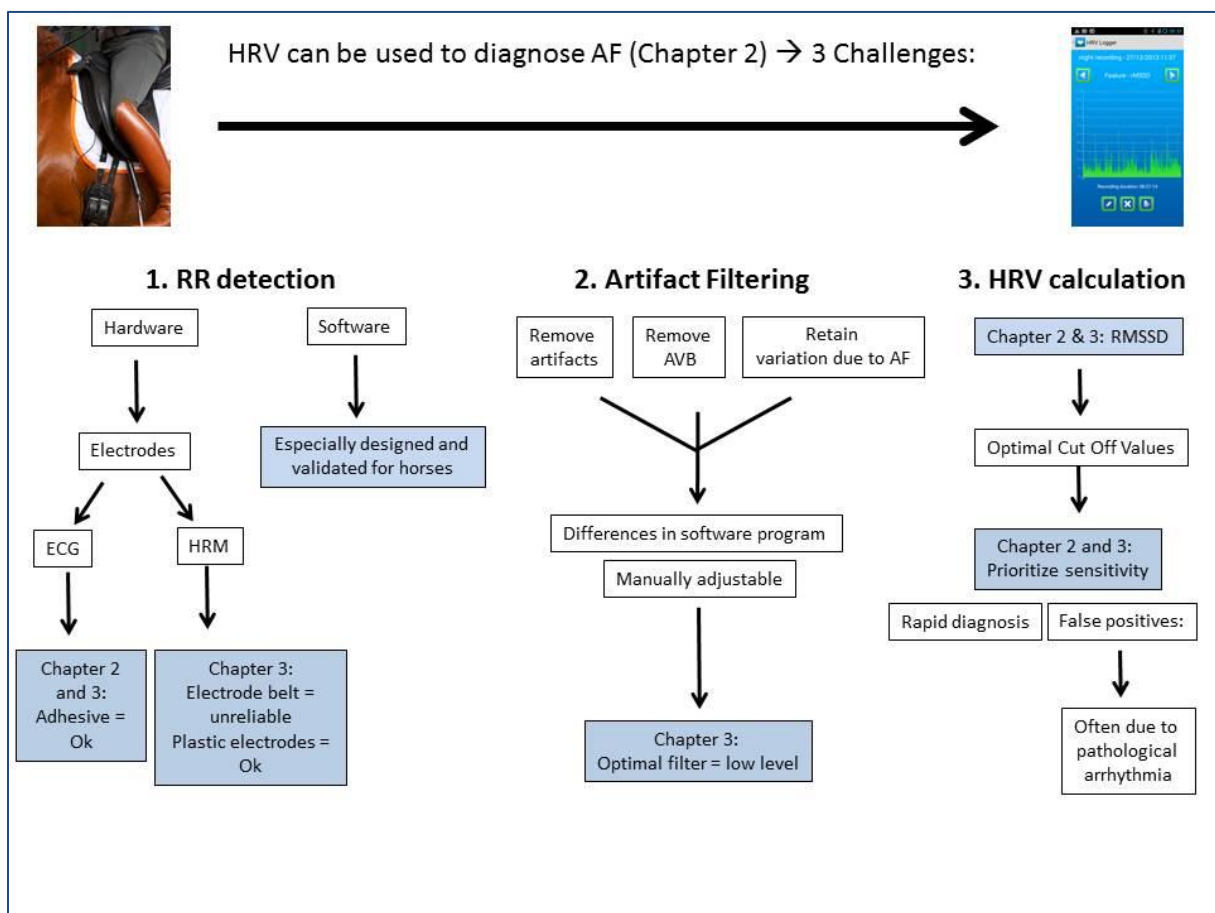

Figure 3: Flowchart of the findings regarding the HRV studies from Chapter 2 and 3.

Conclusion

HRV parameters can be used as a screening tool for AF (Fig.3). When a HRM is used to obtain RR data, care should be taken to ensure correct RR detection by optimizing electrode skin contact and ensuring correct and stable electrode placement. Furthermore, R wave detection software should preferentially be specifically designed for horses. HRV parameters can be obtained from several software programs, smartphone or computer applications. Artifact correction should be set to a low level correction, if possible. In our studies, cut off values for RMSSD were established, but other parameters such as SD1 and SDNN might also be applicable. If cut off values are determined, a high sensitivity should be prioritized.

Pharmacokinetic Profile of Sotalol in Horses

The pharmacokinetic profile of sotalol was less favorable in horses compared to humans or small animals, but better than most other antiarrhythmic drugs in horses (Chapter 4 and 6).

Both oral bioavailability and oral absorption were considerably lower in horses compared to other monogastric species. Although horses have a monogastric digestive system, the lower availability of some drugs can be attributed to their herbivorous diet. Whereas horses do not maintain the full spectrum of cellulolytic gastric bacteria, as do ruminants, they do maintain some fermentative bacteria as part of the normal stomach flora due to their ingestion of a large volume of herbaceous feed. Although the effect of the equine gastric flora on oral stability of drugs is not yet fully understood, it has been shown that some drugs bind to equine ingesta, slowing down their rate of absorption and limiting their bioavailability [20]. While a moderate oral bioavailability of 48% was found in fasted horses, administration of the drug with food led to an important decrease in steady state concentrations. Studies in humans showed variable but rather small effects of food on bioavailability of sotalol, ranging from a 0-20% decrease in absorption [21-23]. In horses however, the influence of feeding on the oral bioavailability of drugs in general is rather variable. Most often the rate of drug absorption is diminished with feeding, resulting in a slower drug uptake and an increase in Tmax, whereas the extent of absorption strongly depends on the formulation of the drug [24,25]. Furthermore, sotalol plasma concentrations in our studies in unfasted horses were variable, not only between horses, but also within horses on different sampling days (Chapter 6). This variability illustrates once more the important effect of feeding and gastrointestinal filling on the oral bioavailability of sotalol. Horses had free access to straw and hay during the day for the entire study period and daily food intake was not monitored. Gastrointestinal filling therefore depended on the feeding behavior of the animals and was probably variable within and between horses. We chose this approach in order to mimic the clinical situation in which this drug would be used.

With an oral bioavailability of 48% in fasted animals, sotalol is less absorbed in horses compared to other species. However, if we compare its bioavailability to other antiarrhythmic drugs in horses, results are promising. Only quinidine, administered by nasogastric tube in fasted horses, has a similar oral bioavailability (48±21%) [26]. Phenytoin has a moderate oral absorption of 35±8% in fasted horses and amiodarone (F=6-33%) and propranolol (F=1-32%) are poorly absorbed [27-29].

The steady state concentrations reached in our studies were well below the therapeutic concentrations aimed for in humans and small animals. In human patients, β-blocking activity starts from a plasma concentration of 0.8 µg/mL and concentrations above 1.4 µg/mL cause class III antiarrhythmic effects, while in dogs β-blocking and class III action were present from a concentration of 0.8 µg/mL and 6 µg/mL, respectively. When such plasma concentrations would be aimed for in horses, predictions using the population pharmacokinetic model indicate that 8 mg/kg bodyweight three times daily would be necessary. However, significant QT and atrial and ventricular ERP lengthening were already present at plasma concentrations around 0.3 µg/mL, indicating class III activity even at low plasma concentrations. Increasing the dose did not lead to significantly higher effects. Therefore, it may not be necessary or beneficial to adjust the dosing schedule and increase the plasma concentrations. In Chapter 7 of this dissertation, β-blocking activity, more specifically a decrease in heart rate, was present after an oral sotalol dose of 2 mg/kg twice daily. Plasma concentrations were not determined in this study, but were probably around 0.3 µg/mL based on the results of our previous studies [30].

While in human medicine a near linear relationship between sotalol plasma concentrations and its electrophysiological effects was observed, this was not shown in our study (Chapter 6). Surprisingly, the electrophysiological class III effects of sotalol, namely ERP and QT lengthening, did not appear to increase with increasing plasma concentrations (Chapter 6). This may possibly be explained by differences in K$^+$ channels between species. The configuration and duration of the cardiac action potentials vary considerably among species[31]. This heterogeneity reflects differences in the type and/or expression patterns of the K$^+$ channels that contribute to the genesis of the cardiac potential. The main targets for sotalol are receptors from the rapidly activating component of the delayed rectifier K$^+$ current, the IKr. Molecular studies have shown that the distribution, the amount and the regulation of the IKr channels are species specific and may differ markedly between species and even within species between individuals [31]. In humans it has been shown that these individual variations in K$^+$ channels may be caused by genetic mutations that may lead to a genetic susceptibility to long QT syndrome and torsade de pointes [32]. Few studies on the distribution and regulation of IKr receptors in horses have been published. Finley *et al.* [33] confirmed the expression of K$^+$ channels and the presence of a delayed rectifier K$^+$ current in horse heart, while Pedersen *et al.* [34] reported a high homology between the human and equine genes encoding the cardiac voltage-gated K$^+$ channel Kv11.1 and responsible for long QT susceptibility in humans. These

studies suggest that, in horses, repolarizing currents and their genetic basis are similar to other species, and that horses are therefore susceptible to both acquired and congenital long QT syndrome. It is possible that horses possess less IKr receptors compared to humans and maximal saturation and clinical response are already obtained at low plasma sotalol concentrations. It is therefore uncertain whether an additional benefit is obtained by increasing the dose or decreasing the dosing interval. Furthermore, in some horses a dose of 2 mg/kg bid already led to increases in QT interval beyond the 20% deemed at increased risk in human patients and the effects of doses higher than 4 mg/kg twice daily were not evaluated in our studies. Therefore, adjusting the dosing schedule in order to obtain plasma concentrations within the range of concentrations aimed for in human medicine and in dogs, may be unnecessary.

Does Sotalol have Real Antiarrhythmic Potential in Horses?

Challenge 1: How Can we Define Antiarrhythmic Effect?

Assessing the antiarrhythmic potential of a drug is often challenging. While we can study the electrophysiological effects of an antiarrhythmic drug *in vitro* on cardiac myocytes and *in vivo* from surface ECG traces or from intracardiac electrophysiological studies, as in Chapter 4, 6 and 7 of this dissertation, the actual potential to reduce arrhythmia is more difficult to assess. When, almost 40 years ago, the dual action of sotalol was first being discovered, a variety of *in vitro* studies were necessary to explain its unique antiarrhythmic properties and the large array of clinical applications this offered. In the following decades, a large number of cohort studies in animal models and later in large groups of human patients were performed. These studies compared groups on placebo, sotalol or other antiarrhythmic treatments for frequency of arrhythmic events, ease of AF cardioversion or recurrence of AF after treatment in order to obtain clinical evidence for the antiarrhythmic efficacy of sotalol [23,35-37]. In horses, such large clinical studies are difficult, due to the smaller study population and practical (transportation, lack of follow-up) and legal (competition, drug approvals and registration) consequences. However, future studies, confirming the antiarrhythmic benefits of sotalol in horses, are necessary. A large prospective study comparing the recurrence rates of horses with and without sotalol treatment post cardioversion could not be included in this dissertation, but would be a valuable future study to assess the clinical antiarrhythmic properties of sotalol. Studying AF susceptibility by intracardiac pacing with and without sotalol, as was recently done for flecainide [38], would also be of clinical interest. The high variability in fibrillation threshold between and within horses, however, complicates this type of studies.

Antiarrhythmic Effects of Sotalol in Horses

Our studies on the electrophysiological effects of sotalol in horses (Fig.4) have shown its potential as an antiarrhythmic drug in horses. Our studies have consistently shown a lengthening of the QT interval on the surface ECG and an increase in both atrial and ventricular refractory periods, illustrating a lengthening of the repolarization phase of the action potential in cardiac tissues and confirming the class III antiarrhythmic action of sotalol. While our studies did not report differences in RR interval or blood pressure in healthy horses, sotalol caused a significant decrease in heart rate in horses with AF, demonstrating its β-blocking activity. In the studies in healthy horses, RR interval was calculated as the mean of 10 measurements, while in the horses with AF the mean resting heart rate over a time frame of 30 minutes was determined, making the latter study results more reliable.
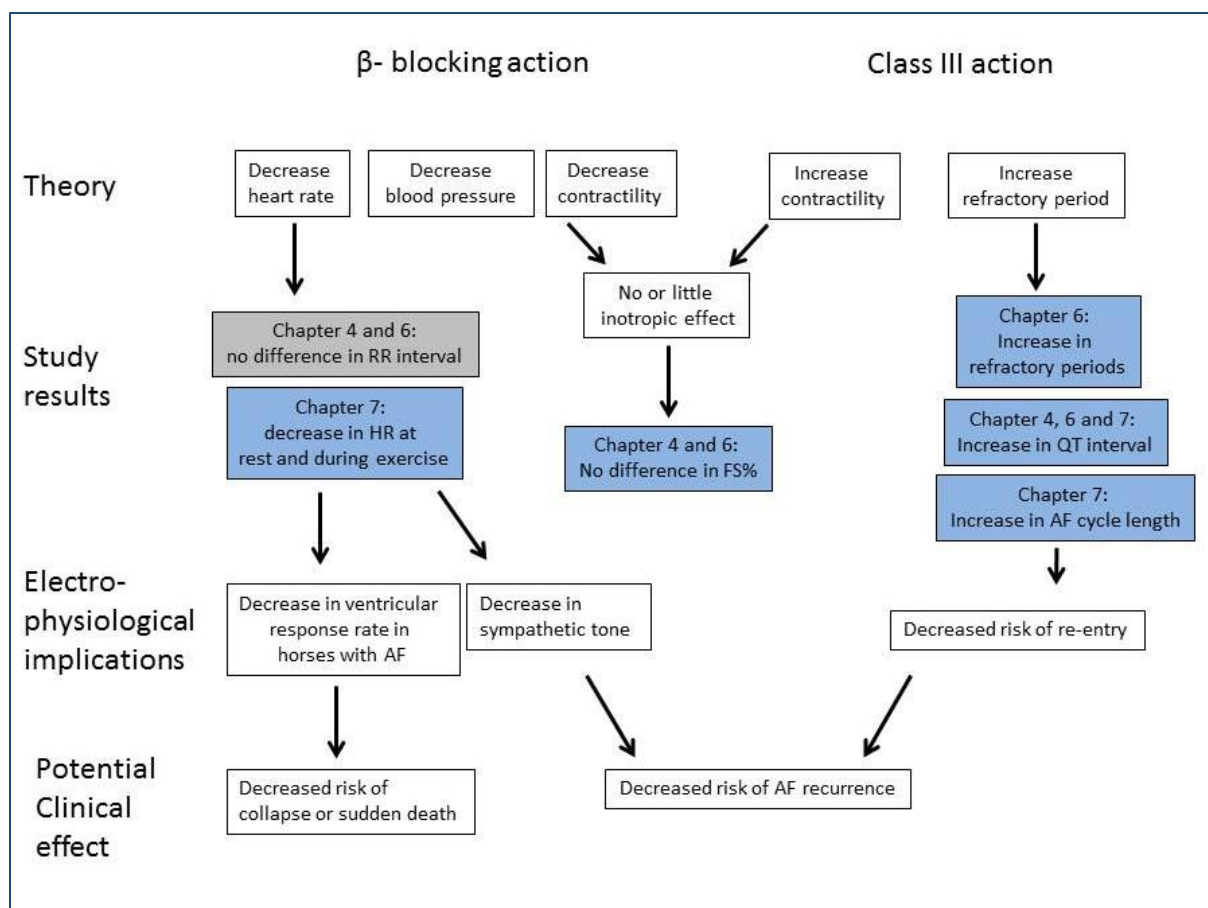
Figure 4: Flowchart of the findings obtained from the sotalol studies in Chapter 4, 6 and 7. HR: heart rate; AF: atrial fibrillation; FS: fractional shortening.

Based on the above findings, sotalol exerted identical electrophysiological effects as in humans and small animals and is therefore expected to have antiarrhythmic activity against supraventricular arrhythmias in horses. Not only does sotalol reduce the sympathetic activity of the heart (β-blocker), thereby possibly decreasing the likelihood of abnormal discharges [39], it also increases the refractoriness of all cardiac tissues. Therefore premature impulses will be less likely to initiate re-entry loops. Furthermore, according to the multiple wavelet theory, AF is maintained through the presence of multiple re-entry circuits meandering chaotically through the atria. It is obvious that, due to an increase in refractoriness, the excitable gap will become smaller and/or the wavelength longer. If sufficient increase in refractoriness occurs, the number of wavelets reduces and/or the head of the depolarization wave will hit its own tail, thereby terminating the re-entry loop. In human patients, the role of sotalol is well established for the maintenance of sinus rhythm after successful conversion of AF, but even when sotalol is used for the pharmacological cardioversion of AF its efficacy was comparable to class Ia and class Ic agents [40].

In our hospital population of horses treated for AF, we have noticed that early recurrence was often preceded by a short period of atrial tachycardia. Before we used sotalol, all the horses that presented periods of atrial tachycardia after cardioversion relapsed into AF in the first days after cardioversion. Since we started using sotalol, 2 horses have shown periods of atrial tachycardia on Holter ECG after cardioversion. Both were treated with sotalol, both remained in sinus rhythm. This could mean that sotalol does effectively reduce AF recurrence, but currently too limited data are available to draw firm conclusions.

Finally, because sotalol, unlike other β-blockers, prolongs the refractoriness of cardiac tissues, it may decrease atrioventricular conduction and, as such, the ventricular response rate in AF patients [35]. Indeed, our study in AF horses on sotalol treatment (Chapter 7) has shown a decrease in heart rate, illustrating its rate control properties. This might be a long-term treatment strategy for horses refractory to AF treatment in order to control heart rate and decrease the risk of collapse during sympathetic stimulation such as stress or exercise. In human medicine, however, sotalol is no longer advised for long-term rate control treatment, because of the risk of torsade de pointes and sudden cardiac death due to excessive QT lengthening. A similar risk might apply to horses and should be further studied.

Challenge 2: Is Sotalol Dangerous in Horses?

The most important side effect of all class III antiarrhythmic drugs is the occurrence of dangerous ventricular tachycardia, especially torsade de pointes, due to excessive QT prolongation. Torsade de pointes is a special form of polymorphic ventricular tachycardia with a characteristic twisting of the QRS complex around the isoelectric baseline (Fig.5). It causes a sudden drop in arterial blood pressure, leading to dizziness and collapse. Most individual episodes of torsade de pointes in humans revert to normal sinus rhythm within a few seconds; however, episodes may also persist and possibly degenerate into ventricular fibrillation, leading to sudden death in the absence of prompt medical intervention. Most pro-arrhythmic events occur in the beginning of treatment, therefore hospitalization in a monitored setting is recommended for initiation of therapy and continued until a steady state drug level is reached [23]. In human medicine the risk of torsade de pointes when on long-term sotalol treatment is estimated between 2 and 4%, increasing with higher dosage [23]. For other drugs the reported risk of torsade de pointes ranges between approximately 0.001% for cisapride to approximately 8% for quinidine [32]. Furthermore, in humans a genetic susceptibility for the development of long QT syndrome and torsade de pointes has been

shown [32]. In experimental models in dogs, proarrhythmia has also been described during sotalol treatment, especially in bradycardic animals [41].

The risk of proarrhythmia is often estimated based on the percentage of QT prolongation. In human medicine, an increase in QT interval of 15-20% is considered dangerous and an indication to reduce the sotalol dose. Moreover, a cumulative daily dose of 320 mg, or 4 mg/kg, results in a sharp increase in the risk of proarrhythmia [42,43]. In all our studies in horses, mean QT lengthening was between 8 and 10%, independent from the dose. Standard deviations were rather small, meaning individual variation was limited. Although the mean QT lengthening in the horses in our studies remained well below 20%, some individuals showed QT lengthening above 20% with one horse even exceeding 30% on a dose of 2mg/kg bid. Plasma concentrations of this particular horse were unfortunately not available. In the sotalol electrophysiology study (Chapter 6), QT intervals did not increase linearly with the sotalol plasma concentrations and horses with high plasma concentrations did not necessarily have the longest QT intervals. This might be explained by a variable individual susceptibility to sotalol, in which the IKr receptor distribution or regulation vary between animals [31] or a genetic susceptibility for long QT syndrome, as was shown in human patients [32] and has been suggested in horses before [34]. In horses it is not known as from which increase in QT interval, the risk for proarrhythmia is significantly increased and no guidelines on cut off values exist.
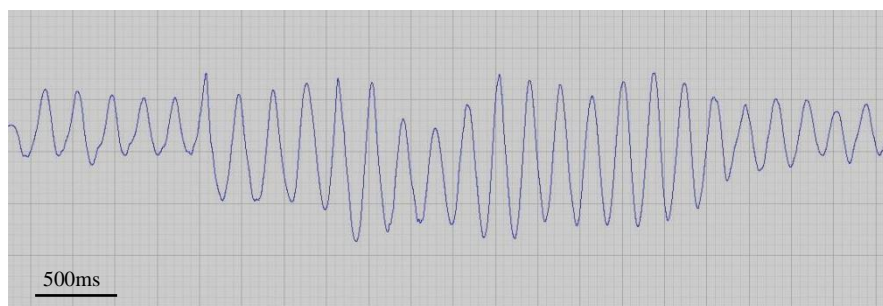
Figure 5: Torsade de Pointes in a horse. Note the QRS complexes turning around the baseline.

During the course of our sotalol studies, no pro-arrhythmic events were noted for doses up to 4 mg/kg two times daily. However, no continuous ECG follow-up was available nor exercise tests were performed in healthy horses, so drawing conclusions regarding safety, especially in horses with AF, is difficult. In analogy with human medicine, when sotalol is administered to horses with hypokalaemia, bradycardia or renal disease or when other QT prolonging drugs are administered concurrently, ECG monitoring until steady state concentrations are reached,

is recommended [44,45]. A preliminary study described an increase in QT interval of 38% in healthy horses under general anaesthesia [46]. When these horses received sotalol, QT interval lengthened even more, with increases above 60% in individual animals [46]. Special care should thus be taken when horses receiving sotalol undergo general anaesthesia and close ECG monitoring or dose reduction might be recommended. Strategies to decrease the chances of proarrhythmia or torsade de pointes include alternative pharmacotherapy, avoiding potentially aggravating drug-drug interactions, bradyarrhythmias or electrolyte abnormalities, and the supplementation of magnesium sulphate [47,48].

During the course of our studies, other, less dangerous side effects were noted. Sweating was regularly encountered, at all dosages and one horse experienced an episode of colic after 1 mg/kg sotalol intravenously. Colic was never observed during the studies with oral sotalol up to a dose of 4 mg/kg bid. Both sweating and abdominal discomfort are known side effects in human medicine and can be explained by the β-blocking activity of the drug. Sweating was most often noted at the start of the sotalol treatment, after the first or second dose. Other side effects often mentioned in human medicine as a reason for discontinuation of treatment are fatigue, bradycardia, dyspnea, weakness and dizziness. In dogs, sotalol is usually well tolerated, rare side effects being lethargy, dyspnea and vomiting [41,49,50]. Dyspnea and bradycardia were not observed in the horses in our studies. However, owners from horses that were on sotalol after cardioversion in our clinic, did occasionally report that their horse was noticeably calmer when receiving sotalol.

Conclusion

Both the β-blocking activity of sotalol and its potential to increase the cardiac repolarization process were demonstrated in horses. Whether these electrophysiological effects also lead to a reduction in supraventricular arrhythmias and decrease the chances of AF recurrence, is likely, but remains to be elucidated in further clinical studies. The dosages used in our studies seemed safe, but care should be taken when sotalol is used concurrently with other QT prolonging drugs, during general anaesthesia or in hemodynamically unstable situations. The fact that sotalol shows β-blocking and class III antiarrhythmic activity, has a moderate oral absorption, is a cheap drug and was well tolerated by our study population, supports its use as a long-term treatment for atrial arrhythmias in horses.

Future Prospects

The ultimate goal of our studies concerning HRV and AF is the development of an easy-to-use home monitoring tool for horses at risk of AF. In order to reach this goal, improvements should be made. Equine specific hardware should be developed with special focus on electrode design and placement. Efforts should be made to optimize skin contact and electrode positioning and retaining the electrodes in place during exercise. Furthermore software for RR detection should be adapted to the ECG wave forms of the horse and an adequate artifact correction filter should be developed in order to remove artifacts and AVB2 whilst maintaining the variation in RR interval due to AF. Finally, when this device is functional, it should be validated, both in a healthy population, a population with various arrhythmias and an AF population, against the gold standard of manually analyzed ECG tracings.

The efficacy of sotalol to prevent AF could be experimentally assessed by measuring AF susceptibility by intracardiac pacing with and without sotalol. However, the large day-to-day variability in burst pacing induced AF in horses makes interpretation of the results difficult.

In analogy with human medicine, the efficacy of sotalol in reducing arrhythmia should be clinically evaluated in large cohort studies in groups of horses with various arrhythmias. These types of studies in horses are complicated due to difficulties in long-term follow-up and issues regarding owner compliance. Furthermore, the large day-to-day variability in arrhythmias on ECG recordings, complicates these types of studies. Drug legislation also hampers the use of sotalol in sport horses. Recently, sotalol was approved by the Fédération Equestre International (FEI) as 'controlled' medication instead of 'banned' medication, meaning that it can be used in competing horses, but only outside the competition period. Unfortunately, high performance horses are the population in which arrhythmias, and especially AF, cause their most important clinical effects and thus the study group in which sotalol treatment would be most indicated. Hopefully the improved legislation together with our study results will lead to further multicenter research in a sufficiently large study population to clinically evaluate the antiarrhythmic potential of sotalol in horses.

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SUMMARY / SAMENVATTING

Summary

Atrial arrhythmias, and especially atrial fibrillation, are rather common disorders in horses. Because of large differences in clinical consequences of atrial arrhythmias, a correct diagnosis is crucial. An early diagnosis is, especially for atrial fibrillation, important in order to improve treatment success and decrease the chances of recurrence. For horses at risk for atrial rhythm disorders, such as horses with an enlarged atrium, moderate to severe mitral valve regurgitation or frequent atrial premature depolarization, regular follow-up is advised. The standard diagnostic tool for heart rhythm disorders is electrocardiography. However, this technique requires specialized equipment and a certain amount of expertise, making regular monitoring often time consuming and expensive. Therefore a cheap and easy to use monitoring tool for arrhythmias, especially atrial fibrillation, would be beneficial. Although short-term treatment of atrial arrhythmias is often successful, long-term success rates are often lower. Especially for atrial fibrillation, chances of relapse after successful treatment are relatively high. Horses at risk for recurrence could benefit from long-term antiarrhythmic treatment in order to suppress premature depolarizations while reverse remodeling takes place. Unfortunately, limited oral antiarrhythmic drugs are available for long-term use in horses. Furthermore, most of these drugs have poor oral availability or may cause important side effects.

The purpose of this PhD thesis was to improve the diagnostic possibilities and pharmacological treatment of atrial rhythm disturbances in horses.

The thesis has been divided into 2 large parts. Part 1 aims to improve the diagnosis of atrial arrhythmias. First we focused on a correct diagnosis of atrial rhythm disorders, especially a correct differentiation between premature depolarizations of an atrial or a ventricular origin (Chapter 1). Therefore the morphology of atrial premature depolarizations was studied in a group of 30 healthy horses. Although it is usually stated that atrial premature depolarizations do not cause changes in the morphology of the QRS complex and the T wave, our study has shown that sometimes they can cause significant changes in QRS complex and T wave morphology. This can make differentiation with premature depolarizations of a ventricular origin challenging. In chapter 2 and 3 the concept of heart rate variability is introduced. Heart rate variability describes and quantifies the variation in heart rate. This variation is larger when arrhythmias are present compared to normal, regular heart rhythms. Therefore, heart rate variability is used in human medicine for the diagnosis of several heart rhythm disorders and especially atrial fibrillation. In horses, heart rate variability is often used in animal

welfare studies assessing pain, stress and fear. In Chapter 2 and 3 of this thesis, heart rate variability is studied as a possible diagnostic tool for atrial fibrillation in horses. In a first study, 6 different heart rate variability parameters were studied in 20 horses before and after treatment of atrial fibrillation and for certain heart rate variability parameters there was a strongly significant difference between horses in sinus rhythms and horses in atrial fibrillation. A second study evaluated whether a commercial heart rate monitor could be used for the diagnosis of atrial fibrillation. In 14 horses, a heart rate monitor automatically calculating heart rate variability was able to differentiate between horses in atrial fibrillation and horses in sinus rhythm. Furthermore, the effect of different artifact correction algorithms was studied. These algorithms are applied before heart rate variability calculations and therefore have an important effect on their results. Artifact corrections levels differ between the different software programs and can sometimes be manually adjusted. Our study has shown that a very low level of filtering already eliminates the most important artifacts whilst maintaining the variability caused by arrhythmia.

The second part of this thesis concerns the treatment of atrial arrhythmias and especially atrial fibrillation. Horses with rhythm disorders sometimes require long-term antiarrhythmic treatment and also horses recently treated for atrial fibrillation may require treatment in order to decrease the chances of recurrence. However, there are currently few antiarrhythmic drugs available for long-term oral use in horses. In human medicine and dogs, sotalol, a β-blocker with additional class III antiarrhythmic properties, is frequently used for the treatment of arrhythmias and the prevention of atrial fibrillation recurrence. Sotalol decreases the excitability of the cardiac tissue and lowers heart rate and blood pressure. Sotalol is already empirically used in horses for the prevention of atrial fibrillation recurrence, but little is known about its pharmacokinetic profile and efficacy in horses. In a first study (Chapter 4), the pharmacokinetics and – dynamics of 1 and 2 mg/kg sotalol were studied in 6 healthy horses. Sotalol had a moderate oral bioavailability in fasted horses and caused a significant prolongation of the QT interval on surface electrocardiogram. This indicates that sotalol also has antiarrhythmic activity in horses. A second study (Chapter 6) revealed the pharmacokinetics, pharmacodynamics and electrophysiological effects of higher doses (3 and 4 mg/kg bid) of sotalol for a longer period (9 days). Furthermore the influence of feeding on the absorption of sotalol could be assessed. Sotalol caused a significant prolongation of the QT interval and the refractory periods, both in the atrium as in the ventricle, indicating some class III antiarrhythmic activity in the horse. For this study, monophasic action potentials

were registered in the standing horse (Chapter 5). This technique is often used in human medicine to study the electrical activity in the heart and the effects of medication. It was the first time this technique was described in horses. Finally, in Chapter 7 the effects of sotalol in horses with atrial fibrillation were studied. In 41 horses with atrial fibrillation, sotalol (2 mg/kg bid for 9 days) caused a decrease in heart rate at rest and during exercise, a prolongation of the QT interval on the surface electrocardiogram and a decrease in atrial fibrillation cycle length. This confirms that sotalol has, aside from its class III effects, also β-blocking action in horses.

In the last chapter of this thesis the results of the different studies are discussed and conclusions are drawn. Which techniques show potential to improve the diagnosis and treatment of atrial rhythm disorders? A first important conclusion was that good guidelines need to be developed in order to improve the correct interpretation of equine electrocardiograms. This would decrease the chance of erroneous diagnoses and treatments. Furthermore, heart rate variability has shown potential as a future diagnostic parameter for atrial fibrillation. Large differences in heart rate variability between horses in sinus rhythm and horses in atrial fibrillation were found. This could lead to the development of an easy-to-use monitoring tool using automatic heart rate variability parameters for an early diagnosis of atrial fibrillation. However, RR detection in horses is not always reliable and needs improvement and an optimal artifact correction algorithm needs to be developed. As a second conclusion we have shown that sotalol exerts both class III antiarrhythmic action and β-blocking activity in the horse, without any clinically significant side effects in the horses in our studies. However, sotalol plasma concentrations were often variable between horses and even within horses on different study days. Furthermore electrophysiological effects were often small and of minor clinical importance and treatment duration was limited to 9 days maximum. Finally, our small population of horses was not permanently monitored for proarrhythmia. For these reasons it is important that further clinical studies assess the efficacy and safety of sotalol as a long-term antiarrhythmic treatment in horses.

Samenvatting

Atriale hartritmestoornissen, en in het bijzonder atriale fibrillatie zijn relatief frequent voorkomende aandoeningen bij het paard. Omdat de klinische implicaties van de verschillende mogelijke atriale ritmestoornissen sterk kunnen verschillen, is een correcte diagnose van groot belang. Bovendien is, voornamelijk voor atriale fibrillatie, een vroege diagnose belangrijk om het succes van de behandeling te verbeteren en de kans op herval te verminderen. Het regelmatig opvolgen van risicopaarden, zoals paarden met een vergroot hart, matig tot ernstige mitraalklepinsufficiëntie of een grote hoeveelheid atriale extrasystolen, wordt daarom steeds aangeraden. Electrocardiografie is de standaard diagnostische techniek voor hartritmestoornissen. Deze techniek vereist echter gespecialiseerde apparatuur en de nodige expertise zodat regelmatige opvolging al snel arbeidsintensief en duur wordt. Er is dus nood aan een eenvoudige, goedkope techniek voor het opsporen van hartritmestoornissen, en in het bijzonder atriale fibrillatie. Hoewel veel ritmestoornissen op korte termijn goed behandeld kunnen worden, is de lange termijn behandeling soms moeilijk. Vooral bij atriale fibrillatie is de kans op herval na een succesvolle behandeling relatief groot. Paarden met een verhoogd risico op herval zouden baat kunnen hebben bij een lange termijn antiaritmische behandeling om de kans op atriale extrasystolen te verminderen terwijl het myocard de kans krijgt zich te herstellen. Er zijn echter zeer weinig orale antiaritmica beschikbaar voor langdurig gebruik bij het paard. De meeste producten hebben een lage orale biologische beschikbaarheid of veroorzaken belangrijke neveneffecten bij het paard.

Het doel van dit doctoraat is daarom het verbeteren van de diagnostische modaliteiten en de farmacologische behandeling van atriale hartritmestoornissen.

De resultaten van dit doctoraat zijn opgedeeld in 2 grote delen. In deel 1 wordt de diagnose van atriale ritmestoornissen van naderbij bekeken. Eerst wordt aandacht besteed aan een correcte diagnose van atriale ritmestoornissen, meer bepaald een correcte differentiatie tussen premature depolarisaties van atriale en van ventriculaire oorsprong (Hoofdstuk 1). De morfologie van atriale premature depolarisaties werd bestudeerd in een studiegroep van 30 paarden. Hoewel steeds werd gedacht dat atriale premature depolarisaties de morfologie van het QRS complex en de T golf niet veranderen, toonde deze studie aan dat er wel degelijk veranderingen in QRS en T golf morfologie kunnen optreden. Dit maakt de differentiatie met premature depolarisaties van ventriculaire origine soms moeilijk. Vervolgens wordt het begrip heart rate variability geïntroduceerd. Heart rate variability parameters kwantificeren de variatie in hartritme. Omdat deze variatie groter is bij hartritmestoornissen in vergelijking met

een normaal, regelmatig ritme, wordt heart rate variability in de humane geneeskunde gebruikt voor de diagnose van verschillende hartritmestoornissen, in het bijzonder atriale fibrillatie. Bij het paard werd heart rate variability tot dusver voornamelijk gebruikt voor het bestuderen van stress en angst bij paarden. In Hoofdstuk 2 en 3 van dit doctoraat wordt het gebruik van heart rate variability voor de diagnose van atriale fibrillatie bij het paard bestudeerd. In een eerste studie werden 6 verschillende heart rate variability parameters bestudeerd bij 20 paarden voor en na behandeling van atriale fibrillatie. Deze studie toonde een sterk significant verschil in bepaalde heart rate variability parameters aan bij paarden met atriale fibrillatie vergeleken met gezonde paarden. In een tweede studie werd vervolgens gekeken of ook een commercieel beschikbare hartslagmonitor kan gebruikt worden voor de detectie van atriale fibrillatie. Bij 14 paarden werd aangetoond dat een hartslagmonitor die automatisch heart rate variability berekent, bruikbaar is voor detectie van atriale fibrillatie. Tevens werd het effect van verschillende artefact correctie filters bestudeerd. Deze filters zijn algoritmen die worden toegepast op de verkregen data betreffende hartritme, alvorens de heart rate variability parameters berekend worden en hebben op die manier een belangrijke invloed op hun resultaat. Ze zijn variabel voor de verschillende beschikbare software programma's en kunnen soms handmatig ingesteld worden. Er werd aangetoond dat een zeer laag niveau van filtering reeds zorgt voor het verwijderen van storende artefacten terwijl de variatie verkregen door de aritmie grotendeels behouden werd.

Het tweede deel van dit doctoraat behelst de behandeling van atriale ritmestoornissen en in het bijzonder atriale fibrillatie. Paarden met ritmestoornissen hebben soms langdurig medicatie nodig en ook paarden behandeld voor atriale fibrillatie kunnen nood hebben aan antiaritmica om de kans op herval te verlagen. Momenteel zijn er weinig antiaritmica beschikbaar voor langdurig, oraal gebruik bij het paard. Bij de mens en de hond wordt sotalol, een β-blocker met klasse III antiaritmische activiteit, frequent gebruikt voor de behandeling van hartritmestoornissen en de preventie van herval bij atriale fibrillatie. Sotalol vermindert de prikkelbaarheid van het hartweefsel en zorgt voor een verlaging van de hartslag en de bloeddruk. Steeds vaker wordt sotalol ook bij het paard gebruikt ter preventie van ritmestoornissen. Er is echter weinig informatie beschikbaar over zijn farmacologisch profiel en zijn werking bij het paard. In een eerste studie (Hoofdstuk 4) werd de farmacokinetiek en -dynamiek van 1 en 2 mg/kg sotalol bij 6 gezonde paarden bestudeerd. Er werd aangetoond dat sotalol een matige orale beschikbaarheid heeft bij uitgevaste paarden. Bovendien veroorzaakte sotalol een significante verlenging van het QT interval op oppervlakte elektrocardiogram en

heeft dus ook bij het paard mogelijks antiaritmische activiteit. In Hoofdstuk 6 werden de farmacokinetiek en –dynamiek van hogere doseringen (3 en 4 mg/kg tweemaal daags gedurende 9 dagen) en de elektrofysiologische effecten van sotalol bekeken, opnieuw bij 6 gezonde paarden. In deze studie werd ook het effect van voeding op de absorptie van sotalol bestudeerd. Sotalol veroorzaakte een significante verlenging van het QT interval en de refractaire perioden, hetgeen zijn klasse III antiaritmische werking op het hartspierweefsel bevestigt. Tijdens deze studie werden ook voor het eerst monofasische actiepotentialen (Hoofdstuk 5) geregistreerd op het staande paard. Deze techniek wordt in de humane geneeskunde hoofdzakelijk gebruikt om de elektrische activiteit in en het effect van medicatie op de verschillende hartkamers te bestuderen. In Hoofdstuk 7 tenslotte, werd het effect van sotalol bij paarden met atriale fibrillatie bekeken. Bij 41 paarden met atriale fibrillatie veroorzaakte sotalol (2 mg/kg tweemaal daags) een daling van het hartritme tijdens rust en inspanning, een verlenging van het QT interval op oppervlakte elektrocardiogram en een daling van de atriale fibrillatie cycluslengte. Sotalol heeft dus, naast de reeds aangetoonde klasse III effecten, ook β-block werking bij het paard.

Tenslotte worden in de discussie van dit doctoraat de resultaten van de verschillende studies samengelegd en besproken. Welke conclusies kan men trekken? Welke technieken hebben potentieel om de diagnose en prognose van atriale ritmestoornissen te verbeteren? Vooreerst is het van belang goede richtlijnen op te stellen voor een correcte interpretatie van het elektrocardiogram bij paarden. Hierdoor kan de kans op foute diagnoses en behandelingen verminderd worden. Voorts is heart rate variability een veelbelovende tool om in de toekomst de diagnose van hartritmestoornissen, in de eerste plaats atriale fibrillatie, op een snelle en gemakkelijke manier te kunnen stellen. Er werden grote verschillen vastgesteld tussen paarden in atriale fibrillatie en paarden in sinusritme. Dit kan er in de toekomst toe leiden dat eenvoudige tools die gebruik maken van automatische heart rate variability berekeningen gebruikt zouden kunnen worden om de diagnose van atriale fibrillatie vroegtijdig te stellen. Echter, RR detectie is bij het paard nog niet optimaal en ook de zoektocht naar een correcte filter methode is lopende. Voorts werd aangetoond dat sotalol ook bij paarden over antiaritmisch potentieel beschikt. Onze verschillende studies toonden zowel de β-block werking als de klasse III antiaritmische werking van sotalol aan bij paard, zonder dat er klinisch belangrijke neveneffecten optraden. De bereikte plasmaconcentraties waren echter vaak variabel zowel tussen de verschillende dieren als binnen één dier op verschillende dagen en de elektrofysiologische effecten waren meestal klein. Bovendien was onze studiegroep

eerder klein, de behandeling vrij kort en werd er geen permanente elektrocardiografische opvolging uitgevoerd. Het is daarom belangrijk dat er verdere, klinische studies gebeuren om de efficiëntie en veiligheid van sotalol als lange termijn antiaritmische behandeling bij het paard te bestuderen.

CURRICULUM VITAE

Barbara Broux werd geboren op 13 februari 1985 te Leuven. Na het beëindigen van het secundair onderwijs aan het Sint Jozefscollege te Aarschot en een uitwisselingsjaar in Zuid-Amerika, startte zij in 2004 aan de studie diergeneeskunde aan de Universiteit Antwerpen. Voor de masteropleiding verhuisde ze naar de Universiteit Gent, waar ze in 2010 het diploma van dierenarts (optie paard) behaalde met onderscheiding. Onmiddellijk na het afstuderen startte Barbara een internship paard, optie Inwendige Ziekten. Daarna trad ze in dienst bij de vakgroep Interne Geneeskunde en Klinische Biologie van de Grote Huisdieren voor een opleiding tot specialist inwendige ziekten paard (ECEIM residency).

Vanaf 1 augustus 2013 is Barbara aangesteld als assistent bij diezelfde vakgroep. Hier is zij betrokken bij het werk in de kliniek en de opleiding van laatstejaarsstudenten en vervolledigt ze haar specialistenopleiding. Dit werd gecombineerd met een doctoraatsstudie betreffende de diagnose en behandeling van ritmestoornissen bij paarden. Tevens vervolledigde zij in 2017 het trainingsprogramma van de Doctoral School of Life Sciences and Medicine aan de Universiteit Gent.

Barbara Broux is auteur en mede-auteur van meerdere wetenschappelijke publicaties en was eveneens spreker op verschillende nationale en internationale congressen. In 2016 won ze de Lluis Monreal Memorial Award voor beste orale presentatie op het ECEIM congres te Helsinki, Finland.

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3. <u>Broux B.,</u> De Clercq D., Vera L., Van Steenkiste G., Decloedt A., Watteyn A., Devreese M., Croubels S., van Loon G. (2016) Pharmacokinetics and Electrophysiological Effects of Different Dosages of Oral Sotalol in Horses: Preliminary Results. 9th Congress of the European College of Equine Internal Medicine, Helsinki, Finland : Abstracts. Oral Presentation.

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5. <u>Broux B.</u>, Lefere L., Verryken K., van Loon G. (2015) Therapeutic Plasma Exchange to Treat Hyperbilirubinemia in Foals with Neonatal Isoerythrolysis. European Veterinary Conference: Voorjaarsdagen Amsterdam, The Netherlands. Oral Presentation.

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