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Basic feasibility studies on interventional cardiology in horses

Ph.D. Thesis

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Table of Contents

List of Abbreviations 5

Summary 6

Összefoglalás 8

Introduction 10

Development of catheter-based interventions in cardiovascular medicine 10

Development of the diagnosis of cardiac arrhythmias in horses 12

The role of echocardiography in interventional methods 14

Pacemakers 17

Electrical cardioversion 17

Electro-anatomical mapping 20

Electrophysiological examination 21

Electrical stimulation methods described in horses 21

Ablation 22

Atrial fibrillation 27

Objectives 31

Chapter I: Pharmacological cardioversion in horses: own experiences 32

Treatment of atrial fibrillation in horses with orally administered quinidine sulphate 32

Successful treatment of ventricular tachycardia with oral propranolol in horses 37

Chapter II: Long-term use of an implantable loop recorder in horses 40

Introduction 41

Materials and methods 42

Results 45

Discussion 46

Conclusion 50

Chapter III: Morphological and histological investigation of the conduction system in the equine atrial muscle sleeve of pulmonary veins 51

Introduction 53
Materials and methods 54
Results 57
Discussion 62
Conclusion 64
New scientific results 66
References 67
The Author's scientific publications 87
Publications forming the basis of the doctoral thesis 87
Other publications 88
Posters and oral presentations 89
Thesis and TDK supervision in undergraduate veterinary training 91
Acknowledgements 92

List of Abbreviations

3D	three-dimensional
AF	atrial fibrillation
AT	atrial tachycardia
ASD	atrial septal defect
AV	atrioventricular
BC	before Christ
CLS	closed loop stimulation
CS	coronary sinus
CT	computed tomography
Cx43	connexin 43
Cx45	connexin 45
ECG	electrocardiogram, electrocardiography
FVT	fast ventricular tachyarrhythmia
ICE	intracardiac echocardiography
ICLC	interstitial Cajal-like cell
ICM	implantable cardiac monitor
IHC	immunohistochemistry
ILR	implantable loop recorder
PV	pulmonary vein
SD	standard deviation
TH	tyrosine hydroxylase
TVEC	transvenous electrical cardioversion
VSD	ventricular septal defect
VT	ventricular tachyarrhythmia

Summary

In horses, both physiological and pathological cardiac arrhythmias occur. Pathological cardiac arrhythmias can develop primarily as conductive disorders or secondarily as a result of changes in the structure of the heart, metabolic and endocrine disorders, systemic inflammation, low blood pressure, bleeding, anaemia, ischaemia, toxicosis, and various drug effects. The examination of arrhythmias is particularly important due to the haemodynamic changes that result from them (reduced blood pressure, decreased flow or perfusion) and the development of electrical instability (fibrillation, sudden cardiac death).

The cardiac rhythm of horses has been studied using electrocardiography (ECG) since the 1910s. The first electrocardiogram obtained from a horse was published in 1913. From the 1960s, with the aid of radiotelemetry devices, it became possible to record electrocardiograms from horses during exercise and, by the 1980s, extensive knowledge was available regarding the physiological and pathological rhythm disorders in horses. Besides pharmacological and transvenous electrical cardioversion (TVEC), interventional methods have been developed in horses lately. There are a lot of technical issues that cause difficulties in the adaptation of human interventional methods in horses. The main differences are the larger size of the equine thorax and heart and the dissimilarities in the electrical properties of the conduction system in the heart.

This dissertation includes three chapters linked to equine cardiology. In the first chapter, our own experiences with pharmacological cardioversion are summarised, highlighting the demand for developing interventional cardiology methods in horses. In the second and third chapters, we discuss two experiments directly related to the field of interventional cardiology. In the first part of the research, we validated the Reveal XT insertable cardiac monitor. The body region where the device should be implanted to generate optimal ECG curves with minimal noise caused by muscular motion considering the normal work of the horse was also investigated. Televet 100 telemetric and Holter ECG equipment used in horses for a long time, was used to validate the cardiac monitor. We examined the possible short- and long-term complications of the implantation and the usability of the device in the field of arrhythmia detection considering overall clinical decision-making issues. As different methods of interventional cardiology are getting more and more widespread in equine medicine, a comprehensive investigation of the histological properties of the equine heart became necessary.

In the second part of the research, 83 cadaver heart specimens were collected for anatomical and histological studies to determine the size of the pulmonary vein ostia to

support one-shot ablation techniques, especially cryoballoon catheter ablation. The second aim of this study was to investigate the conduction system of the pulmonary veins and their antrum with special attention to the myocardial sleeve using histological and immunohistochemical methods. We aimed to identify adrenergic and non-adrenergic nerves, and we investigated cardiac gap junction proteins (connexins) in the equine heart as described previously in humans.

Összefoglalás

A lovakban élettani és kóros ritmuszavarok egyaránt előfordulnak. A kóros szívritmuszavarok elsősorban elsődleges ingerképzési vagy ingerületvezetési zavarokként fordulnak elő, de kialakulhatnak másodlagosan a szív szerkezetének változásai, metabolikus és endokrin rendellenességek, szisztémás gyulladás, alacsony vérnyomás, vérzés, vérszegénység, ischaemia, toxikózis, valamint különféle gyógyszerhatások következményeként is. A szívritmuszavarok vizsgálata különösen fontos az általuk okozott hemodinamikai változások (csökkent vérnyomás, csökkent véráramlás és perfúzió) és az elektromos instabilitás (fibrilláció, hirtelen szívhalál) miatt.

A lovak szívritmusát az 1910-es évek óta elektrokardiográfiával vizsgálják, az első elektrokardiogram lóról 1913-ban készült. A 1960-as évektől kezdve rádiótelemetriás eszközök segítségével lehetőség nyílt lovak elektrokardiogramjainak felvételére mozgás közben, és az 1980-as évekre kiterjedt ismeretek álltak rendelkezésre a lovaknál előforduló fiziológiás és patológiás ritmuszavarokról. A gyógyszeres és a transzvénás elektromos kardioverzió (TVEC) mellett az utóbbi időben intervenciós módszereket is fejlesztettek a lovaknál. Sok technikai probléma nehezíti a humán intervenciós módszerek lovakra adaptálását. Nem csak a nagyobb mellkasi dimenziók és a szív mérete tér el, de jelentősek a szív elektromos vezetési rendszerének különbségei is.

Jelen disszertáció három fejezetből épül fel. Az első fejezetben a saját tapasztalatainkat foglaljuk össze a gyógyszeres kardioverzió lovakban történő alkalmazásáról, kiemelve az intervenciós kardiológiai módszerek fejlesztésének szükségességét lovakban. A második és harmadik fejezetben egy-egy, az intervenciós kardiológia területéhez közvetlenül kapcsolódó kísérletet taglalunk. Az első tanulmány során meghatároztuk, hogy a lovakban ritkán használt Reveal XT szívmonitort mely testtájékra érdemes beültetni, hogy a rögzített EKG-görbék minél több szíveredetű jelet, és minél kevesebb izommozgásból származó zajt ábrázoljanak, figyelembe véve a ló szokásos munkáját. Az eszközt a lovakon már régóta használt Televet 100 telemetrikus és Holter-EKG-készülék egyidejű alkalmazásával validáltuk lóra. Vizsgáltuk, hogy a szívmonitor használatának időtartama alatt jelentkeznek-e komplikációk rövid- és hosszútávon, illetve, hogy az általános klinikai döntéshozatali problémák figyelembevételével, a humán algoritmus mennyire alkalmas a lovakban előforduló ritmuszavarok detektálására. Mivel az intervenciós kardiológia különféle módszerei egyre elterjedtebbek az lógyógyászatban, egyre átfogóbb ismeretekre van szükség a lovak szívének ultrastruktúrájáról.

A kutatás második részében 83 vágóhídi szívet gyűjtöttünk az anatómiai és szövettani vizsgálathoz, hogy meghatározzuk a tüdővénák nyílásának méretét, ezzel támogatva a

one-shot ablációs technikák, különösen a krioballonos katéteres abláció fejlődését. További célunk volt a tüdővénák beszájadzásainak területén az ingerületvezető rendszerszövettani és immunhisztokémiai vizsgálata, különös figyelemmel az ún. „myocardial sleeve” területére. A kutatás során azonosítottuk a myocardiumban előforduló adrenerg és nem-adrenerg idegeket, és megvizsgáltuk a lovak szívében előforduló egyes gap junction fehérjéket (connexineket).

Introduction

Development of catheter-based interventions in cardiovascular medicine

Interventional cardiology is a group of methods that primarily addresses the diagnosis and treatment of heart and vascular diseases using catheter-based techniques. Since cardiology is one of the most technology-demanding fields in medicine, several conditions had to be met for the development of interventional cardiology. Catheter techniques were already being used around 3000 BC in Egypt for the treatment of urinary stones (Mueler R. and Sanborn T., 1995). However, the catheterisation of blood vessels was only mentioned later, around 400 BC, in the writings of Hippocrates, who described examining the function of blood vessels in cadaver models by filling arteries and veins with water and air using copper catheters (Mueler R. and Sanborn T., 1995). Catheter technologies began to significantly develop only in the 17th century (Mueler R. and Sanborn T., 1995). In 1665, Wren administered the first intravenous injection to a dog, and two years later, in 1667, he repeated this procedure in a human. The first recorded insertion of an intravenous catheter occurred in the same year during a blood transfusion from a sheep to a human (Mueler R. and Sanborn T., 1995). The first recorded catheterisation of a horse was performed by an English clergyman, Stephen Hales, in 1705, when he inserted copper tubes into the jugular vein and common carotid artery, connecting them to glass tubes using goose tracheas to measure blood pressure (Figure 1).

Figure 1. Horse catheterisation by Stephen Hales in 1705 (Mueler R. and Sanborn T., 1995)

He repeated this method in dogs and other species (Mueler R. and Sanborn T., 1995; Buchanan J. W., 2013). French physicians Bernard and Magendie referenced their work as cardiac catheterisation in 1844, during which they inserted thermometers into the hearts of horses through the jugular vein and the carotid artery (Mueler R. and Sanborn T., 1995; Buchanan J. W., 2013). In 1847, Bernard performed the first cardiac catheterisation in a dog. In 1931, Forsmann performed the same procedure on a human. These attempts were focused on catheterising the right ventricle because catheterisation of the left ventricle remained unresolved for a long time. Several attempts to reach the left ventricle through the aorta, thoracic wall, or diaphragm had resulted in fatal outcomes. The breakthrough came with the development and spread of radiography and ECG. Lenegre and Maurice made the first endocardial ECG and the first X-ray, confirming their placement of catheter in the pulmonary artery (Mueler R. and Sanborn T., 1995). With the spread of imaging

methods, it became possible to puncture the atrial septum. The first transseptal left ventricular catheterisation was performed by Ross et al. in 1959 (Ross J. JR., 1959). Since the 1960s, this method has been used to access the left ventricle through the foramen ovale from the right atrium in human clinical practice. Since then, developments have shifted towards more advanced catheters and technologies. To reduce radiation exposure, faster and simpler techniques have been developed, protecting the health of both the performing physician and the patient.

Just as in human cardiology, the development of interventional procedures in veterinary cardiology has progressed through similar steps. While the methodological and imaging diagnostic conditions necessary for interventions were established earlier in dogs (Noszczyk-Nowak A. et al., 2011; Santilli R. A. et al., 2009 Wright K. N. et al., 1996; Wright K. N. et al 1999), in horses, due to their herbivorous nature and large size, the development of new methods also became necessary.

Interventional cardiology encompasses imaging and electrophysiological diagnostic and therapeutic methods. In human cardiology, the most commonly performed catheter-based diagnostic methods include intracardiac echocardiography (ICE), electrophysiological mapping, electro-anatomical mapping, while the most common therapeutic methods include ablation techniques, closure of the atrial appendage, coronary artery dilatation, aortic and mitral valve implantation and dilatation, closure of atrial septal defects (ASD) and ventricular septal defects (VSD), as well as closure of the foramen ovale.

Development of the diagnosis of cardiac arrhythmias in horses

In horses, echocardiography, as well as resting, Holter and exercising electrocardiography (ECG) are available for a long time to diagnose anatomical and functional defects of the heart. The cardiac rhythm of horses has been studied using ECG since the 1910s. The first electrocardiogram obtained from a horse was published in 1913 (Nörr J., 1913). From the 1960s, with the aid of radiotelemetry devices, it became possible to record electrocardiograms from horses during exercise (Bassan L. and Ott W. 1968), and by the 1980s, extensive knowledge was available regarding the physiological and pathological rhythm disorders in horses (Vibe-Petersen G. and Nielsen K., 1980). At the inception of electrocardiography, the method was as effective in examining the hearts of horses as in human cases (Holmes J.R. and Darke P.G.G., 1970). However, with technological advancements, differences were discovered between carnivores (and humans) and horses in terms of the conduction properties and modes of impulse propagation of the

heart, making it less informative to interpret electrocardiograms from horses. The anatomy and impulse-forming system of herbivores largely resembles brief but effective ventricular systole in these fast-running prey animals. In horses, the electric impulses originating from various directions simultaneously largely cancel each other, hence the characteristic wavefronts seen on the ECG of carnivores (and humans) do not manifest. Since the surface ECG represents the result of cardiac electrical impulses, the summed effects of the depolarisation of most of the ventricular myocardium are minimally reflected in the ECG. The visible electrical activity mainly originates from the ventricular septum closer to the heart base and a portion of the free wall of the left ventricle (Marr C., 2011)

In the last years, as a consequence of the spreading of smartphones, different application-based ECG devices have become available. (Corradini I. et al., 202; Vezzosi T. et al., 2018; Kraus M. S. et al., 2019, van Loon G et al., 2019). In horses, according to the anatomical properties of the heart, just a few leads are used for obtaining a surface ECG. The Purkinje fibres run deeply in the equine myocardium, contrary to dogs and humans, where they run subendocardially. The consequence of this histological alteration in horses and other herbivores is that it makes fast contraction of the cardiac muscles possible which is essential for the quick escape of these animals. The other specific electrophysiologic characteristic of horses besides their excessive Purkinje network, is the strong vagal tone that influences the rhythm through the AV node conduction velocity. This phenomenon is playing a role in the pathogenesis of different arrhythmias, like atrial fibrillation (Marr C. and Bowen M., 2011).

Electrophysiological methods can be performed only with the support of a 12-lead ECG, which makes the determination of the electric axis of the heart and the exact origin of arrhythmias possible (Van Steenkiste G., 2020; Hesselkilde et al., 2021). Besides the 12-lead ECG, the use of insertable cardiac monitors (ICMs, or loop recorders ILRs) is another innovation linked to the development of interventional cardiology. Loop recorder implanting is often used for detecting subtle arrhythmias or after performing interventional methods on patients. These modern ILRs are continuously monitoring the ECG sign and record it in case of the detection of abnormalities instead of recording the whole ECG track as the Holter monitors. The programming of an ILR can be performed by a so-called programmer device and the detection is running according to the preset parameters. Bradyarrhythmia, tachyarrhythmia and asystole are detected by the comparing of the R-R intervals. For detecting atrial fibrillation, the device examines the variability of R-R intervals during a 2-minute period. The suspicious ECGs are stored by the device and can be read later by the clinician (Hindricks et al., 2010). In human cardiology, loop recorders are implanted aseptically in a subcutaneous pocket. The battery life of an ICM is between 2-3 years

depending on the manufacturer and the type. The investigation of using loop recorders has already started and the successful application in the detection of induced atrial fibrillation (Buhl et al., 2021a; Buhl et al., 2021b) and for the examination of Standardbreds showing poor performance was reported earlier (Buhl et al., 2021b).

The role of echocardiography in interventional methods

Introducing different types of catheters is the base of each interventional method. As a consequence of large thoracic dimensions, computed tomography (CT), radiography and fluoroscopy are not applicable imaging methods for the detection of catheter tips during interventional methods in horses, therefore ultrasonography remains the only tool for this purpose. Transthoracic three-dimensional (3D) echocardiography can be performed for the detection of catheter tip placement in the heart (Vandecasteele et al., 2016; Vandecasteele et al., 2019; van Loon G., 2021), and it was performed successfully earlier (Redpath el al., 2020; McElhinney A., 2019). In the field of standard two-dimensional echocardiography, the revision of standard views became necessary to provide better visualisation of the left side of the heart, especially the left atrium and pulmonary vein ostia (Figure 2).

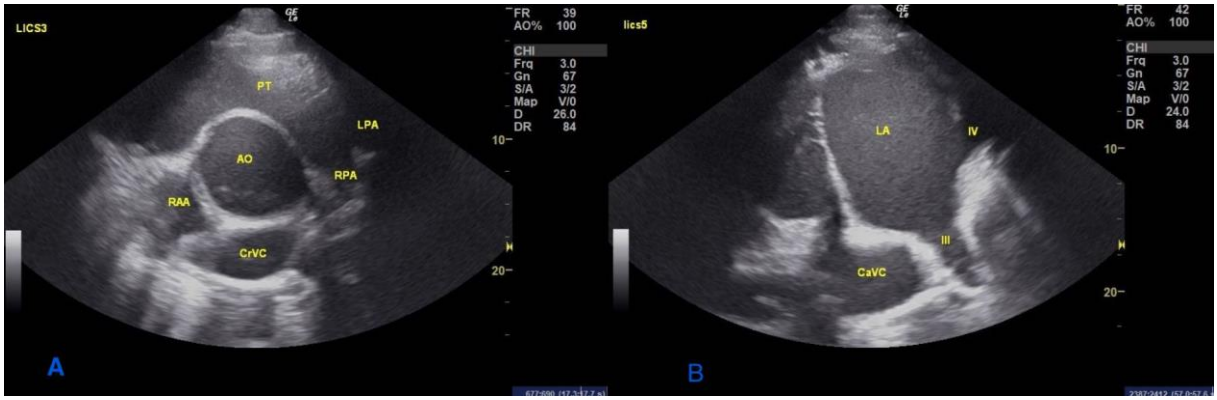

Figure 2. Equine echocardiograms using non-standard views to visualise atrial structures. A) Left third intercostal space: RAA: right atrial appendage, AO: aorta, CrVC: cranial vena cava, PT: pulmonary trunk, LPA: left pulmonary artery, RPA: right pulmonary artery B) Left fifth intercostal space. LA: left atrium, CaVC: caudal vena cava, III: pulmonary vein ostium III, IV: pulmonary vein ostium IV

In human medicine, transoesophageal ultrasonography is also available for the imaging of the left heart, but the commercially accessible oesophageal transducers are only suitable

for foals or adult miniature horses because of the short cable length (Fries R. C. et al., 2020). Intracardiac ultrasonography has an important diagnostic potential in human cardiology. The small ultrasound transducer is built in a catheter tip, and it can visualise the delicate anatomical structures, for example,, the crista terminalis, the fossa ovalis and the pulmonary vein ostia, which are all important for intracardiac methods. The tightness of the catheter-tissue junction during ablation methods can be also visualised by intracardiac ultrasonography. Intracardiac ultrasound catheters can communicate well with 3D mapping systems.

During our preliminary echocardiographic examinations (unpublished) performed on horses, we managed to image the ostia of the pulmonary veins, along with other anatomical structures necessary for guiding different catheters into the heart. (Figure 3-5).

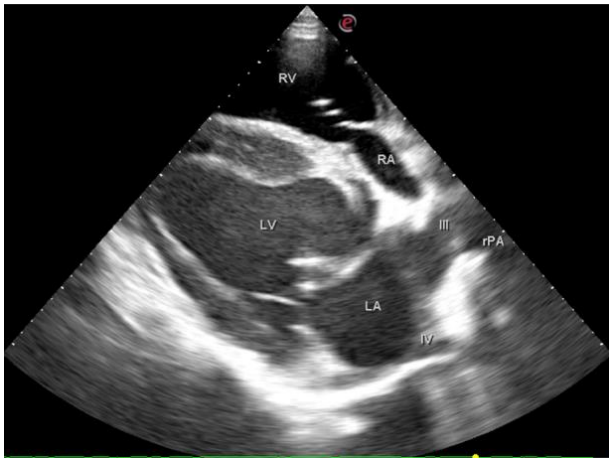

Figure 3. Echocardiography of the atria and the surrounding structures, from the right side, in parasternal, long-axis, four-chamber view, examined from the fourth intercostal space. The image shows the right ventricle (RV), the right atrium (RA), the left ventricle (LV), the left atrium (LA), and the openings of the third (III) and fourth (IV) pulmonary veins.

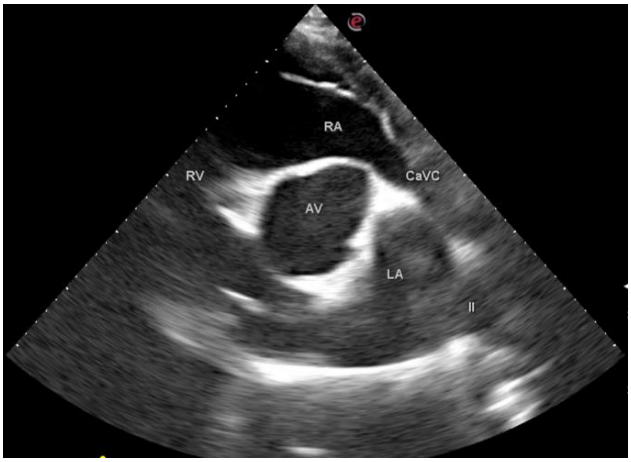

Figure 4. Echocardiography of the atria and the surrounding structures, from the right side, in parasternal, short-axis view at the level of the aortic valve, examined from the fourth intercostal space. The image shows the right ventricle (RV), the right atrium (RA), the aortic valve (AV), the vena cava caudalis (CaVC), the left atrium (LA) and the ostium of the second pulmonary vein (II).

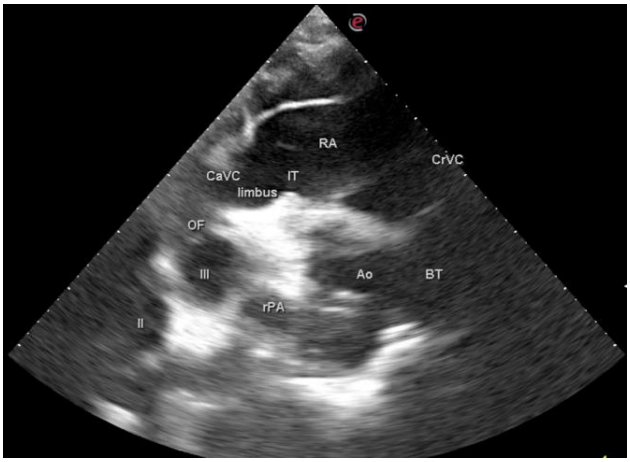

Figure 5. Echocardiography of the atria and the surrounding structures, from the right side, in parasternal, short-axis view, examined from the fourth intercostal space. The image shows the the right atrium (RA), the vena cava cranialis (CrVC) and caudalis (CaVC) separated by the tuberculum intervenosum (IT), the fossa ovalis (OF) and the limbus (limbus), the aorta (Ao), the truncus brachicephalicus (BT), the right pulmonary artery (rPA) aortic valve (AV), the vena cava caudalis (CaVC), the left atrium (LA) and the ostium of the second (II) and the third (III) pulmonary veins.

Pacemakers

Pathological bradyarrhythmias are relatively rare abnormalities in horses, but these cases require therapy (Keen J. A., 2020). These conditions can be treated by implanted pacemaker devices, like in human patients. The pacemaker is monitoring the heart rate and rhythm constantly and forwarding electric impulse towards the myocardium through an electrode which is implanted in the myocardium. The device can be one-, two-, three- or four-chamber according to its operation, but one- and two-chamber devices are the most widespread. The type of the pacemaker device is labelled by the so-called NGB-code system, with 4 or 5 letters, according to the type of operation. The first and second letters are labelling the affected chambers, the third letter signs the reaction of the device to intrinsic beat sensing. The fourth letter shows the way of programming. The rate adaptive “R” and closed-loop stimulation (CLS) devices are already used successfully in horses and dogs (van Loon G et al. 2020; Moise N. S. et al., 2021a; Moise N. S. et al., 2021b). In horses, the first pacemaker device was implanted in a horse suffering from Adams-Stokes syndrome in 1967 (Taylor D. H. and Mero M. A., 1967), then Reef et al. implanted a two-chamber pacemaker in a horse suffering from third degree AV-block which started competing again after the intervention (Reef V. B. et al., 1986). The implantation can be performed through the cephalic vein and via the jugular veins (van Loon G. et al., 2001; Petchdee S et al., 2018). The first pacemaker was implanted in a donkey in 1993 (Pibarot et al., 1993), then D-eLange et al. implanted a rate adaptive and a CLS pacemaker in miniature donkeys (De Lange et al., 2019). Sedlinská et al. described the use of a unipolar single-chamber pacemaker in the same species for 18 years (Sedlinská et al., 2021).

Electrical cardioversion

Electrical cardioversion is suitable for treating focal and macro re-entry atrial tachycardias (Van Steenkiste G. et al., 2019). Transcutaneous electrical cardioversion is not applicable in horses because of the vast body dimensions and the significant impedance of the thoracic wall. As an alternative method, only pharmacological cardioversion was available for a long time. Pharmacological cardioversion is usually performed by oral administration of quinidine sulphate, but because of the excessive toxicity of this drug, transvenous electrical cardioversion (TVEC) has been developed (McGurrin M. K. et al., 2005). The comparison of the efficacy and successful treatment ratio of atrial fibrillation by TVEC and quinidine sulphate was not investigated earlier, but TVEC could be effective in cases where the arrhythmia is not responsive to quinidine therapy (McGurrin M. K. et al., 2005,

McGurrin M. K. et al., 2008). With this method, more than 95% of horses suffering from atrial fibrillation can be successfully converted to sinus rhythm (van Loon G. et al, 2020). The procedure requires an aseptic surgical environment, general anaesthesia, and continuous ECG monitoring. The 180 cm long catheter pair (Digitimer Ltd.) (Figure 6) required for the procedure needs to be connected to a Lifepak 12 or 15 type defibrillator device (Physio-Control Corporation) (Figure 7).

Figure 6. One pair of transvenous electrical cardioversion (TVEC) catheters for treating atrial fibrillation in horses (Digitimer Ltd.)

Figure 7. Lifepak 15 defibrillator (Physio-Control Corporation)

To reach the largest possible area of the myocardium, the catheters are equipped with a 12 cm long surface for delivering electrical impulses at their tips (van Loon G. et al, 2020). During electrical cardioversion, the catheters are introduced in standing position under sedation. The position of the catheters is checked by echocardiography or radiography. After the horse is anaesthetised, the position of the catheters is re-checked by echocardiography, and it can be modified at this stage. One of the catheters is placed in the left pulmonary artery and the tip of the other catheter is placed in the right atrium. During the procedure, biphasic shock is generated and conducted through the catheters to the myocardium with an escalating energy impulse in case of failure. Shock delivery can begin with an energy of 50 J (McGurrin et al., 2008), but in some centres, the starting energy is 150 J (Van Steenkiste et al., 2019). When delivering a shock over 250 J, there is a greater risk of developing ventricular fibrillation or a ‘torsades de pointes’ arrhythmia. To avoid ventricular fibrillation, shock delivery should be synchronized with the R waves appearing on the ECG (McGurrin et al., 2008). If cardioversion does not occur even at maximum impulse energy, the procedure is repeated after repositioning the catheters (McGurrin et al., 2006; van Loon G. et al., 2020). The likelihood of successful cardioversion can be increased by administering an infusion containing amiodarone (6.52 mg/kg/h) during and after the procedure, and to prevent recurrence, frequent monitoring of heart rhythm along with the administration of sotalol or propafenone is recommended in the weeks following cardioversion (Van Steenkiste et al., 2019).

Electro-anatomical mapping

Using electro-anatomical mapping methods, we can determine the origin of various cardiac arrhythmias with extreme precision. In human medicine, electro-anatomical mapping is routinely performed under fluoroscopy guidance using standard, 4-lead catheters, most commonly in the right atrium. In horses, due to the large thoracic dimensions and the thickness of the chest wall, an image that would provide useful guidance for maneuvering the catheters is not available, making 4-lead catheter mapping initially impractical in this species. The solution is provided by 3D mapping systems based on the principle of magnetic impedance, which analyses impedance changes and changes in magnetic field, offering a hybrid solution (van Loon G. et al., 2020).

Although the procedure has already been performed under sedation in standing position, demonstrating the role of abnormal impulses originating from the pulmonary veins in the development of atrial fibrillation (Linz D. et al., 2020), the minimal motion in the standing, sedated horse may complicate the precise localisation of abnormal impulses. Therefore, according to the currently accepted methodological description (van Loon G. et al., 2020), electro-anatomical mapping should be performed under general anaesthesia. Similar to TVEC, during the process, catheters need to be introduced in standing position and guided by ultrasonography into the lower third of the left jugular vein. First, a sheath facilitating the insertion of catheters is introduced through the jugular vein to the entrance of the coronary sinus, followed by the insertion of a decapolar electrode catheter through the sheath into the coronary sinus. With local anaesthesia, a preformed, flexible sheath is then guided cranially from the previous insertion site into the vein, allowing later access for the mapping catheter to the right cardiac chambers. Before catheter insertion, 12-lead ECG electrodes are placed to monitor the horse during mapping. After catheter insertion and induction of general anaesthesia, a device creating a magnetic field is placed on the horse. During mapping, the mapping catheter is first guided into the right atrium and then into the right ventricle, recording intracardiac ECGs on the endocardium while the software creates a spatial representation of the heart chambers and continuously marks the position of the catheter with the help of the magnetic field. The mapping of the left atrium and ventricle is performed with a decapolar catheter inserted into the coronary sinus, compared to the mapping catheter. During mapping, ventricular depolarisation and impedance are compared to the 12-lead ECG signals (van Loon G. et al., 2020; Van Steenkiste G. et al., 2020).

Electrophysiological examination

During the electrophysiological examination, myocardial electrophysiological properties and the origin and/or mechanism of any arrhythmias are examined using endocardial ECG recording and stimulation (Fazekas et al., 1999; Van Steenkiste G. et al., 2020). Electrophysiological examination is currently a prerequisite for ablation therapies. During the intervention, catheters are introduced into the cardiac chambers via the femoral artery or vein in humans and via the jugular veins in horses using the Seldinger technique (van Loon G. et al., 2020). Stimulation of the endocardium can be performed from any cardiac chamber, but it is most commonly done from the right atrium, right ventricle, and the coronary sinus. Multipolar electrode catheters (2-20 electrodes) inserted into the heart allow the recording of intracardiac electrograms and/or the electrical stimulation of the heart in contact with the endocardium (Fazekas et al., 1999; van Loon G. et al., 2020). The number of electrode catheters is determined by the type of examination. For the investigation of supraventricular tachycardias, 4 multipolar catheters are introduced into the heart; for the examination of ventricular tachycardias, the introduction of 1 or 2 electrodes may be sufficient (Fazekas et al., 1999). Before visualisation, the endocardial electrograms are processed by a software, which optimally filters and amplifies the appropriate signal. The recorded signal can originate from a unipolar recording, which occurs between a positive pole of a catheter electrode and a negative pole of a body surface electrode, or from a bipolar recording, which records the signal between adjacent intracardiac electrodes. Unipolar recording provides information about the local electrical activity of the endocardium, while bipolar recording provides information about a larger area of the endocardium. Although in human cardiology bipolar recording is routinely used with occasional unipolar supplementation, in horses, only the unipolar recording method has been described so far (van Loon G. et al., 2020). Electrical stimulation aims to initiate or reproduce clinical arrhythmias, assess the interruptibility of these, study the pacemaker and conductive tissues of the heart, determine the effective refractory period, and determine the mechanism of arrhythmias (Fazekas et al., 1999).

Electrical stimulation methods described in horses

Overdrive pacing beside the sinus node

By slightly increasing the frequency of stimulation next to the sinus node compared to the sinus rhythm, the sinus node recovery time can be determined. The test is based on the

assumption that the overdrive stimulus recovers more slowly if the sinus node does not function properly (van Loon G. et al., 2020).

The extra stimulus test

During the extra stimulus test, after a series of stimuli at a given frequency, called "drive," a gradually shortened coupling interval stimulus is given. Using this method, similar to human interventions, the effective refractory period was determined in horses (van Loon G. et al., 2020), which is the longest coupling interval at which the myocardium cannot be stimulated. In healthy ponies and horses, with a pacing frequency of 1000 ms, the atrial effective refractory period is 200-300 ms, and the ventricular effective refractory period is 270-440 ms. The length of the effective refractory period shortens with increasing frequency, allowing to study the electrophysiological adaptation of the myocardium, the vulnerability of arrhythmias, and the degree of electrical remodelling at different pacing frequencies (van Loon G. et al., 2020).

In human cardiology, in addition to the above, incremental pacing is an important electrophysiological examination method in which the pacing frequency is increased to determine the highest frequency at which atrioventricular conduction is 1:1. This test examines the function of the atrioventricular node. They also perform continuous pacing mapping, where they stimulate the heart at a frequency matching the tachycardia to determine the exact localisation of the origin of the tachycardia. During the method, they look for the point where they obtain an electrocardiogram matching the pacing from the endocardium. Before the implantation of intracardiac pacemakers, it is essential to examine the interruptibility of tachycardias, examining the effectiveness of the extra stimulus in the localisation of tachycardia. It is important to note that in the myocardium affected by electrical remodelling, extra stimuli may lead to tachyarrhythmias; therefore, electrophysiological examination requires full defibrillation and resuscitation readiness. This method allows the experimental investigation of tachyarrhythmias, especially the most common one in horses, atrial fibrillation (van Loon G. et al., 2020).

Ablation

The aim of ablation procedures is to catheter-basely eliminate functional disorders occurring in the electrical impulse generation and conduction system of the heart. The precursor to catheter ablations was an open-heart surgery during which longitudinal incisions on the endocardium were attempted to eliminate the diagnosed arrhythmia. During transcatheter ablation, the ablation catheter is guided to an area (for example the

pulmonary vein ostia or a a myocardial injury) suspected to cause arrhythmia, also known as the arrhythmia substrate, and then the conduction around this area is blocked. Ablation is considered successful if the lesion necessary to eliminate the arrhythmia has been created, the desired arrhythmia has ceased, and during follow-up processes, it could not be induced again.

Ablation energy sources

The first catheter ablations were performed in human patients using the heat generated by the voltage and current between the catheter inserted into the heart and the defibrillator patch electrode placed under the left scapula. The main damage to the myocardium is caused by coagulation necrosis due to the vapour bubble formed at the tip of the catheter, tissue damage caused by the high-pressure shock waves formed, and sarcolemma damage caused by the electric field. Disadvantages of the method included pain, tissue rupture due to uncontrolled energy release, and the formation of arrhythmia foci due to the inhomogeneity of lesion formation. The method was replaced by the most widely used radiofrequency ablation before its spread, which operates with the delivery of current between 30-1000 kHz frequency and a catheter with a single tip. Radiofrequency energy affects the myocardium by passing through the tissue, with ions following the direction of the current, converting this electromagnetic energy into kinetic energy and heat (Fazekas et al., 1999). This effect, called resistive heating, causes tissue lesions 2 mm away from the electrode tip, but deeper tissue penetration of heat occurs at a depth of an additional 3-5 mm due to heat conduction (Fazekas et al., 1999). The factor of heat conduction must always be considered to avoid tissue rupture. Catheters equipped with temperature control replaced initially used catheters with current strength regulation due to the formation of excessively large lesions. At temperatures above one hundred degrees, sudden vapour formation at the tip of the catheters can cause crater-like lesions, thrombosis, and tissue rupture, so catheters with cooling heads were developed to create larger lesions (Fazekas et al., 1999).

Although radiofrequency ablation is the most widespread worldwide, cryoablation is used for ablation within vessels, around vessel junctions, and thinner tissues to reduce procedure-related pain and the risk of coagulation and tissue rupture. In addition, tissue damage does not occur immediately unlike radiofrequency ablation. During cooling, reversible changes occur in the tissues at minus 20 °C, and conductivity temporarily ceases, and if the tissue is reheated in this early phase, it regains its original function. Reversible cooling allows the application of so-called cryomapping. If it is possible to

eliminate the arrhythmia by cooling the tissue to minus 20 °C, cooling can be continued to minus 40 to minus 70 °C; if not, reheating the tissues can be attempted for a more accurate search for the arrhythmia substrate (Fazekas et al., 1999). Another advantage of cryoablation is that the catheter tip adheres to the tissue at the moment of freezing, so it is not necessary to continuously monitor the catheter position during the freezing period, reducing the radiation exposure caused by fluoroscopy to the patient and the operating staff. With single-tip catheters, point-like, linear, and circumferential lesions can be created, and focal ablation, segmental isolation, targeted ablation of local electrograms, isolation of pulmonary vein entries, or ablation of vegetative parasympathetic ganglia can be performed (Fazekas et al., 1999).

Pulmonary vein ablation

Among atrial tachycardias, atrial fibrillation has the greatest clinical significance in horses. In both humans and horses, it has been proven that the abnormal impulses causing arrhythmia spread from the pulmonary veins. In the venous junctions, myocardial tissue and vein tissue fuse like glove fingers. This tissue arrangement allows the development of pathological automaticity (Tagawa et al., 2001; Linz et al., 2020).

The isolation of pulmonary vein entries in humans is done circumferentially by "circumscribing" the ostia. Specifically designed for this task are single-shot techniques, with cryoballoon ablation being the most widespread. The essence of the procedure is to achieve a circular lesion covering the entire venous junction with a single positioning of the ablation catheter, which is simpler and significantly faster than the previous point-to-point isolation of junctions (Nobre-Menezes et al., 2014).

The next-generation cryoballoon ablation method, which utilises liquid nitrous oxide, has become extremely widespread worldwide, with several thousand successful interventions performed in our country as well (Máté V. et al., 2021). The cryoballoon catheter is inserted into the pulmonary vein, and then the balloon at the tip of the catheter is filled with the cooling nitrous oxide. The expanded balloon seals off the venous junction and adheres circularly to the vein wall. The prerequisite for a successful intervention is the complete sealing of the antrum because if the balloon does not adequately seal off the venous junction, the blood flowing nearby will heat the balloon, preventing it from cooling to the desired extent. Similarly to cryoballoon catheters, radiofrequency catheters have also

been developed for one-shot isolation of pulmonary vein entries, but these are not currently part of routine therapy.

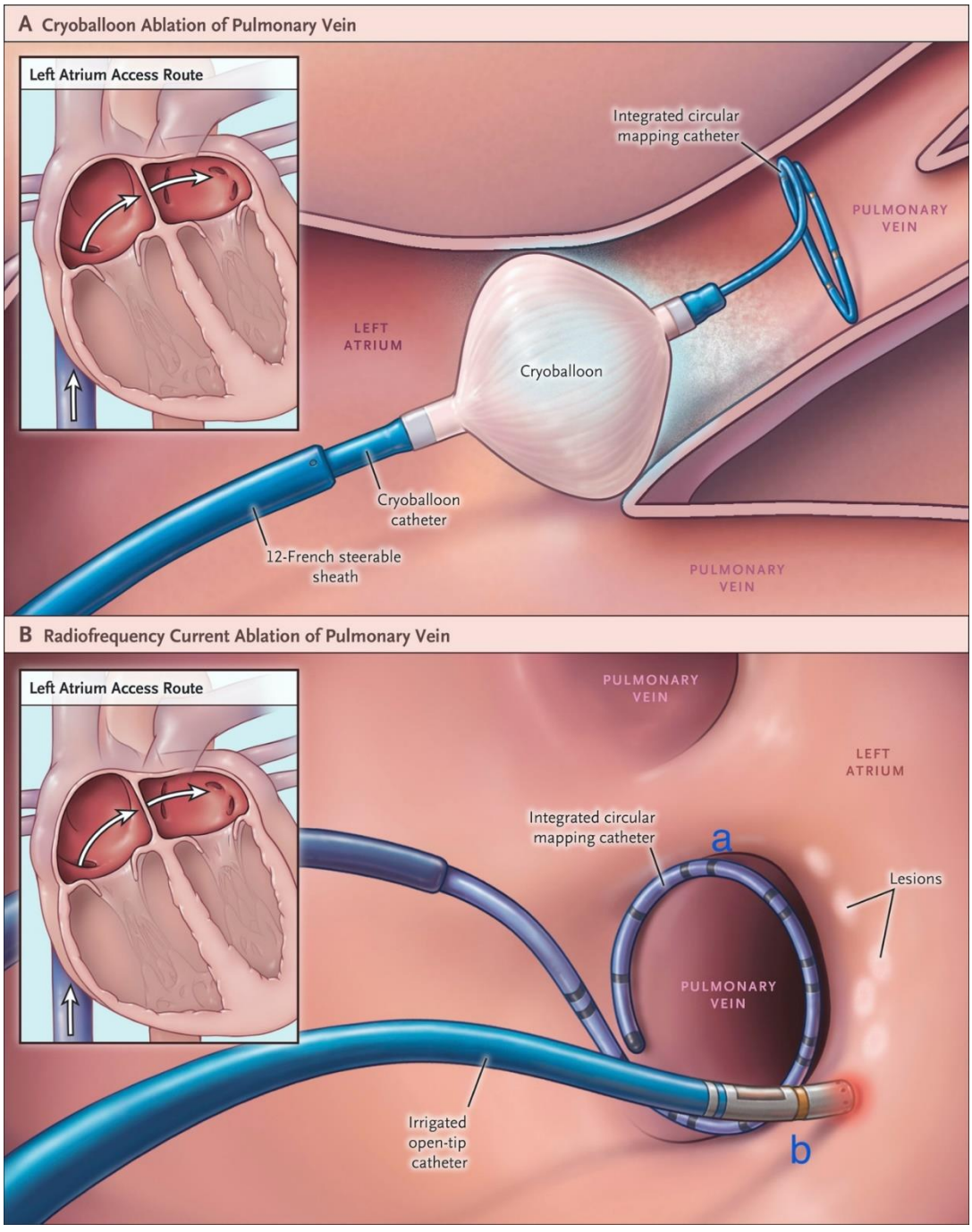

Figure 8. Different methods on pulmonary vein ablation. A) Cryoballoon ablation B) Radiofrequency ablation a) mapping catheter b) radiofrequency catheter (Kuck et al. 2018, Fire and Ice study)

To compare the advantages and disadvantages of radiofrequency and cryoballoon ablation, Medtronic Ltd. initiated a global survey called "Fire and Ice," which compared cryoballoon catheters to radiofrequency single-tip catheters (Figure 8), considering aspects such as the number of successful interventions, fatal or non-fatal adverse effects, thrombogenicity, and the percentage of recurrence. The research concluded that neither method was inferior to the other (Kuck et al., 2018). It seems that future ablation techniques will not be radiofrequency or cryo-based. Although not yet routine, pulsed-field ablation has begun to be applied worldwide, where the catheter tip delivers microsecond-scale electrical impulses, causing sarcolemma electroporation. The greatest advantage of this method, besides being able to perform irreversible "test" ablations, is its tissue specificity. It easily causes damage in myocardial tissue, but the surrounding tissues, which are the most common sources of complications, such as the oesophagus, the walls of large vessels, and the phrenic nerve, are less vulnerable (Reddy et al., 2019).

Ablation in horses

The first successful radiofrequency ablation was described in 2018 (Van Steenkiste et al., 2018) The intervention was performed on a show jumper horse that had already undergone TVEC treatment twice but still suffered from an atrial tachyarrhythmia. The intervention was performed under general anaesthesia, and a decapolar catheter, as well as two TVEC catheters for managing potential complications, were introduced through the left jugular vein into the coronary sinus, right atrium, and left pulmonary artery. Additionally, a 3D electro-anatomical mapping catheter was introduced into the right jugular vein. Focal atrial tachycardia was diagnosed during mapping and ablated with a radiofrequency catheter. The treatment of atrial tachycardia by radiofrequency ablation was further described in 8 horses in 2021. Among the successfully treated horses over 7 weeks, 3 suffered from focal atrial tachycardia, and the disease in 4 horses was caused by local re-entry. Recurrence occurred in 3 cases within one year. According to the authors, more precise anatomical mapping before ablation is the solution to these problems (Van Steenkiste et al., 2021). In addition to the first ablation of right atrial tachycardias, the first transseptal punctures through the oval fossa were performed to access the left heart chambers (van Loon G., 2021). Ablation in horses is complicated by the difficulty of holding the catheters at a single point in the large, highly mobile heart, and diagnostic imaging

methods assisting catheter manoeuvring are limited. Radiography, fluoroscopy, and CT scan used in human cardiology are not available due to the large chest size (van Loon G., 2021).

In both human and equine cardiology, both qualitatively and quantitatively, interventional and electrophysiological procedures are developing most rapidly. In the field of electrophysiology in human medicine, the primary challenge is reducing waiting lists and protecting patients and health care professionals from radiation, as it often results in cancer affecting them. The advancement of technology and the use of artificial intelligence in medicine predicts that in the future, we will be able to solve complex arrhythmias robotically, using 3D impedance and anatomy-based mapping systems with single-shot techniques, with energy that is effective and non-invasive. In the field of pacemakers, technologies are expected that are not implantable devices, but chip-based and rechargeable at any time, and can be implanted anywhere in the heart. In the future, perhaps we will be able to deliver artificially produced cells to the appropriate location that do not require any device, wire, or battery usage. The occurrence of sudden cardiac death in the human population has significantly decreased with the use of deployed automated defibrillators; in the future, these devices will likely be operable from smartphones, further reducing the risk of fatalities (Brugada J., 2022). With the development of genetic therapies, there is potential for a completely new approach to treating heart diseases. In equine cardiology, preliminary research has shown that although technological development is much slower in veterinary medicine due to financial and ethical significance, in this species, we also have the opportunity to replicate the changes that have occurred in the human field. Among interventional cardiology methods, loop recorder implantation in horses has been carried out at the Department and Clinic of Equine Medicine of the University of Veterinary Medicine Budapest in Hungary. The technical conditions for transvenous electrical cardioversion are also already in place, so the procedure will soon be available for clinical cases suffering from atrial fibrillation.

Atrial fibrillation

Arrhythmias are common in horses, and although many of them are physiological, and hence do not require treatment, there are a number of pathological ones causing poor performance or exercise intolerance, and a few of them are potentially life-threatening. Atrial fibrillation is the most common rhythm disorder in horses causing poor performance. Estimated occurrence is approximately 0.3-2.5%. Because of its clinical and economic

significance, the most important aim in the development of interventional methods is to establish new therapies to successfully treat atrial fibrillation in race- and sport horses.

In horses doing light work, it usually does not cause clinical signs and often presents as an incidental finding during physical examination. The most common clinical sign in working horses is decreased performance, fatigue, and slower than usual recovery following physical exercise (Bentz et al., 2002). Epistaxis, colic, and tachypnoea may also rarely occur. If atrial fibrillation is caused by heart disease, symptoms of heart failure (dyspnoea, subcutaneous oedema, collapse) may also manifest (Bertone J. J., 1984).

During atrial fibrillation, the coordinated electrical and mechanical activity of the atria ceases. The numerous small and uncoordinated contractions are unable to effectively pump blood from the atria into the ventricles during diastole. Atrial fibrillation can be caused by abnormal automaticity and re-entry. For re-entry to occur, it is necessary that both conduction block and cells in different electrophysiological states are present around the blocked area. The fulfilment of these conditions is facilitated by larger anatomical size and autonomic nervous system effects that further strengthen the observed inhomogeneity in the functioning of ion channels in the myocardium. Therefore, it is understandable why this arrhythmia occurs so frequently in horses with physiologically large atria and strong parasympathetic dominance.

During physical examination, a normal or rapid, irregularly irregular rhythm can be heard, and the fourth heart sound is not audible. Pulse waves may vary in size, and occasionally, they may be absent (pulse deficit). Atrial fibrillation occurring at normal heart rates is referred to as "lone" atrial fibrillation, but the underlying electrophysiological processes of the arrhythmia are not yet understood (Verheyen T. et al., 2013). Sometimes, heart murmurs may also be heard over certain heart valves as a consequence of atrial fibrillation or the underlying heart disease causing atrial fibrillation.

Atrial fibrillation can occur acutely or chronically. It can be continuous but may also be temporary or paroxysmal (Holmes et al., 1986). It may occur with normal heart rate or with tachycardia. Among the triggering factors are congenital or acquired morphological (associated with atrial dilation) heart disease, extracardiac diseases (e.g., diseases of the abdominal organs, respiratory diseases, sepsis, poisoning) or it can be an autonomous atrial fibrillation. The clinical signs of atrial fibrillation are caused by the lack of coordinated atrial contractions and, in the case of tachycardia, by the tachycardia itself. Paroxysmal atrial fibrillation often occurs in racehorses but can also occur in other breeds (Holmes et al., 1986).

The diagnosis of atrial fibrillation in horses, as in other animal species, is based on the absence of P waves and the occurrence of irregular R-R intervals. Fibrillation f waves can also be seen on the baseline, with frequencies reaching 275-500 beats per minute.

A relatively common cause of decreased performance in racehorses is paroxysmal atrial fibrillation which can spontaneously revert to normal sinus rhythm within 24-48 hours of onset. Because of this, another ECG examination should be performed which may yield negative results. However, if many early atrial complexes are observed with a 24-hour Holter monitor, it confirms the suspected diagnosis of paroxysmal atrial fibrillation.

Following the diagnosis of atrial fibrillation, echocardiography is used to identify or rule out underlying morphological heart disease. The echocardiographic findings are often normal, but congenital heart disease or possible valvular or myocardial disorders may be observed. As a consequence of atrial fibrillation, the left atrium may enlarge. If the pathological arrhythmia has caused significant changes in heart structure, there is less chance to convert the animal to sinus rhythm using cardioversion methods. If echocardiographic findings are normal, a thorough clinical examination involving all organ system is required to identify the cause of atrial fibrillation. Only if all these examinations are negative can it be conclusively stated that the horse has autonomous atrial fibrillation. From a therapeutic point of view, it is important to determine whether the atrial fibrillation is primary or secondary and how long the condition has been present.

The aim of the therapy is to achieve normal sinus rhythm and a normal heart rate. The tachycardic form is relatively rare in horses, so the focus is primarily on restoring sinus rhythm. The treatment of atrial fibrillation should only begin 24-48 hours after the onset of the disease, as during this period, the abnormal arrhythmia may spontaneously revert to sinus rhythm. In cases of secondary causes (heart disease, extracardiac diseases), therapy is initially limited to treating the underlying condition. Many medications (e.g., quinidine) are contraindicated for secondary atrial fibrillation caused by other heart disease. The prognosis for horses showing clinical signs and suffering from heart failure is extremely poor (Morris D. D. and Fregin G. F., 1982). When making therapeutic decisions, it is important to consider the frequency and intensity of work intended for the horse. Some horses with atrial fibrillation are able to perform light work, but in cases where the heart rate rises to 220 beats per minute during work, cardioversion is necessary. If this is not financially feasible, then retirement of the horse is necessary. There are two traditional techniques for cardioversion in horses. One is pharmacological which is usually performed using quinidine, and the other is transvenous electrical cardioversion (TVEC) (McGurrin M. K. et al.,2005a; McGurrin M. K. et al.,2005b). Treatment was previously done

exclusively with quinidine sulphate administered orally or quinidine gluconate given intravenously. The efficacy of quinidine therapy depends on the time elapsed since arrhythmia development, the factors causing the arrhythmia, and may involve dose- and individual-dependent toxic side effects that may prevent the implementation of the method in some horses (Muir W. W. et al., 1987). Other drugs, such as amiodarone (De Clercq D. et al., 2006), flecainide (Risberg A.I. et al., 2006), and propafenone (De Clercq D. et al., 2009), have not lived up to their expectations. Since pharmacological cardioversion is not feasible in every horse due to the reasons mentioned above, the transvenous catheter method of electrical cardioversion, which is developed in humans since the 1960s, has been adapted for horses.The advantage of pharmacological cardioversion is that it does not require expensive equipment and general anaesthesia, while the advantage of the TVEC method is that horses suffering from continuous atrial fibrillation for several years can be successfully treated with this method (De Clercq D. et al., 2014).

Currently, the main direction in the development of treatment is the adaptation of human catheter-based ablation methods in horses. The essence of these techniques is to use a mapping system to identify the exact location of abnormal impulses within the heart and to eliminate them via a transcatheter method. As mentioned earlier, radiofrequency ablation has already been done in a few horses (Van Steenkiste et al., 2018). Our original goal was to establish the basics of second-generation cryoballoon ablation in horses, but unfortunately, the unfavourable economic conditions made it impossible to acquire the necessary equipment.

Objectives

Three topics are covered by this dissertation with the aims as follows.

Chapter I: in this chapter we discuss our own experiences about pharmacological cardioversion in two different arrhythmias, highlighting the importance of developing interventional cardiology methods in horses.

Chapter II: insertable cardiac monitors are often implanted to diagnose temporary arrhythmias, following catheter-based interventions to detect the possible recurrence of arrhythmias or to differentiate between neurological and cardiac disease in horses with syncope. In this feasibility study, we validated an implantable cardiac monitor in horses by concurrently using the internationally accepted Televet 100 telemetric and Holter ECG device (Jorgen Kruuse A/S, Langeskov). We investigated the development of short- and long-term complications as well as the diagnostic capabilities of the device in horses used in different disciplines.

Chapter III: as electrophysiological studies and catheter-based interventions in horses has been developing, an extensive comprehension of the histological properties of the equine heart became necessary. The aim of this study was to investigate the conduction system of the pulmonary veins and their antrum with special attention to the myocardial sleeve using anatomical, histological and immunohistochemical methods. We aimed to identify adrenergic and non-adrenergic nerves, and we investigated cardiac gap junction proteins (connexins) in the equine heart as described previously in humans.

Chapter I: Pharmacological cardioversion in horses: own experiences

The main reason for the development of interventional cardiology methods in mostly humans, but of course in all species, is the demand for effective treatment of arrhythmias, and the life-threatening situation caused by them. Before the invention of the TVEC method in 2005 in horses (McGurrin et al., 2005), the only option for treating arrhythmias in horses was pharmacological cardioversion. These treatments are based on the systemic effect of an orally or an intravenously administered agent. As a consequence of the generalised effects of a drug, sometimes it has side effects, which can be toxic or even fatal or it can be “too generalized”, which means that it is just unable to block or solve the arrhythmia, because some structural or conductional issue is laying under the rhythm disturbance. Our own experiences of pharmacological cardioversion are concluded in the followings.

Treatment of atrial fibrillation in horses with orally administered quinidine sulphate

Case reports

Z Bakos, S Kovacs - Magyar Állatorvosok Lapja, 2019

Our own experiences regarding to the pharmacological cardioversion of atrial fibrillation are concluded in the followings.

In our study, five horses suffering from atrial fibrillation were treated with quinidine sulphate, which is a class Ia antiarrhythmic drug that blocks sodium channels and certain potassium channels, reduces the activity of Purkinje fibres, and decreases pacemaker activity. Its effects lead to reduced cardiac excitability and conduction, and the prolongation of the refractory period, making it suitable for the treatment of atrial fibrillation and other re-entry based arrhythmias (Galfi P. et al., 2011; Sage A. et Mogg T. B., 2010).

Quinidine can be administered orally as quinidine sulphate or intravenously as quinidine gluconate (Kimberly M. and McGurrin J., 2015). The latter has the advantage of rapidly achieving the desired plasma concentration through continuous infusion or repeated injections without directly irritating the gastrointestinal tract (Kimberly M. and McGurrin J., 2015). For treating atrial flutter that has developed within the past two months, this method is the most effective (Morris D.D. et Fregin G.F. 1982; Shwarzwald C.C., 2018). However, it is more expensive, and less accessible in some or completely unavailable in other countries. Conversely, oral quinidine sulphate is cheaper, more accessible, and well

absorbed from the stomach. It is administered via a nasogastric tube, as it has a very bitter taste and irritates the oral mucosa. (Kimberly M. and McGurrin J., 2015, Reef V.B. et al., 1995). To treat the common toxic side effects of quinidine, it is advisable to place an intravenous catheter in the jugular vein of the horse at the start of the therapy for easy administration of prepared medications in case of an emergency (Shwarzwald C.C., 2018). Cardiac monitoring should be performed using telemetric ECG (Reef V.B. et Bonagura J., 2011) (Figure 1).

During therapy, 22 mg/kg body weight of quinidine sulphate is administered every 2 hours until conversion occurs, toxic side effects (detailed below) develop, or the horse has received four doses. Most treated horses cannot tolerate more than four doses without showing toxic signs. If conversion does not occur and atrial fibrillation persists, the plasma concentration of quinidine should be measured under optimal conditions (therapeutic concentration: 2-5 µg/ml), although this is not always possible. If the plasma concentration exceeds 4 µg/ml or cannot be measured, administration should continue every 6 hours until sinus rhythm is restored, toxic side effects occur, or the cumulative dose reaches 176 mg/kg. If the horse does not convert to sinus rhythm after 24 hours of treatment, cardioversion can be supplemented with oral digoxin (0.0055-0.011 mg/kg twice daily) for the next 24-48 hours (Shwarzwald C.C., 2018). Digoxin should be administered for longer only if its plasma concentration can be measured. This is important because quinidine and digoxin competitively bind to plasma proteins, nearly doubling the digoxin concentration and leading to overdose symptoms (lethargy, anorexia, colic, other arrhythmias) (Reef V.B., 2010).

Cardiological side effects of quinidine include tachycardia, atrioventricular conduction disorders, widening of the QRS complex, prolongation of the Q-T interval, and “torsades de pointes” morphology, where QRS complexes and T waves broaden and appear to mirror around the isoelectric line, making the ECG trace resemble a sine wave (Jesty S.A., 2014; Shwarzwald C.C., 2007). Other potential issues include hypotension, negative inotropism, congestive heart failure, cardiovascular collapse, or even sudden cardiac death. Non-cardiac side effects, which are more common, include depression, sweating, urticaria, paraphimosis, nasal mucosal swelling, colic (accompanied by flatulence and/or diarrhoea), ataxia, and laminitis (Shwarzwald C.C., 2018). These side effects are frequent, so they should be anticipated with every horse by the 2nd or 3rd dose.

The primary goal in treating atrial fibrillation is to restore sinus rhythm through cardioversion unless congestive heart failure has developed, the horse's overall condition is inadequate, or it is too old for cardioversion (Shwarzwald C.C., 2018). The therapeutic

decision should also consider the horse's intended activity level. Many horses can perform low to moderate intensity work despite atrial fibrillation, but for safety reasons, they are usually retired if treatment is not an option (Reef V.B. et Bonagura J., 2014). Some horses return to their previous work after successful cardioversion (Reef et al., 1988). In some cases, atrial flutter may occur after successful conversion, necessitating repeated cardioversion (Decloedt et al., 2015). If the periods in sinus rhythm become progressively shorter, repeated treatment is not worthwhile, and retirement should be considered (Reef V.B. et al., 1995). Horses diagnosed with atrial flutter require ongoing monitoring and regular ECG checks, even if they show no symptoms. If the horse is not retired, the rider and handlers should be informed about the condition and potential symptoms for safety reasons (Reef V.B. et Bonagura J., 2014).

In our study, five adult horses with suspected atrial fibrillation based on cardiac auscultation were referred for further investigation and therapy. Four of them showed exercise intolerance as well. Based on previous physical examinations, haematology and biochemistry, systemic diseases were excluded. On presentation, all horses were bright, alert and responsive, and they did not show signs of congestive heart failure. Atrial fibrillation was confirmed by resting electrocardiography using base-apex lead in all cases. Echocardiography revealed normal findings in three horses, mild left ventricular dilation and aortic regurgitation in one animal, and moderate aortic root and left ventricular dilation in another one. Fractional shortening of the left ventricle was decreased in this horse.
The medical history was consistent in four horses in that the owners observed a decrease in performance. In the fifth horse, this was presumably not noticed because the horse was subjected to minimal exertion. Referring veterinarians detected arrhythmia during physical examinations and suspected atrial fibrillation. Based on the medical history, all five cases had primary heart disease, without systemic illness, although blood tests were not repeated. The results of our physical examinations were very similar across all five horses. Clinical baseline values, except for the heart rate of the last horse, fell within the normal range, and besides from auscultation of the heart, physical examination revealed no pathological abnormalities. Auscultation clearly revealed an irregularly irregular rhythm, and the absence of the fourth heart sound was also suspected. ECG examination definitively indicated atrial fibrillation in our cases. The absence of P waves and the presence of abnormal f-waves from fibrillation were recognised, and the irregularly irregular rhythm detected by auscultation was confirmed by the significant variability in R-R intervals. Echocardiography in the 1st, 2nd, and 4th cases showed no pathological changes. In the 3rd horse, the diameters of the aortic root and left ventricle were increased with reduced left ventricular contractility. Despite repeated auscultation and careful

Doppler examination, regurgitation could not be detected. In the 5th horse, measurements showed only minor deviations, but morphological changes and regurgitation were confirmed.

In most horses, atrial fibrillation develops without structural heart disease (Reef V.B., 2010), which is certainly true for three of our cases. In these horses, the likely cause of atrial fibrillation development was the "re-entry" phenomenon. In the 3rd and 5th cases, it is difficult to determine the exact cause of the arrhythmia, though re-entry cannot be ruled out either. However, no atrial dilation or congestive heart failure was found in any animal, so these causes can be excluded. Paroxysmal atrial fibrillation may spontaneously convert to normal sinus rhythm within 24-48 hours of onset. In such cases, another ECG should be performed a few days after the detection of clinical signs, which may be negative, but if many premature atrial complexes are seen with a 24-hour Holter procedure, it confirms the suspected diagnosis of paroxysmal atrial fibrillation. In our cases, the duration of atrial fibrillation was unknown but certainly exceeded 48 hours. Early diagnosis of atrial fibrillation is difficult, often impossible, in sport and leisure horses performing less intense work compared to racehorses, as the poor performance may not present as a leading clinical sign. In the five cases described, the chronicity of the disease could not be determined, but atrial fibrillation likely persisted in each horse for weeks. This information would have been crucial for estimating the success of cardioversion, as the more chronic the disease, the lower the chance of restoring sinus rhythm.

Oral administration of quinidine sulphate is a long-established method for treating atrial fibrillation. In two of the five cases we treated (1st and 2nd), we successfully restored sinus rhythm. In the 3rd case, cardioversion was only temporary, and in the 4th horse, treatment was discontinued at the owner's request due to side effects. This success rate is significantly lower than literature data (Kimberly M. and McGurrin J., 2015). One explanation could be the low number of cases, but it is also possible that the disease had been present for months, and cardioversion failed due to electrical remodelling of the atria. In the 3rd case, an attempt could have been made to use other drugs (e.g. amiodarone or flecainide) or transvenous electrical cardioversion, but the owner declined these options. Among the common and usually harmless side effects, we observed nasal mucosal swelling and mild paraphimosis in geldings. The 3rd horse also showed colic symptoms. Cardiovascular side effects included tachycardia and QRS complex widening in the 3rd case, and only tachycardia in the 4th case. After successful cardioversion, we followed and monitored the 1st horse for four weeks and the 2nd for eight weeks with ECG. To our knowledge, they remained in sinus rhythm and returned to their original workload. In the 3rd and 4th cases, the intensity of work had to be reduced due to unsuccessful

cardioversion. The 5th horse was already performing very light work before atrial fibrillation was diagnosed, so no significant changes were needed.mAfter unsuccessful pharmacological cardioversion in the 3rd and 4th cases, other methods could have been attempted to restore sinus rhythm. The first option is transvenous electrical cardioversion (TVEC). Among other drugs, amiodarone and flecainide are options, although there is significantly less experience with these compared to quinidine, and these drugs can also cause severe, even fatal side effects (De Clerq D. et van Loon G., 2007 20, 22).
The five cases we have presented highlight that atrial fibrillation should always be considered in horses showing decreased performance. The disease can be suspected in every case with routine auscultation, and after confirmation with ECG, most cases can be successfully treated, allowing these horses to return to their original work. However, pharmacological cardioversion requires considerable expertise due to the numerous severe side effects, so it is advisable to involve a prepared specialist in the treatment.

Our findings highlight the importance of performing cardiac auscultation and ECG on horses with exercise intolerance.

Successful treatment of ventricular tachycardia with oral propranolol in three horses

S Kovács, J Dixon, Z Bakos - Magyar Állatorvosok Lapja, 2019

Compared to supraventricular arrhythmias, ventricular rhythm disturbances are much less common in horses (De Clercq D. et al., 2007; Reef V.B., 2010; Reimer et al., 1992). Following the analogy in human medicine, ventricular tachycardia is defined by the occurrence of more than three consecutive premature ventricular complexes. This can be either paroxysmal (lasting less than 30 seconds) or sustained (Shwarzwald C.C., 2018). Ventricular tachycardia is a clinically significant arrhythmia, caused by primary cardiac issues (myocardial fibrosis or inflammation) (Machida N. et al., 1992; Reef V.B., 2010, Reimer et al., 1992; Stern et al., 2012) or systemic diseases (electrolyte imbalances, acid-base disturbances, gastrointestinal disease, toxicity, trauma, or drug side effects) (13, Reef V.B., 2010, Reimer et al., 1992), and it has pathological haemodynamic effects. Acute gastrointestinal diseases are particularly important to highlight, as they can often lead to ventricular tachycardia and may even be the direct cause of death (Reef V.B., 2010). Sustained tachycardia exceeding 120 beats per minute can lead to symptoms of congestive heart failure, and at rates over 150 beats per minute, collapse can occur (Jesty S.A., 2014). Ventricular tachycardia can easily progress to ventricular flutter or fibrillation, conditions that lead to cardiac arrest. On an electrocardiogram, ventricular tachycardia typically presents with QRS complexes of abnormal morphology and T waves in the opposite direction to normal (Reef V.B., 2010). P waves may be absent (if concurrent atrial fibrillation is present) or can be identified independently of the QRS complexes. A special form of polymorphic ventricular tachycardia is "torsades de pointes," where the QRS complexes and T waves widen, appear to mirror along the isoelectric line, and the ECG trace takes on a sinusoidal pattern (Jesty S.A., 2014; Reef V.B., 2010).

Treatment for horses with ventricular tachycardia should begin immediately upon diagnosis. The treatment aims to reduce heart rate and support electrical stability of the heart while conducting further investigations to identify the underlying cause.

Lidocaine is the first-choice drug for treating ventricular tachycardia, as it is readily available and typically has few side effects (Bradley et al., 2017).

Three horses with elevated heart rate and colic like signs were referred to our equine clinics for further investigation and therapy. After excluding gastrointestinal and systemic diseases, uniform ventricular tachycardia was confirmed by resting electrocardiography using base-apex lead. Other ancillary diagnostic tests including echocardiography, measurement of serum cardiac troponin I concentration were used to reveal signs of structural cardiac abnormalities and possible myocardial injuries. With the help of echocardiography, both the etiology and the morphological changes resulting from arrhythmia can be examined. In the cases presented, we were unable to detect any congenital or acquired morphological abnormalities. Among the heart measurements obtained with two-dimensional and M-mode examination, the left ventricular diameters were either normal or only slightly elevated. The fractional shortening of the left ventricle is the most commonly used calculated value for characterizing left ventricular systolic function. If its value is lower than the lower limit of the normal range, the left ventricle is no longer able to pump blood adequately, and congestive heart failure may develop. In our cases, the fractional shortening values were reduced, which were severe in the first horse.Cardioversion in all cases was started with lidocaine first administered in bolus, then as a continuous rate of infusion.

Compared to a bolus, continuous drip infusion of lidocaine more effectively achieves the plasma concentration necessary for conversion (Sage A. et Mogg T.B., 2010). Lidocaine is a class IB antiarrhythmic agent that blocks sodium channels and shortens the action potential, reducing cardiac excitability, the speed of intraventricular conduction, and the likelihood of abnormal automaticity. We administered the lidocaine drip infusion for 5-12 hours, but none of the three horses described in this report achieved sinus rhythm with either bolus or continuous drip infusion of lidocaine, so treatment continued with oral propranolol. This treatment did slow the rate temporarily but was unable to convert the rhythm. As soon as the continuous rate infusion was withdrawn, a quick and spontaneous return to a marked tachycardia occurred.

Therefore, following human literature and reported dose rates in horses, propranolol was administered orally. Propranolol was chosen from the other potential drugs because it is suitable for treating both supraventricular and ventricular tachycardias, which is crucial in cases where their definitive differentiation is challenging. Additionally, propranolol has a dose established for horses, unlike other beta-blockers, and it was readily available for treating horses.

Propranolol is a non-selective β-adrenoreceptor antagonist with a negative inotropic effect, classified as a class II antiarrhythmic. It can be administered orally, but its bioavailability varies between individuals. Therefore, an individual dose must be determined for each horse, and administration should begin under ECG monitoring. Potential side effects include depression, lethargy, weakness, bradycardia, atrioventricular blocks, hypotension, negative inotropism, congestive heart failure, and bronchoconstriction (contraindicated in cases of heaves) (Schwarzwald C.C., 2018). Propranolol reduces sympathetic activity of the heart by decreasing heart rate and myocardial contractility, but it also reduces myocardial oxygenation (Sage A. et Mogg T.B., 2010). By blocking sodium channels, it stabilises cell membranes throughout the body, the systemic significance of which is being studied in human research (Boyer N. et al., 2017; Herndon D. et al., 2018, Montazeri M. et al., 2016). However, with cautious use and strict monitoring of cardiac function, propranolol can be used to treat ventricular tachycardia in horses (Bradley et al., 2017; Reef V.B., 2010; Schwarzwald C.C., 2018).

In the three cases we examined, the horses were given propranolol orally in varying doses and for different durations. Administration occurred three times daily (every 8 hours). When determining the dosage, we had to consider the significantly reduced shortening fraction, which suggested poor left ventricular function in the first case, and in the last case, we had to consider the mare's pregnancy alongside the moderately reduced shortening fraction. In both cases, we had to attempt conversion to sinus rhythm with a lower dose. After terminating the ventricular tachycardia and restoring the heart's electrical stability, propranolol was discontinued after varying periods for each horse, with repeated ECG examinations. All horses received crystalloid fluids intravenously and other supportive treatment. Intravenous dexamethasone sodium phosphate was also given to two animals. After the conversion and successful recovery, horses were discharged, and the oral propranolol therapy was continued at home for different duration between eight days and one month. Follow-up cardiac examination four to eight weeks later revealed sinus rhythm in all cases.

In human medicine, ventricular tachycardia is also a life-threatening arrhythmia. In emergency cases, to prevent ventricular fibrillation, external defibrillator is used for the cardioversion. For the treatment of persistent or intermittent ventricular tachycardia, an internal cardiac defibrillator can be implanted, or catheter ablation can be performed (Tung R., et al., 2010)

Chapter II

Long-term use of an implantable loop recorder for ECG recording in horses

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Abstract

Potential arrhythmias recorded by cardiac monitors using the built-in human algorithm are as follows: number of total episodes, ventricular tachycardia, asystole, bradycardia, atrial tachycardia, atrial fibrillation, and the percentage of time spent in atrial tachycardia or atrial fibrillation. Long-term use of an insertable cardiac monitor (loop recorder) has been reported in one horse earlier, however its accurate role as a diagnostic tool is still unclear. The aim of the present study was to investigate the long-term applicability of the Reveal XT cardiac monitor for recording cardiac arrhythmias in adult horses. The Reveal XT cardiac monitor was implanted in 12 horses under sedation in standing position. Median duration of data recording calculated for the population was 1169 days. The number of false positive detections of asystole and bradycardia was extremely high in all horses. For atrial fibrillation, false positive detection occurred in five, and false negative detection occurred in one horse. The present study showed that long-term use of the Reveal XT cardiac monitor is feasible, well-tolerated, and the devices worked reliably, without complications. The human algorithm could not be used for automatic detection of arrhythmic episodes in the study population. The device could detect atrial fibrillation in horses, but the recorded AF burden was inaccurate, and the stored ECGs had to be manually interpreted. Because the human analyser algorithm of this cardiac monitor fails to identify bradyarrhythmias in horses based on our results, this instrument is not capable for accurately determining the aetiology of episodic collapse in this species.

Keywords: equine, cardiac monitoring, arrhythmia, atrial fibrillation, electrocardiography

Introduction

Although normal heart rate and rhythm can be evaluated by cardiac auscultation, electrocardiography (ECG) has an important role in the detection of cardiac arrhythmias in human and veterinary patients (Ioannidis et al., 2001; Bacharova et al., 2014; Lorello et al. 2019). The clinical use of ECG is influenced by the anatomical properties of the equine heart. One of these is that the extensive Purkinje network penetrates deeply in the myocardium to facilitate the myocardial contraction which is inevitable in the quick escape of the animal. Besides the anatomy of Purkinje fibres, the atrioventricular conduction delay is strongly influenced by the vagal tone. Because of these anatomical and functional variations, routine ECGs are obtained by a few leads, which is enough to diagnose the common arrhythmias in this species. Besides resting ECG, Holter and exercise ECGs are frequently used (Trachsel et al., 2010; Vezzosi et al., 2019). Smartphone application-based ECGs, wearable devices, and 12-lead ECGs are still under investigation (Ter Woort et al., 2023; Corradini et al. 2020; Hesselkilde et al., 2021; Van Steenkiste et al., 2018; Vezzosi et al., 2018; Brloznik et al., 2019; Nath et al., 2021). Implantable loop recorders (ILRs) are small, battery-powered electric devices which are able to detect and store abnormal ECG events by continuous monitoring of subcutaneous ECG. Device programming, and data collection is wireless. Usability of ILRs in horses suffering from collapse has been reported (Lyle et al., 2010). Buhl et al. investigated the usefulness of ILRs in the detection of atrial fibrillation (AF) in horses with induced AF and in a population of Standardbreds suffering from reduced performance (Buhl et al., 2021a). Besides horses (Lyle et al., 2010a; Lyle et al, 2010b; Buhl et al, 2021a; Buhl et al 2021b, Vernemmen et al., 2024), application of ILRs have been investigated in other domesticated species like dogs (Santilli et al., 2010) and cats (Willis et al., 2003). In human medicine, implantable loop recorders are used in the diagnosis of syncope, collapse, or intermittent weakness (MacKie et al., 2010) and they provide help in the therapeutic decision making after cardiac interventions and monitoring the appearance or recurrence of arrhythmias (Poole et al., 2020; Simantirakis et al., 2018). They are also implanted for the diagnosis of transient loss of consciousness, palpitations, syncope, and are suitable for monitoring myocardial infarction, some congenital heart diseases, and cardiomyopathy (Kwok et al., 2022). The proper use of ILRs in humans is still under investigation. Although real-time recognition of suspected arrhythmias is facilitated, the subcutaneous nature of these devices causing over-sensing, which leads to falsely detected episodes. According to a multicentre study of 1470 human patients, 59.8% of detected episodes were false-positive (O'Shea et al., 2021). The implantation and the remote reading of the device offers continuous heart rhythm monitoring of the patient, avoiding the cost of Holter-monitoring lasting from 24

hours to one week, but besides the cost of the device, it requires an invasive implantation procedure. The aim of our study was to investigate the long-term usability of the Reveal XT loop recorder in horses, including the determination of the ideal body region, the long-term tolerability of the device, and the evaluation of arrhythmia detection.

Materials and methods

Animals

Twelve horses (6 mares and 6 geldings) were used in the study. These were from different breeds: Nonius (4), Hungarian Sport Horse (3), Hungarian Half-bred (2), Shagya Arabian (1), Standardbred (1), and Zweibrücker (1). They varied in age from 10 to 19 years (mean ± standard deviation: 13.7 ± 3.0 years), and in body weight from 460 to 680 kg (mean ± standard deviation: 552.5±87.1 kg). Before inclusion, all horses underwent physical examination with special regard to the cardiovascular system as well as two-dimensional, M mode and colour flow Doppler echocardiography, including standardised measurements (Marr, 2011) as well as 10 minutes resting and then 20 minutes exercising ECG (5-minutes walk, 5-minutes trot, 5-minutes canter, 5-minutes rest) done by a Televet II electrocardiograph (Engel Engineering Services GmbH, Germany).

Device and implantation

The loop recorders were provided by Medtronic Hungary Ltd. The 62 mm x 19 mm x 8 mm Reveal XT device has a weight of 15 g. The distance between the electrodes is 40 mm and the battery life yields approximately 3 years. This loop recorder is optimised for detecting the subcutaneous R wave of the electrocardiogram. Differentiation between normal rhythms and pathological arrhythmias is based on the sensed RR intervals. Detection of atrial fibrillation is based on the ventricular response to the abnormal atrial rhythm. Differences in the RR intervals are evaluated during a two-minute period to detect irregularity of the ventricular rhythm. Considering the everyday life and work of the animals, different anatomical sites (lateral aspect of neck, thoracic inlet, and ventral abdomen) were tested before the implantation. ECG signals were checked by using single lead surface electrodes (Vector Check Tool, Medtronic, USA) which allowed testing to obtain the highest recordable R wave amplitude. Based on these results, we implanted the loop recorders in a ventral position to avoid artefacts from the motion of the shoulders and girth area. The exact site for implantation was the ventral body wall, approximately 5 cm from the midline on the left side, just behind the girth area. Twelve devices were implanted in twelve horses in standing position, under sedation using intravenous romifidine (20 µg/kg,

Sedivet inj., Boehringer Ingelheim Vetmedica GmbH, Germany), detomidine (10 μg/kg, Domosedan inj., Orion Pharma Animal Health A/S, Finland) butorphanol (10 μg/kg, Nalgosed inj., Bioveta a. s., Czech Republic) and xylazine (0.5 mg/kg, CP-Xylazin 2%, Produlab Pharma b. v., The Netherlands) , as well as local anaesthesia (10 ml lidocaine, Lidobel inj. 20 mg/ml, Bela-Pharm Gmbh & Co, Germany). After clipping, the skin was prepared aseptically. Following local infiltration of the skin and subcutaneous tissue with lidocaine, a 2.5 cm long incision was made on the skinperpendicularly to the horse's longitudinal axis with a number 20 scalpel blade, after which we created a pocket (approximately 2.5x6.5 cm in size) between the subcutis and the fascia using Mayo scissors. After activation, the device was placed into this pocket in a way that the electrodes on the device faced towards the surface of the skin. After implantation, the device was secured through the suture holes found on its connector block with non-absorbable suture material (USP 1 Prolene, Johnson & Johnson Medical GmbH, Germany), and then the incision was closed with USP 1 Ethilon suture material (Johnson & Johnson Medical Ltd, United Kingdom), using interrupted, vertical mattress suture pattern. The wound was covered with a sterile dressing (sterile gauze swab and Snögg Animal Polster, Orkla Wound Care AB, Norway) for 17 days, during which the horses were kept in stall rest. The skin sutures were removed on day 14. After 17 days, the horses returned to their usual routine.

Programming

The human algorithm of Reveal XT can detect atrial tachyarrhythmia (AT), atrial fibrillation, ventricular tachyarrhythmia (VT), fast ventricular tachyarrhythmia (FVT), bradyarrhythmia, asystole and the number of total episodes as well as the percentage of time spent in AT/AF can be obtained. The device can store a total of 49.5 minutes of electrocardiograms, which consist of a 22.5 (3x7.5 minutes, 6.5 minutes prior and 1 minute after activation) minutes of patient activated recording and 27 minutes of automatic activation up to 27 episodes with one minute duration each. Automatic recordings are classified by the algorithm according to arrhythmia types which are stored separately. After the memory of the device is full, the oldest recordings of each type of arrhythmia are overwritten by newly detected episodes. The Reveal XT can store the data of an additional 30 detected episodes without ECGs, hence these are not available for manual evaluation. A dedicated programmer device (CareLink 2090 Programmer, Medtronic, USA) was used to set the parameters of the loop recorders immediately after implantation. The default sensing threshold of the R wave is 0.035 mV. Because of the thicker thoracic wall of horses, the device is farther from the heart, than in humans, therefore we lowered the sensing threshold to the minimum (0.025 mV). Because lowered sensing threshold caused double sensing, the sensing of T

waves as R waves, we changed the blanking decay (the minimal time before a new R wave can be sensed) from 150 milliseconds (ms) to 500 ms. We programmed the loop recorders to detect all types of arrhythmias: atrial tachyarrhythmia/atrial fibrillation (AT/AF), ventricular tachyarrhythmia (VT), fast ventricular tachyarrhythmia (FVT), bradyarrhythmia, and asystole. The device has an AF detection algorithm which continuously examines the regularity of RR intervals and detects AF, when the duration of irregularity is above two minutes. VT and FVT episodes are sensed according to the preset thresholds. The interval was set to 260 ms (231 beats/minute) with a duration of 30 beats for FVT. The same values for VT were 340 ms (176 beats/minute) and 16 beats. FVT episodes are supervised by an algorithm which is examining the noise level of the obtained ECG. If the noise level is high, the episodes are not stored. An FVT episode ends when one of eight consecutive R waves are detected with an interval equal to or longer than the programmed VT interval, or the median ventricular interval is equal to or longer than the programmed VT interval during a period of 20 seconds, or no R wave is detected during a period of 10 seconds. Bradyarrhythmia is detected, when the number of R waves in the examined interval is lower than the preset one. In our study, the interval was set to the maximum (2000 ms) which equals to a heart rate of 30 beats/minute. Bradyarrhythmia sensing stops when four consecutive R waves are detected at the preset or shorter interval, or if no R wave is detected for 10 seconds. In this case, asystole is detected. Besides this, the device detects an asystole episode when the interval from the previous ventricular sense to the current event exceeds the programmed asystole duration. This parameter was set to the maximum, 4.5 seconds in our study. The asystole episode is suspended, after 12 sensed ventricular events.

After implantation, 8 horses were exercised on lunge, under saddle and four horses were driven. All horses were exercised regularly. The operability of the devices was controlled by a Televet II ECG unit during data collection. The graphical display of long-term electrocardiograms are usually based on RR intervals. The data export capability of the two devices used in the study (Televet II ECG and Reveal XT loop recorder) are technically different. The RR intervals were exported as Microsoft Excel files from the desktop software of the Televet system. From these RR intervals, Lorenz plots could be created. In these plots, each RR interval was plotted against the next RR interval (Jingxiu et al., 2021) The software of the programmer of the loop recorder does not support the export of RR intervals. Instead, the programmer itself creates plots of the individual ECG events which were exported as pdf files. Due to the different nature of the graphs, it was impossible to create completely matching plots, but the tendencies were obvious (**Figure**

1). Data from the loop recorders were collected wirelessly using the same programmer every 8 weeks.

Data analysis

Statistical analysis was done by Microsoft Excel for Microsoft 365, IBM SPSS 22 and Wizard 2 software programs. Shapiro-Wilk statistic was used to assess normality of variables. Descriptive statistics were calculated. The mean ± standard deviation was used to describe normally distributed data and the median ± interquartile range was calculated for not normally distributed variables.

Results

Animals and feasibility

Physical examination revealed normal cardiovascular findings in 11 horses. One horse had an irregular and chaotic cardiac rhythm with palpable pulse deficit on cardiac auscultation. Echocardiography showed no morphological abnormalities. Two-dimensional and M-mode measurements of different cardiac structures were within the reference intervals. Colour flow Doppler echocardiography did not confirm regurgitation or turbulent flow at the valve areas or other blood flow abnormalities e.g. subclinical congenital cardiac shunts. Resting and exercise electrocardiograms did not show abnormalities in 11 horses and confirmed atrial fibrillation in one horse. Both resting and exercising heart rate of this animal was within normal limits. The exact previous duration of the arrhythmia in this animal was unknown, but resting electrocardiography demonstrated atrial fibrillation years before the beginning of the present study. Based on this history and our results, this horse suffered from persistent atrial fibrillation. No treatment was attempted previously and during the study period.

Tolerability of the device

After the implantation, the incisions healed primarily in all horses within 14 days. A purulent discharge appeared around the suture site after 135 days of implantation in one animal. The device was removed three days later, after 138 days of recording. The discharge originated from the interior non-absorbable sutures. All the remaining 11 devices worked reliably during our investigation without causing local complications. Three horses were sold during the study period therefore the loop recorders were removed earlier (175 days after implantation in two horses, and 271 days following implantation in one animal). The devices in the remaining eight horses were kept in until the programmer indicated that they reached the end of their service periods.

ECG data obtained by the loop recorders

Median ± interquartile range of duration of data recording was 1169±1085 days (minimum: 138 days, maximum: 1373 days). The number of total episodes was 1,163,068 (median ± interquartile range: 42,791±182,737 per horse). As the duration of data recording was extremely variable between horses, the number of episodes per day was also calculated for better comparison. Interestingly, these values also showed extreme variability (median ± interquartile range: 46.35±119). The number of atrial tachycardia (AT) episodes was 2,091 (0.18% of all episodes). Atrial fibrillation (AF) occurred more frequently (31,408 episodes, 2.7%). The mean time spent in AT/AF was 2.1%. Evaluating this parameter separately in the 11 healthy horses and in the horse affected by AF, the values were 0.35% and 16.33%, respectively. Total number of the detected fast ventricular tachycardias (FVTs) was 1671 (0.14% of all episodes). Total number of VTs was even lower (149, 0.013% of all episodes). Asystole (when no R wave is detected for 10 seconds or when the interval from the previous ventricular sense to the current event exceeds 4.5 seconds) was the most frequently detected alteration (908,359 episodes, 78.1%) followed by bradycardia (219,390 episodes, 18.86%).

Discussion

In human medicine, implantable loop recorders are subcutaneous devices, which can be used for continuous monitoring of arrhythmias. In the past years, these devices were miniaturised, and the algorithms have become sophisticated, providing ECG monitoring up to 3 years (Bisignani et al., 2019; Kwok et al., 2022). The main benefit of the use of ILRs in clinical practice is the all-time remote monitoring of patients, improving the diagnostic method and decreasing the costs of healthcare at once (Bisignani et al., 2019). Besides the automated recording system, the monitors are usually equipped with a patient activator device, allowing the human patients to record ECGs during the symptoms (Bisignani et al., 2019). The main limitation of the use of ILRs is the recording of a subcutaneous ECG, which can lead to false positive and false negative detection of arrhythmias.

In humans, loop recorders are used in the diagnosis of transient loss of consciousness, recurrent falls, unexplained syncope, postural orthostatic tachycardia syndrome, palpitations, cryptogenic stroke, and obstructive sleep apnea. It can also be implanted after heart failure, myocardial infarction, AF ablation, or coronary artery bypass graft. ILRs can also be useful in the evaluation and modification of antiarrhythmic therapy (Pezawas, 2023; Bisignani et al. 2019; Kwok et al., 2022). Previous studies using ILR's in horses

focused on the diagnosis of paroxysmal atrial fibrillation based on Standardbred racehorses in work suffering from poor performance (Buhl et al., 2021a) and among research conditions also using Standardbreds (Buhl et al., 2021b). According to the type of device, Buhl et al. have used Reveal Linq cardiac monitor, which is also from the Reveal series of Medtronic Ltd, but a smaller device. Data from a single Warmblood horse with multiple episodes of paroxysmal atrial fibrillation (Kjeldsen et al., 2023) and a tutorial on an unknown horse were also reported (Lyle et al., 2010b). In these reports, only short-term results were published. The aim of the present study was to investigate the long-term application of loop recorders in adult horses with a focus on the clinical usability in the diagnosis of different arrhythmias. In our population, horses were from different breeds, use and daily activity. The usual implantation sites for ILRs are the pectoral region (Lyle et al., 2010a; Lyle et al., 2010b) and the girth area (Buhl et al., 2021b). The use of the xiphoidal region was described previously in one horse (Buhl et al., 2021a). This was very similar to the site we used. Although the left cardiac region would have provided better quality ECGs at rest, motion artefacts during exercise could have significantly decreased the signal to noise ratio, hence we chose a different implantation site on the ventral abdomen behind the xiphoid cartilage which did not interfere with the tack independently from the type of exercise (lungeing, riding or driving). During rest and exercise, ILRs were well tolerated by all horses without any signs of discomfort similarly to previous studies (Buhl et al., 2021a; Kjeldsen et al., 2023). The duration of the implantations published earlier varied between a few weeks and 3 years (Buhl et al., 2021a; Buhl et al., 2021b; Kjeldsen et al., 2023; Lyle et al., 2010a; Lyle et al., 2010b; Vernemmen et al., 2024).

In humans, the implantation of ILRs should be done using an aseptic protocol independently from the size of the device (Gunda et al., 2015). The human implantation method required minimal modification in horses. In our study, the implantation and wound healing were free of complications in all, but one case. From this horse, the device was removed 138 days after implantation. There was no visible injury within or around the area, but a mild, odourless purulent discharge appeared from the position of the former skin sutures. When the recorder was removed, it seemed that the inflammation was induced by one of the two non-absorbable sutures holding the device in place. Bacterial culture of the discharge provided negative result. The recording period was more than 3 years in eight horses which was enough to evaluate if the technique was equally safe both for the device and the horse. No complications were noted during the recording time in these 8 horses.

The devices were programmed with the human algorithm, but we changed the default parameters of sensing used by the previous studies in horses (Lyle et al., 2010a; Lyle et

al., 2010b; Buhl et al., 2021a; Buhl et al., 2021b). Tachyarrhythmia detection data were more precise with the new settings compared to the original default ones because it was more sensitive with the exclusion of episodes caused by double sensing (sensing of T waves as R waves and counting them as separate cardiac cycles). With the human algorithm, the device was not able to detect real bradyarrhythmia in horses. With the modification of the human algorithm by lowering the heart rate sensing, it would be easily feasible in the future. Because of the longitudinal nature of the study, and the extremely high number of detected episodes, it was impossible to check all episodes individually. In order to draw conclusions, 100 episodes of each type were interpreted manually. Comparison of our results with those of other studies was difficult because of the different devices, implantation sites and recording intervals. Lyle et al. used implantable cardiac monitors for the examination of episodic collapse (Lyle et al., 2010a; Lyle et al., 2010b). During the investigation of 4 horses in two publications, arrhythmias have not been revealed (Lyle et al., 2010a; Lyle et al., 2010b). Paroxysmal atrial fibrillation has been diagnosed in Standardbreds and Warmblood horses by loop recorders earlier, but these publications did not report other ECG abnormalities (Buhl et al., 2021a; Buhl et al., 2021b; Kjeldsen et al., 2023; Vernemmen et al., 2024).

The quality of the recorded ECGs was good in most cases, although traces recorded during exercise had a high noise ratio caused by motion artefacts. Atrial fibrillation episodes were detected correctly in the affected horse. However, there were false episodes both in the affected horse and in the healthy animals. The false detection was caused by motion artefacts, heart rate variability and double sensing. The persistent atrial fibrillation was detected correctly 28,816 times out of 31,408 total AF episodes (91.7%), although when the heart rate was low in this horse, false bradycardia or asystole were recorded, but in fact AF was still present (**Figure 2**). Because of this anomaly, in this animal, the maximum time spent in AF between two interrogations, as detected by the loop recorder was only 34.7%. According to previous human studies as well as to our current investigations, atrial fibrillation can be successfully detected by loop recorders. However, human studies and our data showed the over-detection of this arrhythmia (Afzal et al., 2020; O'Shea et al., 2021). Based on the manual interpretation of detected episodes in our population, AF was falsely detected in normal horses because of high heart rate variability or temporary double sensing. A recent systematic review reported a high (59.7%) false positive alarms of the overall remote monitoring transmissions in human patients (Covino and Russo, 2024). In their study, the most frequent type of false positive alarms was for atrial fibrillation, mainly caused by premature atrial and ventricular complexes (Covino and Russo, 2024). In our equine population, false detection of AF was

much lower (2592 out of 31408 episodes, 8.3%) compared to their data. In humans, the detection of AT episodes and the time spent in atrial tachycardia or atrial fibrillation is relevant after electrophysiological interventions, when the AF burden can predict the recurrence of the arrhythmia. With the development of interventional techniques in horses (van Loon et al., 2020; Buschmann et al., 2023), the implantation of cardiac monitors with an equine-specific algorithm after ablation methods will have the same clinical significance in horses. Fast ventricular tachyarrhythmia (heart rate above 231 beats/minute) and ventricular tachyarrhythmia (heart rate above 176 beats/minute) were detected falsely in all manually checked ECGs. The main reasons of these errors were exercise (sinus tachycardia), motion artefacts and double sensing. Although, the most frequently detected abnormalities were bradyarrhythmia and asystole, only one study has been published about these arrhythmias earlier (Nissen et al., 2022) According to our results, the causes of this extremely high false positive detection were the low resting heart rate of the animals and the presence of physiological sinus as well as second-degree atrioventricular blocks caused by high vagal tones. In the Reveal XT cardiac monitor, the maximum programmable RR interval was 2000 ms (30 beats/minute), but all horses had lower heart rates than this value. Although the physiological resting heart rate of horses is considered between 28-42 beats/minute, in reality, without human interactions and the placement of electrodes and an external ECG device, the true resting heart rate is significantly lower. In the manually interpreted electrocardiograms, the lowest heart rate was 12 beats/minute when sinus blocks were present, which is lower than the previously published heart rate of a Quarter Horse (Nissen et al., 2022). Heart rates between 20-30 beats/minute were very common in all horses, consequently it cannot be evaluated as a pathological rhythm disturbance.

The use of implantable loop recorders is limited even in human medicine. For most conditions, cost effective and non-invasive methods such as Holter-monitoring, external loop recorders and patient monitors as AliveCor or Apple Watch are available (Kwok et al., 2022). Because these devices cannot take actions in case of arrhythmia detection, for life threatening arrhythmias in-site monitoring or implantation of a pacemaker is inevitable. The use of ILRs is most appropriate in human patients with mild disease for detection of infrequent symptomatic or asymptomatic arrhythmias and in patients with high risk of developing arrhythmias. In horses, similarly to human indications, these instruments are more successful in the detection of infrequent arrhythmias compared to Holter-monitoring or telemetric devices. Because atrial and ventricular tachycardias can be evaluated by manual inspection of the recorded ECG traces in horses, the devices with the current human algorithm may be convenient for the follow up of the medical treatment of

tachyarrhythmias. However, as the human algorithm fails to recognise bradyarrhythmias in horses, the device is not capable for the identification of the aetiology of episodic collapse. At the same time, it can provide information about tachycardias and the possible concurrent motion of the animal during collapse (Lyle et al., 2010b). With the modification of the human algorithm for the equine heart rate, heart rate variability and ECG features, the use of ILRs can be extended to the general investigation of infrequent arrhythmias as suggested in the present study.

Limitations of our study were the relatively low number of horses, the fact that only one horse had pathologic arrhythmia in the population, and the limited number of manually checked electrocardiograms compared to the total number of detected episodes. These lacks might be corrected in further studies performed on a larger equine population including more healthy horses and those with pathological arrhythmias. However, the extension of the number of cases can create hindrances, regarding the implanting technique as a semi-invasive method.

Conclusions

The present study showed that the use of the Reveal XT loop recorder is feasible, well-tolerated, and the devices worked reliably without complications even for long-term ECG recordings. At present, the human algorithm cannot be used for automatic detection of all arrhythmic episodes in horses. The device could detect atrial fibrillation in horses, but the recorded AF burden was inaccurate, and the stored ECGs had to be manually interpreted. Implantable loop recorders are currently suitable for diagnosing tachyarrhythmias in clinical equine patients if the use of Holter, smartphone, or wearable devices is not feasible. Development of ablation techniques in horses will require the routine use of intracardiac loop recorders. Our results may serve as an aid in the development of an equine algorithm, which automates the interpretation of electrocardiograms recorded by these devices.

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The study was approved by the Institutional Animal Care and Use Ethical Committee of the University of Veterinary Medicine Budapest, Hungary (reference: PE/EA/1442-7/2019).

Chapter III

Morphological and histological investigation of the conduction system in the equine atrial muscle sleeve of pulmonary veins

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Declarations

Authorship

S. Kovacs and Z. Bakos participated in study design, study execution, data analysis and interpretation, and preparation of the manuscript. S. Kovacs and Z. Bakos have full access to the data and take responsibility for the integrity and accuracy of data. B. Racz and P. Sotonyi participated in study execution, data analysis and interpretation, and preparation of the manuscript. All authors gave their final approval of the manuscript.

Source of funding

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Competing interests

No competing interests have been declared.

Ethical animal research

The study was approved by the Institutional Animal Care and Use Ethical Committee of the University of Veterinary Medicine Budapest, Hungary (reference: PE/EA/1442-7/2019).

Informed consent

All tissue samples were derived from a commercial abattoir thus informed consents from the previous owners were not required (Author Guidelines section 5.4.iv).

Data accessibility statement

Data are available from the corresponding author upon reasonable request.

Summary

Background: Atrial fibrillation is the most common arrhythmia in horses causing poor performance. The role of pulmonary vein triggers in the pathogenesis has been identified in horses. Ablation methods have been investigated, but the available information on anatomical, histological and immunohistochemical assessment of the pulmonary vein ostia and the conduction system of the myocardial sleeve is still limited.

Objectives: The aim of the study was to describe the morphological properties of the myocardial sleeve in healthy horses.

Study design: cross-sectional.

Methods: Eighty-three equine hearts were dissected. The number and diameters of pulmonary vein ostia were determined, and anatomical localisation was described. Fifty-eight tissue samples were collected for histology (haematoxylin and eosin stain) and twelve of these were used for immunohistochemistry (S100; tyrosine hydroxylase [TH], and connexin [Cx] 43, 45 antibodies).

Results: The mean number of pulmonary vein ostia was 4.5 (4 veins: 46 horses, 5 veins: 31 horses, 6 veins: 6 horses). Diameters (mean ± SD) of the main ostia were as follows: vein I: 20.2±7.0 mm, vein II: 32.7±7.1 mm, vein III: 33.4±5.9 mm, vein IV: 18.1±4.5 mm. Diameters of supernumerary vein ostia varied between 3.0 and 28.0 mm (mean ± SD: 11.5±5.5 mm). Early branching was found in 26 horses (31.3%) and 30 veins (vein I: 14, vein II: 9, vein III: 5, vein IV: 2). Histology confirmed the presence of a muscle sleeve composed of myocardial tissue in each pulmonary vein. S100 and TH positivity was detected in each vein, and it confirmed the presence of adrenergic and non-adrenergic nerve fibres within the myocardial sleeve. Cx43 and 45 positivity were also found in each vein indicating the presence of gap junctions.

Main limitations: Low number of immunohistochemistry samples.

Conclusions: Future ablation techniques should consider that conductive tissue is present in the entire myocardial sleeve in all pulmonary vein ostia.

Introduction

Atrial fibrillation (AF) is the most common pathological arrhythmia in horses, causing poor performance or exercise intolerance (Schwarzwald C.C., 2018; Slack J. et al., 2015; Deem D.A. and Fregin G.F., 1982). The role of pulmonary vein (PV) as trigger in the pathogenesis of AF was identified in humans more than two decades ago, and it has recently been confirmed in horses (Haissaguerre M. et al., 1998; Linz D. et al., 2020). In humans, electro-anatomical cardiac mapping and ablation methods are routinely used. These techniques have also been investigated in horses and they will likely become accessible in equine cardiology (Linz D. et al., 2020; Hesselkilde et al., 2020; Van Steenkiste et al., 2020a; Van Steenkiste et al., 2020b). Mapping of the whole heart was performed in 2020 (Van Steenkiste et al., 2020c), and mapping and radiofrequency ablation of atrial tachycardia and an accessory pathway was also described earlier (Van Steenkiste et al., 2022; Buschmann E. et al; 2023). However, the available information on anatomical, histological and immunohistochemical assessment of the pulmonary vein ostia and the conduction system of the myocardial sleeve is still obscure in this species (Vandecasteele T. et al., 2016; Vandecasteele T. et al., 2018; Vandecasteele T. et al., 2019).

After the common pulmonary vein is canalised during human heart development, it attaches to the left atrium initially with one single orifice. As the atrial wall expands, it reaches second-order bifurcation tributaries of the pulmonary vein, and these branches incorporate into the wall of the left atrium, forming four main pulmonary vein ostia (Sherif H.M., 2013). As in humans, four main PVs have been identified in horses (Vandecasteele T. et al., 2016), but in humans only 43.5-82% of the cases had normal drainage pattern (Cronin P. et al., 2007; Altinkaynak D. et al., 2019; Prasanna L. C. et al., 2014; Klimek-Piotrowska W. et al., 2016). Other patterns varied by supernumerary vein ostia and/or early branching (Cronin P. et al., 2007). Variations of drainage patterns are becoming more important as cardiac surgery and ablation methods are developing (Prasanna L. C. et al., 2014; Marom E. M. et al., 2004). The origin of the atrial wall surrounding the pulmonary vein ostia is still under investigation, but it definitely differs from the working myocardium in the trabeculated left atrial appendage (Douglas Y. L. et al., 2006). In view of published data focusing on the equine atrial wall, an extensive network of Purkinje fibres in the subendocardium was observed previously which is also penetrating the myocardium (Bishop S.P. and Cole C.R., 1967; Kiryu K. et al., 1981). Similar to humans (Hinescu et al., 2005; Hinescu et al., 2006), telocytes have been found in the wall of the pulmonary veins at the veno-atrial junction of the horse, but no neuronal cell bodies were detected in the same study (Vandecasteele T. et al., 2018). To establish the correct placement of

ablation lines and encircling methods in the horse, the overall histological assessment of the type, location, and extension of the conducting system in the atrium and the veno-atrial junction is necessary, which may help in designing future catheter ablation techniques.

Connexins are transmembrane proteins; six of these transmembrane protein units set up a connexon, which is a hemichannel of a gap junction between two cardiomyocytes. The ion passage through the gap junctions provides the cell-to-cell continuity of depolarisation. The dysfunction and impaired spatial distributions of connexins likely play a pivotal role in atrial fibrillation in human (Iwasaki et al., 2011; Kato et al., 2012). In human myocardial sleeves, telocytes – previously called interstitial Cajal like cells (ICLCs) – are located in the interstitium between the wall of pulmonary veins and the myocardial sleeves. The long cytoplasmatic processes of these cells connect cardiomyocytes, nerves, blood vessels and interstitial immune cells, and they also play role in the tensional integration of pulmonary veins. Previous studies suggest that telocytes play an unknown role in the development of atrial fibrillation (Sukhacheva T. V. et al., 2021; Gherghiceanu et al., 2011; Hostiuc S. et al., 2018).

The aim of our study was to investigate the conduction system of the pulmonary veins and their antrum with special attention to the myocardial sleeve using anatomical, histological and immunohistochemical methods. We aimed to identify adrenergic and non-adrenergic nerves, and we investigated cardiac gap junction proteins (connexins) in the equine heart as described previously in humans (Pérez-Lugones A. et al., 2003; van der Velden H. M. et al., 2002; Kugler S. et al., 2018).

Materials and methods

Study population

Eighty-three dissected normal equine hearts purchased from an abattoir were studied. An ante mortem physical examination was performed in each case with special attention to the cardiovascular and respiratory systems. Only hearts of horses without cardiac murmurs, arrhythmias (except first and physiological second-degree atrioventricular blocks) and pathological lung sounds were included in the study. The horse population consisted of 46 mares, 12 stallions and 25 geldings which varied in age from 1 to 25 years (mean ± standard deviation [SD]: 13±8 years), body weight from 335 to 660 kg (mean ± SD: 489±65 kg). The most common breed was Hungarian Half-bred (55.4%) which was followed by Hungarian Sport Horse (12%), Nonius (8.4%), Lipizzaner (7.2%), Furioso (4.8%), Standardbred (4.8%), Thoroughbred (3.6%) and Arabian (3.6%).

Gross anatomy

All hearts were taken out from the thorax together with the intact lungs, pulmonary trunk, and longer sections of the aorta, cranial vena cava and caudal vena cava. Peripheral lung tissue not containing pulmonary veins (PVs) was removed. All specimens were transported to the Department and Clinic of Equine Medicine and examined on the day of slaughter. The ventricles and the right atrium were removed to get an extensive sight of the dorsal wall of the left atrium. All PV orifices were directly visualised, the number of veins were counted, their anatomical location, and early branching were recorded and photographed. Supernumerary ostia were defined in case they appeared completely separated from the main ostia. Early branching was defined as smaller ostia inside the lumen of one of the main openings slightly apart from the atrial wall. Diameter of each ostium was measured as follows. A regular pair of compasses was inserted in the ostium and opened to a maximum distance which still allowed to rotate the compass 360 degrees. The tips of the compass were touching the wall of the ostium which was very slightly distended mimicking the effect of a cryoablation balloon catheter. Then the compass was withdrawn and the distance between the tips was determined. This measuring technique was chosen to save time and human resources. It was validated on six equine hearts. A 30F Foley catheter was used as a validation tool. Following the measurement of the diameter of the pulmonary veins with the pair of compasses, the Foley catheter was introduced into the ostia, and then the balloon was inflated by air using a syringe. The balloon made the pulmonary vein wall tight, but not significantly overinflated. The catheter was removed, and a vernier calliper was used to measure the diameter of the balloon. The values obtained from the two measurement techniques followed normal distribution, and were compared by independent samples T-test, and no significant differences were found between the two methods ($p=0.6$). Tissue samples were collected from each ostium in a way that all samples enclosed the veno-atrial junction (a minimum of 5 mm sections of both atrial and venous tissues). The distance between the sampling site and the left atrial myocardium was variable as the length of the myocardial sleeve was also variable. This made standardised sampling (i.e. standard distance from the edge of the atrial myocardium) impossible. The samples were placed immediately in 10% buffered paraformaldehyde solution for fixation.

Histology

Fifty-eight tissue samples were randomly chosen for routine histology. Samples were washed for 30 minutes in running tap-water, then dehydrated in ascending ethanol series followed by xylene and infiltrated with paraffin wax (Histoplast, Thermo Fisher, Germany). Tissue blocks were made with a HistoDream embedding station (Milestone, Italy), and from these blocks 3-4 µm thick sections were cut with a Leica HistoCore Multicut microtome (Leica, Germany). Sections were mounted on glass slides and stained with routine haematoxylin and eosin (Sigma, Germany) for general histological evaluation.

Immunohistochemistry (IHC)

Twelve tissue blocks were randomly chosen (four main PVs from three different horses) for immunohistochemistry. Selection of antibodies was based on previous publications reporting successful detection of tissue elements of vegetative innervation, as well as impulse-forming and conducting structures in other mammals and humans (see Table 1.) (Bishop S.P. and Cole C.R., 1967; Gherghiceanu et al., 2011; Hostiuc S. et al., 2018; Pérez-Lugones A. et al., 2003; Matsuyama T. et al., 2006).

Briefly, sections were microwaved at 800 Watts (W) and then at 180 W with Dako Envision Flex pH 9.0 solution to retrieve antigens. Duration of antigen-retrieval by microwave was 5 minutes for all antibodies. Heating at 180 W was done for 10 minutes for S100, and 15 minutes for tyrosine hydroxylase, connexin 43 and 45. Non-specific peroxidase-activity was blocked with EnVision FLEX Peroxidase-Blocking reagent. After washing in PBS, sections were incubated with EnVision FLEX/HRP. The immunopositive structures were visualised by the EnVision Flex DAB+Chromogen diluted in EnVision Flex Substrate Buffer. Gill II Haematoxylin (Leica) was used for nuclear staining, followed by washing in PBS, dehydration in ascending ethanol series, xylene, and cover slipping with DPX mountant (Sigma). Slides were scanned with Pannoramic MIDI digital slide scanner (3D Histech Ltd., Hungary), and digital images were captured using the CaseViewer 2.4 Software (3D Histech Ltd., Hungary).

Data analysis

G*Power software was used for a priori sample size calculation. As published data were not available for the current topic, calculation was based on a Cohen’s d effect size value of 0.65 (between medium and large) which resulted in a sample size of 78 horses. IBM

SPSS 22 and Microsoft Excel for Microsoft 365 software programs were used for further statistical analysis. Shapiro-Wilk statistic was used to assess normality of variables. Descriptive statistics were done, and independent samples T-test, one-way ANOVA, and Pearson's correlation analysis were used to assess the relationship between population variables, number of PV ostia, and PV diameters. The level of significance was set at $p<0.05$.

Results

Anatomy

The four main PV ostia were identified in all 83 dissected hearts similarly to humans and other mammals. Based on the definitions of supernumerary ostia and early branching, 375 PV ostia were identified in 83 hearts. Four openings were observed in 46 (55.4%), five in 31 (37.3%), and six in 6 (7.2%) hearts. Mean number ± SD of the PV ostia was 4.5±0.6. To definitely identify the individual main openings, they were numbered from I to IV from the left auricle towards the interatrial septum as reported earlier (Figure 12) (Van Steenkiste et al., 2020b). Ostia I to III were located approximately in a line, ostium I being the most caudal and closest to the left auricle. Ostium II was to the right of ostium I, and ostium III was the rightmost opening. Its right wall joined to the interatrial septum. Ostium IV was located cranial to the others in a slightly variable position, nearer to ostium I in some hearts, and nearer to ostium II in other hearts.

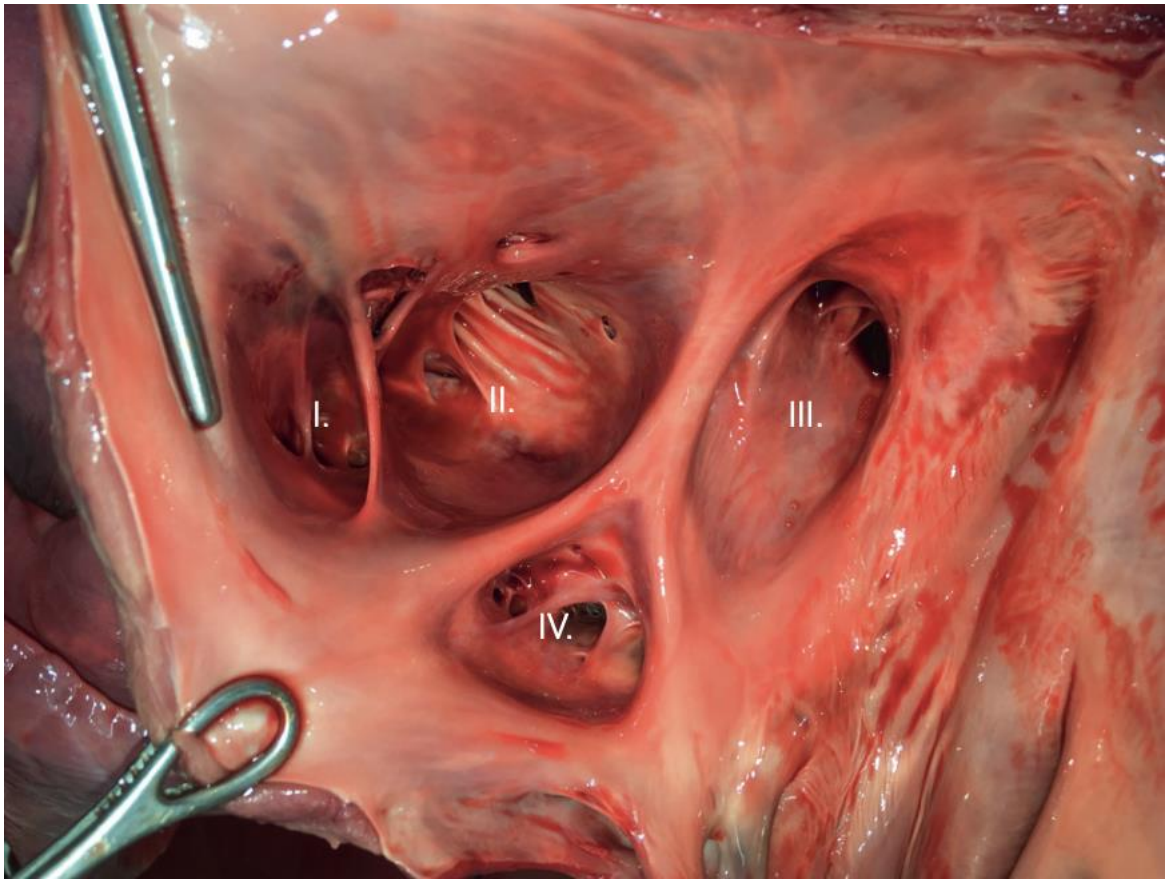

Figure 12. The four main pulmonary vein ostia in the left atrium of a normal horse. The left auricle is located on the left, and the interatrial septum is on the right.

The diameters of the main PV ostia are shown in Table 2. These values substantially varied but were normally distributed (p values obtained by Shapiro-Wilk statistic for ostia I to IV were 0.8, 0.2, 0.3 and 0.4, respectively). Ostia II and III had larger diameters, their mean values were close to each other with a difference of 0.7 mm. The diameters of ostia I and IV were smaller, and also similar to each other. The mean diameter of ostium I was larger by 2.1 mm than that of ostium IV.

In those hearts where five or six ostia were found, the diameter of the first (closer to the left auricle) supernumerary opening varied between 3 and 25 mm (mean ± SD: 11.1±5.0 mm). These values for the second additional ostium were 5 to 28 mm (mean ± SD: 14.2±7.9 mm). When one supernumerary opening was identified, it was near to ostium I in 25 cases (out of 31, 80.7%), close to ostium II in 4 hearts (12.9%), and near ostium III in one heart, and IV in another one (3.2% each). The second additional opening was positioned next to ostium I in three hearts (out of 6, 50%), and in one case near ostia II, III and IV (16.67% each).

Early branching was detected in 26 hearts (31.3%) and in 30 main ostia. One early branching was found in 22 cases, and two in four hearts. Ostium I branched in 14, II in 9, III in 5, and IV in 2 cases. The number of early branches was two in 25 horses, three in 4 hearts, and four in one case.

Age and body weight of the horses also showed normal distribution (p values were 0.2 and 0.7, respectively). Correlation analysis was done to show whether age had any effect on the diameters of PV ostia, but no significant relationship was found (p values for ostia I to IV were 0.8, 0.2, 0.3 and 0.6, respectively). One-way ANOVA was performed to investigate the effect of breed on the number and diameter of the openings. No significant differences were identified between the data of different breeds. Independent sample T-test was used to explore the relationship between sex and the number and diameters of PV ostia. Stallions and geldings were considered as male horses, and their data were compared to those of mares. No significant association was observed between sex and the number of openings (p=0.5), and the diameters of ostia I to IV (p values were 0.4, 0.8, 0.2, and 0.05, respectively). Two methods were used to assess the effect of body weight on the diameter of PVs. Pearson's correlation analysis did not reveal any significant association between these variables. In the other analysis, six weight groups (≤400 kg, 401-450 kg, 451-500 kg, 501-550 kg, 551-600 kg, >600 kg) were formed, and the PV diameters of these were compared by one-way analysis of variance, but no significant differences were discovered. The same method was used to investigate if the number of ostia (the presence of supernumerary openings) had any impact on the diameters. Ostium I was significantly smaller (mean ± SD: 17.8±4.9 mm) in those hearts where five ostia were present (p=0.04) compared to those with four (mean ± SD: 21.7±7.9 mm) or six ostia (mean ± SD: 20.8±5.8 mm).

Histology and immunohistochemistry

Statistical analysis of histological and immunohistochemical results was not possible because of the qualitative nature of these findings. The evaluation of the slides produced from the tissue samples (n=58) unequivocally showed that atrial myocardium and the tissue of the walls of the pulmonary veins were circularly overlapping each other. The myocardium extended into the antrum of the pulmonary veins in every studied occasion which supported the presence of the myocardial sleeve in this species (Figure 13).

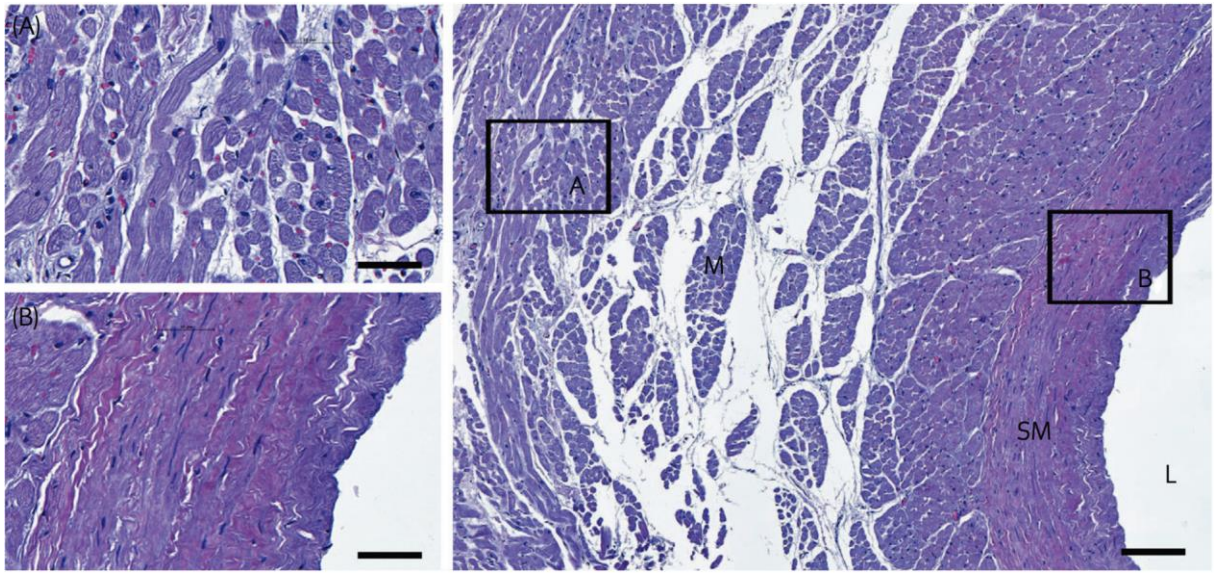

Figure 13. The myocardial sleeve in the antrum of a pulmonary vein of a normal horse. Haematoxylin-eosin stain, scale bar: 50 µm for A and B, and 250 µm for the right panel with inserts. L: lumen of the vein; M: myocardium; SM: smooth muscle in the venous wall.

Length of the myocardial sleeve varied between 0.4 and 4.8 cm (mean: 1.7 cm). The antrum was always fully surrounded by the sleeve. The sleeve extended into early branches to a variable depth. In slightly more peripheral bifurcations, no sleeve was seen.

Cx43 and Cx45 positivity was also observed in these samples which showed that gap junctions were also present in PV ostia. S100-positive and TH-positive, as well as S100-positive and TH-negative fibres were detected in all 12 tissue samples chosen for immunohistochemistry, confirming the presence of adrenergic and non-adrenergic vegetative fibres within the myocardial sleeve of horses (Figure 14).

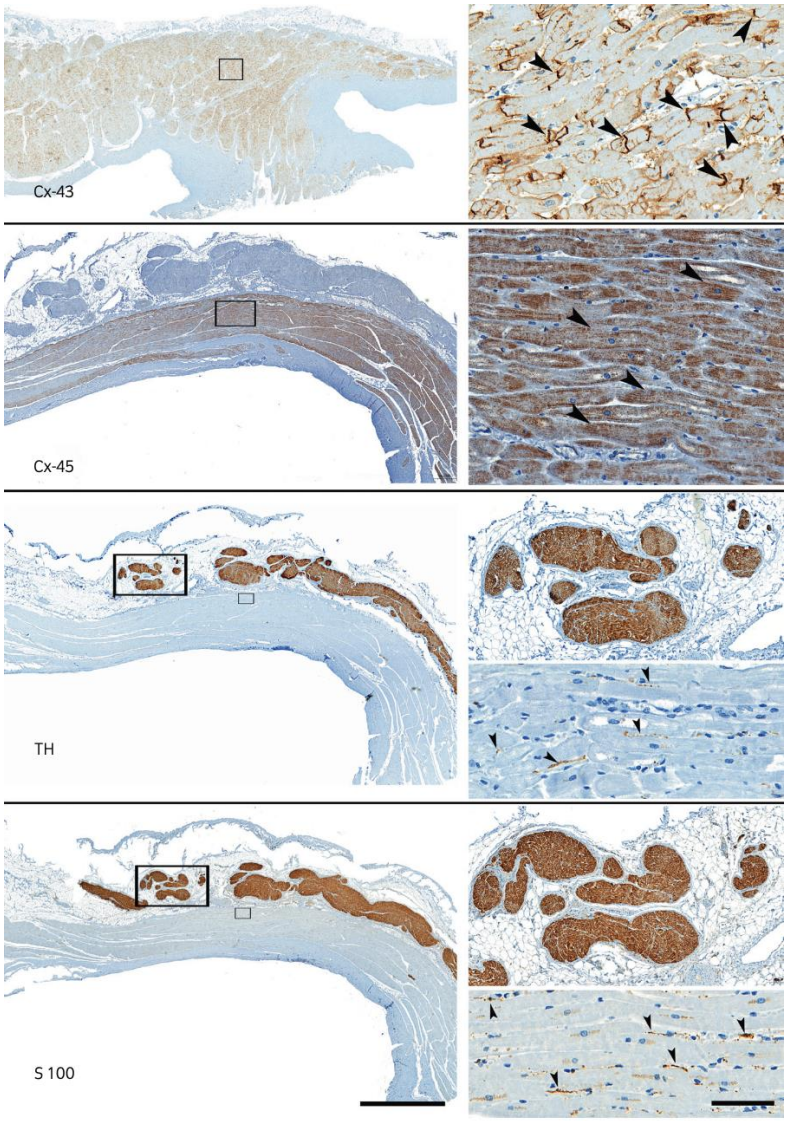

Figure 14. Representative micrographs from the ostia of pulmonary veins of normal horses showing multiple immunohistochemical stainings (please note, that white colour is background because of tissue separation). Panels show connexin 43 (Cx-43) immunostaining revealing putative gap junctions (arrowheads pointing to immunopositive structures between cardiomyocytes), and cardiomyocytes immunopositive for connexin 45 (Cx-45, arrowheads). In addition, we also detected Tyrosine hydroxylase (TH) and S100 immunopositivity of vegetative nerve fibres in the ostium of a pulmonary vein of a normal horse. Arrowheads pointing to immunopositive punctate and patchy staining (likely varicose nerves and nerve endings) among longitudinally sectioned profiles of cardiomyocytes. Scale bar: 3 mm for the low magnification views and 500µm for the high magnification panels.

Discussion

Anatomical location and number of pulmonary vein ostia have been described in human studies. These reports confirmed a relatively high variety in the human heart which should be considered during ablation therapies, because it may impede the successful execution of these procedures in certain cases (Prasanna L. C. et al., 2014; Marom E. M. et al., 2004). In a recent study, 130 randomly selected adult human hearts were dissected, and only 70.8% of these hearts showed the classical four PV ostia pattern (left and right superior and inferior). Eight more varieties have been found in the remaining 29.2% (Klimek-Piotrowska W. et al., 2016). In contrast, available data is limited in the veterinary literature, especially in horses. In an earlier study, 35 hearts from large mammals were dissected (17 sheep, 9 cattle, 5 pigs, 2 donkeys, 1 horse and 1 camel). It was concluded that generally three main ostia (left superior and inferior, and right pulmonary veins) could be identified in these animals. In some cases, two right veins (superior and inferior) were present (Nathan H. and Gloobe H., 1970). Other sources about horses reported that five to seven (Fehér G., 2000) and seven to nine ostia (Marr C. and Bowen M., 2011) could be found in this species. There is only one recent publication which included data about the dissection of 17 equine hearts and reported about the anatomical features of the left atrium and pulmonary veins in the horse (Vandecasteele T. et al., 2016). Similarly to this study, four main ostia were identified in that population. The anatomical pattern of these structures is identical to this study. The left atrial orifice of the vein draining the accessory lung lobe near ostium I was not considered as a separate ostium in that study. It was not stated if this opening was detected in each heart, and if it was completely separated from ostium I. Three main ostia were found in one horse in that study which differs from our results as all hearts had four main orifices in our population. In 37.4% of the horses (31 out of 83), a fifth ostium was found in this study which was located most frequently near ostium I (25/31, 80.7%). In six hearts (7.2%), a sixth ostium was also identified, 50% of these were positioned close to ostium I as well. These supernumerary openings were considered separate ostia in the present study, as they represent a separate interventional area when performing ablation. The anatomical variations may complicate the execution of ablation procedures in adult horses, as there are limitations to image these structures compared to humans where computed tomography and fluoroscopy are available. Further electrophysiological studies are required to examine whether pathological impulses causing atrial arrhythmias may arise from these structures.

There are no available numerical data about the diameters of pulmonary vein ostia in horses, hence our results cannot be compared to previous studies. A recent study has reported about the two-dimensional echocardiographic imaging technique of atrial-related

structures including pulmonary vein ostia in ten normal horses. However, results of linear measurement of these vessels were not published. Based on the diameters determined by this study, the currently available human cryoballoon catheters would be suitable to ablate ostium I in approximately 90% of the horses, and ostium IV in all horses. However, considering the maximal diameters, the procedure would be feasible for ostium II in only one third, and for ostium III, in one quarter of the horses.

The significance of early branching during ablation is that the cardiologist must decide whether it is possible to electrically isolate the entire ostium in front of the bifurcations, or a substantially more time-consuming isolation is required when the lumens of the branches are treated separately. Early branching may make the ablation difficult when it impedes the insertion of the balloon catheter. In the examined population, almost one third of the horses (26/83, 31.3%) showed this anatomical variation, affecting mainly ostium I.

Considering the effect of sex on the diameters of PV ostia, ostium IV was significantly smaller in mares compared to stallions. The mean difference was 2.5 mm which probably has no clinical importance from an interventional cardiology point of view. Unexpectedly, no significant effect of body weight on PV dimensions was found. This is in contrast to previous studies reporting that cardiac variables including aortic and pulmonary artery diameters depend on body weight (Berthoud D. and Schwarzwald C. C., 2021; Trachsel et al., 2016). A possible explanation of this might be the variable number of veins. The effect of body weight was investigated in a different way when six groups were formed. The clinical importance of this was to observe whether smaller horses would need currently available smaller balloon catheters for ablation procedures, but no significant association was found. A significant association was detected between the number of ostia and the diameters of those. In horses with five orifices, ostium I was smaller compared to horses with four or six ostia. As the supernumerary veins were located most frequently near ostium I, it is logical that the presence of an additional opening may decrease the diameter of the main ostium. However, hearts with six orifices did not show this alteration which may be explained by the low number of horses (n=6) with six ostia.

Earlier histological studies did not investigate the myocardial sleeve in horses (Bishop S.P. and Cole C. R., 1967; Bikou et al., 2011), but in one recent study, tissue samples were taken from 11 equine hearts (one sample from each heart) (Vandecasteele et al., 2018).The myocardial sleeve was identified in each sample which is in accordance with our results based on the analysis of 58 samples. This may morphologically confirm the assumption of a recent electrophysiological case report which presented evidence that pulmonary vein triggers induced atrial fibrillation in a horse (Linz et al., 2020).

Immunohistochemical investigation of the conducting system in equine myocardial sleeves has not been reported so far. Four proteins (Cx43, Cx45, TH, and S100) previously described in other mammals to demonstrate the presence of tissue structures that potentially play a role in the induction and maintenance of atrial fibrillation were studied (Sukhacheva T. V. et al., 2021; Hostiuc S. et al., 2018; Chaldoupi S. M., 2009). Adrenergic and non-adrenergic vegetative fibres, gap junction proteins, and telocytes were detected in our samples.

Limitations of the study

The number of horses from certain breeds was too low to draw reliable conclusions. Working cardiomyocytes could have been differentiated by further stainings from impulse-forming and conducting cardiomyocytes. Immunohistochemistry could have been done on a larger number of tissue samples which would have provided a more comprehensive view about the presence and distribution of the above-mentioned tissue structures.

Conclusion

This study aimed to provide numerical data of the diameters of pulmonary vein ostia in a larger normal equine population to help planning future ablation techniques. Although cryoballoon catheter ablation would be a technically easier and significantly shorter procedure than point-by-point radiofrequency ablation to isolate pulmonary vein ostia in horses with atrial arrhythmias, the maximal balloon diameter of currently available catheters represents a significant limitation in feasibility in many horses. Histological results confirmed the existence of myocardial sleeves in the antrum of all pulmonary veins. Based on our immunohistochemical findings, ablation methods should consider that conductive tissue is present in the entire myocardial sleeve in all pulmonary vein ostia.

Table 1. Types, manufacturers, dilutions, incubation times, and positive controls of antibodies used for immunohistochemistry

Antibody	Type, manufacturer, dilution	Incubation time (minutes)	Positive control
S100	S100 rabbit polyclonal antibody, DAKO, z0311, 1:200	60	Canine heart tissue
Tyrosine hydroxylase	Tyrosine hydroxylase monoclonal antibody, Thermo Scientific, TH-16 MA5-32984, 1:100	60	Canine substantia nigra
Connexin 43	Connexin 43 antibody, Santa Cruz, F-7 sc-271837, 1:200	30	Canine heart tissue
Connexin 45	Connexin 45 antibody, Santa Cruz, G-7 sc-374354, 1:200)	30	Canine heart tissue and canine substantia nigra

Table 2. The diameters of the four main pulmonary vein ostia in normal horses

	Ostium I	Ostium II	Ostium III	Ostium IV
Minimum (mm)	6.0	18.0	16.0	9.0
Maximum (mm)	53.0	56.0	48.0	29.0
Mean (mm)	20.2	32.7	33.4	18.1
Standard deviation (mm)	7.0	7.1	5.9	4.5

New scientific results

1. Long-term in vivo use of the Reveal XT loop recorder in horses is feasible, well-tolerated, and the devices worked reliably without complications.

2. The human algorithm could not be used for automatic detection of arrhythmic episodes in horses. The device could detect atrial fibrillation in horses, but the recorded AF burden was inaccurate, and the stored ECGs had to be manually interpreted.

3. Extensive numerical data on the pulmonary vein ostia is described in horses, including the exact diameters of the ostia and the changes in early branching.

4. Histological results confirmed the existence of myocardial sleeves in the antrum of all pulmonary veins of horses

5. Based on our immunohistochemical findings, conductive tissue is present in the entire myocardial sleeve in all pulmonary vein ostia of horses.

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Kovacs Sz., Bakos Z. Dr. Kovács Szilvia: Intervenciós kardiológiai módszerek lovakban – hazai lehetőségek, XXIX. Lógyógyászati Kongresszus 2021.11.26

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Kovacs S., Bakos Z., Racz B. Kardiális őssejtek és telociták vizsgálata egészséges lovak szívében. MTA Állatorvos-tudományi Bizottsága Akadémiai Beszámolóján 2023., Budapest

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