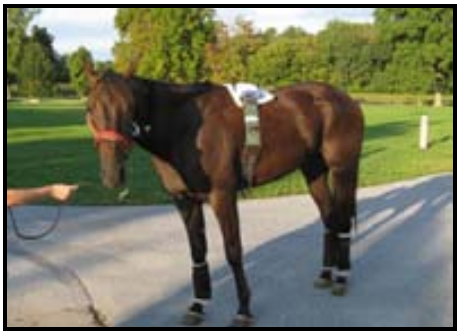
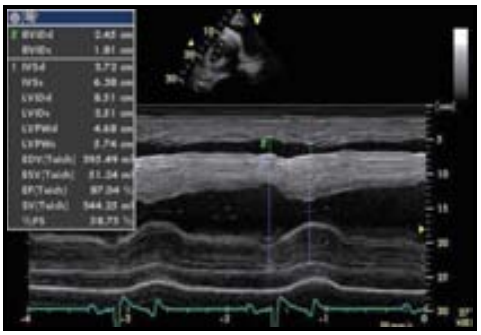
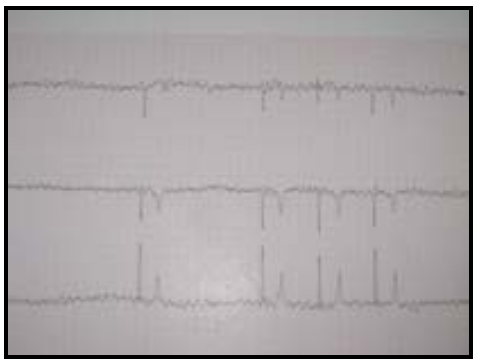
STUDIES IN EQUINE

CARDIOLOGY







PhD Thesis

Cristobal Navas de Solís

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Departament de Medicina i Cirurgia Animals

Facultat de Veterinària

Universitat Autònoma de Barcelona

ESTUDIOS EN CARDIOLOGÍA EQUINA

Memòria presentada per:

Cristobal Navas de Solís

per a optar al grau de

Doctor en Veterinària

Barcelona, Setembre 2013

Director de la tesi: Eduard Jose Cunilleras

Departament de Medicina i Cirurgia Animals

Facultat de Veterinària

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STUDIES IN EQUINE CARDIOLOGY

Thesis work presented by

Cristobal Navas de Solís

to qualify for the degree of

PhD in Veterinary Medicine

Barcelona, September 2013

Thesis director: Eduard Jose Cunilleras

Eduard Jose Cunilleras, professor lector del Departament de Medicina i Cirurgia Animals de la Facultat de Veterinària de la Universitat Autònoma de Barcelona,

CERTIFICO:

Que la tesi doctoral que porta per títol "Estudios en cardiología equina", de la que n'és autor el llicenciat en Veterinària Cristobal Navas de Solís, s'ha realitzat a la Facultat de Veterinària de la Universitat Autònoma de Barcelona i a la Universitat de Pennsylvania, sota la meva direcció.

I per a què així consti, a efectes de ser presentada coma a Tesi Doctoral per a optar al títol de Doctor en Veterinària, signo aquest certificat a Bellaterra, de Setembre de 2013.

Eduard Jose Cunilleras

AGRADECIMIENTOS

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INDEX

INDEX

INTRODUCTION

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1. Relevance of cardiac disease and monitoring in equine medicine and for the equine industry

Equine cardiology is a fascinating field relevant to veterinary clinicians, scientists, horse owners, trainers and riders. Knowledge in this field can help physiologists and academicians understand equine health, disease or performance and provide veterinary practitioners with the information necessary to make clinical decisions both in the equine patients with heart disease that they treat and in the healthy athletes they oversee. A horse's health and wellbeing can be affected by many different cardiac diseases. Equine cardiology is important to the equine industry from the economic stand point as cardiac diseases can require therapy, rehabilitation and cause horses to lose training days or be declared not apt to compete. All of these things, can cause losses for trainers, riders and owners. Cardiac adverse events during exercise can be dramatic and have serious consequences for horses and their riders or drivers. Accidents during racing or equestrian sports are often widely broadcasted by the media and impact the public image of the sport and the society's perception of animal welfare. Most importantly the safety of horses, riders and caretakers can be jeopardized by cardiac adverse events such as cardiovascular collapse or sudden cardiac death.

The studies presented in this document were designed with the common underlying motivation of advancing the understanding of equine cardiology. More specifically, these investigations sought to increase the knowledge of three cardiac problems that had received little or no attention in the previous literature: 'Myocardial insult and arrhythmias after acute hemorrhage', 'Hypertensive cardiomyopathy' and the 'Evaluation of coagulation and fibrinolysis in horses with atrial fibrillation'. These studies provide information to researchers, academicians and clinicians that can potentially impact horses' health, human safety and the equine industry. The three conditions studied are naturally occurring cardiac problems that can affect horses and humans. Many aspects of these diseases differ between species but many others are shared. This gives the research presented here translational potential that adds a layer of relevance to the studies. Myocardial injury in the intensive care unit, the thromboembolic risk of patients with atrial fibrillation and the cardiac consequences of hypertension are complex topics of active research that affect millions of human beings worldwide and in which many questions and controversies remain.

2. Cardiac evaluation and monitoring methods

Thorough histories, physical examinations and more specifically, careful cardiac auscultations, are the mainstay of the evaluation and monitoring of horses with suspected disease of the cardiovascular system. These provide clinicians with the most important information to diagnose and manage equine cardiac diseases. Different diagnostic aids can further characterize the cardiovascular disease and help provide more precise diagnoses, therapeutic regimens, monitoring plans and prognoses. Echocardiograms and electrocardiogramss (ECGs) have been

used for decades to evaluate equine health and performance.[1,2] In recent years many other diagnostic aids, used in humans and other veterinary species, have been described in equine medicine. Measurements of cardiac biomarkers, exercising and continuous ECGs, advanced echocardiographic techniques, such as tissue Doppler imaging (TDI) or two dimensional speckle tracking (2DST) are nowadays available to equine practitioners and are progressively becoming more widely used in specialized equine facilities. These techniques have allowed advances in the understanding of equine cardiac health and disease but despite its use in many institutions gaps in knowledge and in the understanding of their usefulness and limitations currently exist.

2.1 Continuous ECGs

The surface ECG records the electrical potential differences between the areas of the skin where electrodes are placed. The electrodes detect changes in the electrical field present around the heart during the cardiac cycle[3] allowing clinicians to determine the heart rhythm and identify arrhythmias or conduction disturbances. The study of the ECG is critical to the evaluation and monitoring of horses with primary cardiac disease and in horses with severe systemic illness, endotoxemia, electrolyte or acid base disturbances, poor performance or during general anesthesia.

Traditionally short electrocardiographic tracings have been printed and analyzed when a rhythm abnormality was suspected based on auscultation, compatible clinical signs or when evaluation of the cardiac rhythm was considered indicated for other reasons. Some authors have suggested

that the equine heart rate (HR), sympathetic response or rhythm can be affected by handling and these authors have suggested that the optimal way of evaluating rhythm in horses may be by means of ambulatory ECGs.[4-6] Moreover, many arrhythmias may be intermittent and a standard recording period of twenty-four hours is recommended when a short electrocardiographic tracing does not provide enough information to make clinical decisions.[4] Many analogue and digital systems have been used with both telemetry (real time) and holter (recording) capabilities. Some of the current devices may offer both options simultaneously and the differentiation between telemetric and holter monitoring may become semantic in the near future. More recently, devices that transmit the electrocardiographic signal via Internet, blue-tooth technology or mobile phone have allowed intensive rhythm monitoring and specialized care to be more accessible to many clinicians. Newer digital systems that use adhesive electrodes can be easily adapted to an elastic surcingle, or glued and are easy and quick to place. These systems provide high quality ECGs and are well tolerated by horses. The main limitation of these electrocardiographic units is that twelve lead ECGs cannot be obtained with the currently available systems. Fortunately in many cases a base apex ECG provides all the information needed for rhythm interpretation in horses.[a]

Ambulatory continuous ECGs have been described in normal horses at rest, during and after exercise or anesthesia and in horses with different medical conditions such as endotoxemia, envenomation or intoxications.[4, 6-16] Despite the description of the normal electrocardiographic recording, at rest and during exercise, the clinical relevance of many rhythm disturbances such as post-exercise ventricular arrhythmias, occasional atrial or ventricular exercising premature contractions, accelerated idioventricular rhythms, certain types of advanced second degree atrioventricular block or ventricular ectopy/aberrant ventricular conduction in the face of atrial

fibrillation remains uncertain.[a] The presence of arrhythmias at rest and particularly during exercise are a relevant topic to horse health and human safety as certain arrhythmias can cause collapse or sudden cardiac death.[17-20] Determining the importance of exercising arrhythmias is complex and controversial in humans.[21] The understanding of exercising arrhythmias, and in general of the effects of cardiac abnormalities in equine athletes, is in infancy. Big strides have been made in the last few years due to the efforts of researchers in many institutions in Europe, North America and Japan.[11-13, 18,] The international veterinary community has recognized the importance of cardiac disease and arrhythmias in the performance horse and a panel of experts worked during 2012 and 2013 to release a consensus statement jointly sponsored by the American College of Veterinary Internal Medicine and the European College of Equine Inernal Medicine that will define current recommendations and will try to establish a platform from where future research can arise.[a]

2.2 Echocardiograms

Echocardiography has been a diagnostic aid and research tool in equine cardiology for over 30 years.[2,23] Two-dimensional echocardiography, M-mode echocardiography, contrast echocardiography, pulsed wave Doppler, continuous wave Doppler and color-coded Doppler are the traditionally used modalities.[24] Newer modalities such as TDI or 2DST have been introduced in recent years and are promising tools for the equine cardiologist.[25-29] Echocardiography can give very valuable information while assessing horses with different types of cardiovascular problems such as valvular disease, cardiomyopathy, pericardial disease, arrhythmias or congenital heart disease. The normal appearance of the equine heart in different breeds,[6,23,30-32]

the potential applications of echocardiography of horses,[23] and the changes associated with many different types of cardiac or extracardiac conditions,[b, 33-45] training,[46-48] or performance ability[49] have been described.

2.3 Blood pressure monitoring

Blood pressure is often measured in the veterinary intensive care unit as a marker of tissue perfusion. Flow (cardiac output [CO] in the case of the vascular system) can be described as the product of pressure (mean arterial pressure) and resistance (systemic vascular resistance [SVR]) and is undoubtedly a superior marker of perfusion when compared with blood pressure. Changes in vascular resistance can affect CO, as implied by the above-mentioned definition, and therefore blood pressure is not the only determinant of tissue perfusion. Because measuring CO requires specialized equipment and expertise it can be cumbersome in the clinical setting[50] and measuring blood pressures non-invasively is a fair alternative to gain knowledge about potential flow. Arterial blood pressure measurements have been obtained traditionally in horses in which hypotension or poor perfusion are suspected.[51, 52] On the other hand, systemic hypertension is very rarely cited as a concern in the equine literature.[53-55]

Arterial blood pressure can be measured by invasive (direct) and non-invasive (indirect) methods. Invasive blood pressure (IBP) measurements are the gold standard but require catheterization of an artery. Placement and maintenance of an arterial catheter is common practice during equine general anesthesia and can be achieved in the standing horse. However, this may be technically difficult in non-anaesthetized fractious horses and maintaining arterial

catheters and fluid lines for prolonged periods of time to obtain frequent direct blood pressure readings can be time consuming and challenging. This makes direct blood pressure monitoring less practical for the routine, non-research setting.[51, 52, 56-58]

Techniques for the measurement of non-invasive blood pressure (NIBP) include auscultatory, ultrasonic Doppler, and oscillometric methods. Oscillometric techniques provide systolic, diastolic, and mean arterial pressures, whereas other indirect methods do not provide mean arterial pressure.[52] As mean arterial pressure (MAP) is a better estimationr of tissue perfusion than systolic or diastolic pressures[51] oscillometric methods are preferred on the clinic floor.[51, 52] The capability of quickly obtaining MAP have made oscillometric blood pressure monitors the currently most commonly used blood pressure devices in equine medicine.[59] Furthermore, MAP has been reported to be less sensitive to changes in recording technique[52] , an advantage that can increase the reliability of the results.

Measurement of NIBP by oscillometric monitors has been proven to be highly correlated with invasive methods and provide acceptable estimations for invasive blood pressure (IBP) in foals and adults. In foals, a mean bias of 0.5-4.5mmHg, depending on the monitor used, has been described.[52, 56] The agreement between NIBP and IBP measurements appears to be better in foals than in adult horses and some controversy remains regarding the accuracy of indirect blood pressure measurements in adult standing horses.[c, d] Gay et al. reported indirect systolic and diastolic values to be close to invasive measurements and have a high correlation (r = 0.978 and 0.975 for systolic and diastolic pressures respectively) using a Doppler ultrasonic technique.[60] Branson and collaborators found the average difference between IBP and oscillometric blood

pressures in anesthetized horses to be 18, 9, and 11 mmHg respectively for the systolic, diastolic and mean arterial blood pressures and the correlation coefficient to be 0.93 with the NIBP consistently underestimating invasive measurements.[61] Latshaw et al., in a study designed to determine the effect of cuff sizes on oscillometric blood pressure measurements, concluded that the oscillometric method correlated well (correlation coefficients of 0.8-0.99 depending on the cuff size) with direct measurements in anesthetized and standing horses although there was a difference between the values obtained using the two methodologies. Non-corrected tail cuff measurements most consistently underestimated the actual blood pressure in several studies. [c, 60] Similarly to that reported in small animals, it is likely that non-invasive methods will continue to be the method of choice in the clinical setting.[59] The equine veterinary community would benefit from standardization and validation of methods and equipment when measuring NIBP especially in adult standing horses.

When obtaining indirect blood pressure recordings, cuff size needs to be taken into consideration as cuffs with bladder widths that are too wide result in falsely low readings and cuffs that are too small result in falsely high readings.[51,62]A bladder width to tail girth circumference ratio of 0.34 for systolic pressure and 0.98 for diastolic pressure have been recommended[62] for adult horses when using Doppler ultrasound techniques. When using oscillometric techniques, recommended bladder width to tail circumference ratios are 0.2-0.25 in adults.[51] However little variation (5% approximately) has been reported with ratios up to 0.5 when obtaining measurements on the middle coccygeal artery. The bladder width reportedly affects measurements more in adult horses compared to foals. Bladder width to tail girth circumference ratios between 0.3 and 0.9 seem to be adequate and do not significantly affect results of oscillometric measurements in

foals.[52,56]

Blood pressure recordings should be corrected to heart level or described as 'coccygeal uncorrected values'.[51,62] In the standing horse the tail is above the level of the heart (20-30 cm in the average size horse) and therefore the displayed NIBP will be lower than the actual blood pressure (15-20 mmHg approximately). A horse under anesthesia and in dorsal recumbency has its tail below the level of the heart and therefore the readings displayed by the blood pressure monitors, when obtaining non-invasive recordings at the coccygeal artery on a horse in dorsal recumbency will be higher than the true blood pressure.[51] A correction factor of 0.7-0.77 mmHg/cm of vertical distance between the base of the heart (point of the shoulder) and the base of the tail can be used[60-62] to account for the difference of recording position and the different size of horses. The result of multiplying this correction factor by the vertical distance from the base of the tail to the point of the shoulder should be added to the pressure readings in the case of the standing horse, and subtracted in the case of the horse in dorsal recumbency.

Reference ranges for IBP in normal adult horses are 126 to 168 mmHg (systolic) over 85 to 116 mm Hg (diastolic), with a mean of 110 to 133 mmHg.[51] Reference ranges for NIBP in normal adult horses vary depending on the method and correction factors. Normal values described in a large population of standing horses studied by Parry et al. were 80-144 mmHg , 49-105 mmHg and 60-116 mmHg for systolic, diastolic and mean pressures respectively. These values were uncorrected and obtained using an oscillometric technique[62] and a cuff with bladder width approximating half the tail girth circumference placed at the base of the tail (uncorrected coccygeal artery pressure). The cited study provides reference ranges for corrected values and

different correction factors for bladder width in addition to the above-mentioned correction factor for the vertical distance between cuff placement site and heart base.

2.4 Cardiac biomarkers- cardiac troponin I

Troponins are proteins part of the contractile apparatus of skeletal and cardiac muscle that regulate muscle contraction.[64] Cardiac troponins are attached to tropomyosin and regulate the interaction between this protein and actin in the cardiac myofibrils.[65, 66] Troponins and tropomyosin, lie between actin filaments and in the relaxed muscle block the attachment site for the myosin crossbridge preventing muscle contraction. At the beginning of systole, calcium channels open and calcium attaches to troponin causing a conformation change and exposing actin binding sites for myosin. Tropomyosin binding to actin activates the thin filaments allowing crossbridging of myosin and muscle contraction.[66]

The troponin complex consists of three different proteins (troponin I, T, and C). Troponin C has the calcium binding sites and troponin I has the inhibitory subunit that prevents contraction if calcium is not bound to the troponin C. Troponin T attaches troponin I to tropomyosin and actin filaments. Different isoenzimes of troponins I and T are present in different muscular tissues (skeletal and cardiac) and smooth muscle does not have troponins. Cardiac troponins I (cTnI) and T (cTnT) are therefore specific proteins of the cardiac muscle. The amino acid sequence and structure of cardiac troponin C is homologous to the troponin C present in skeletal muscle which makes troponin C a less desirable marker of myocardial injury. A small percentage of troponins are free in the cytoplasm of the skeletal and cardiac muscle while most of them are bound to the

contractile apparatus. Cardiac troponins are currently considered the biomarker of choice for detection of myocardial injury[64] and have replaced other markers of myocardial injury, such as myocardial bound creatine kinase (CK-MB), lactate dehydrogenase and a-hydroxybutyrate dehydrogenase[67] due its high sensitivity and specificity.

In the event of myocardial injury the troponins present in the cytoplasm are released first and rapidly. This initial release of free troponins will be followed by a slower, but larger in magnitude, release of the troponins bound to the contractile apparatus.[64] The amount of free troponins is small compared to the amount of bound troponins and this causes an average 12-48 (and up to 72)[67] hour delay between myocardial insult and the peak plasma concentration of troponin. The time to peak concentration of cardiac troponins during myocardial disease may also be different depending on the type of injury, the presence of comorbidities that could compound the myocardial injury or the presence or absence of ongoing damage after the initial insult. [64,68-74]

The elimination half-life of intravenously administered cTnI in horses is very short (0.47 hours in average) and elimination is reportedly renal.[75] In the clinical scenario of myocardial injury the plasma concentration of cTnI often remains elevated for longer than would be expected for an enzyme with such a short elimination half-life, possibly due to the presence of ongoing myocardial injury in many cases.

The increase in concentration of cardiac troponins in humans is proportional to the myocardial damage in the scenario of myocardial infarction[76] and proportional to the severity of disease in

the critical care setting.[77] Myocardial infarction is a rare occurrence in horses[78] and cardiac troponins will be measured more commonly in this species in the event of arrhythmias, toxic insult or if myocardial injury secondary to extracardiac disease is suspected in the critical care unit.[79-82] It is plausible, or likely, that the degree of myocardial injury in horses is proportional to the increase in plasma/serum concentration of cardiac troponins but this has not been formally proven. In a recent study by Nath et al., evaluating the plasma concentration of cTnI in horses with primary cardiac disease the median cTnI concentration of non-survivors was higher than the concentration for survivors suggesting that the degree of increase in plasma concentration may be associated with prognosis in horses with cardiac disease. In the equine critical care unit the plasma concentration of cardiac biomarkers has also been suggested to have prognostic significance and this was proven by two different groups in horses with colic.[81,82] However in other clinical scenarios, such as the foal with neonatal sepsis the same conclusions could not be achieved by measuring cTnT.[80] The overlap in plasma concentrations of cardiac biomarkers between survivors and non-survivors and presence of outliers may preclude the use of cardiac troponins as an absolute prognostic indicator in horses with cardiac disease or critical illness although in some clinical scenarios it may be an useful marker of severity of disease.

There is currently no gold standard for the measurement of concentrations of cardiac troponins. Different assays (often enzyme-linked immunosorbent assays [ELISA]) have been used in horses,[67, 80, 83] including point of care analyzers.[68] When using different assays or analyzers it is important to recognize that the different methods have different analytical sensitivity, precision, limits of detection and reference ranges. Assays designed to measure cTnI or cTnT in humans can be used in horses and other species due to the highly conserved structure of these enzymes

between species.[84, 85] Ultrasensitive cTnI assays are capable of detecting lower troponin concentrations and could potentially be useful to more accurately assess mild myocardial injury, poor performance or progression of disease due to the superior analytical sensitivity. To our knowledge these assays have not been used in horses and are unlikely to be widely used in equine medicine in the near future due to their high cost.

There are many causes of primary and secondary myocardial damage reported to cause increase in cardiac troponins in humans and animals. The relevance and differential diagnoses for an increase in cardiac troponins was recently reviewed by Wells and Sleeper[64] and cited the following as potential causes of increased troponins: ischemic heart disease, congestive heart failure, coronary vasospasm, hypertrophic cardiomyopathy, inflammatory disease involving the heart (e.g., myocarditis, pericarditis), infiltrative disease of the myocardium (e.g., sarcoidosis, amyloidosis), aortic insufficiency jet lesions, toxins (e.g., doxorubicin, cantharadin, white snake root or oleander), babesiosis, ehrlichiosis, third degree heart block, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy in Boxers, mitral valve disease, pericardial effusion, subaortic stenosis, blunt chest trauma, cardiac surgery or myocardial biopsy, drug toxicity, electrical cardioversion, pacing, implantable cardioverter defibrillators, snake envenomation, hypotension/hypovolemia, hypertension, sepsis, pulmonary embolism and pulmonary hypertension, rhabdomyolysis with cardiac injury, extreme or endurance exercise, sympathomimetic activity (e.g., cocaine use, massive catecholamine release such as in head trauma or stroke), prolonged tachycardias, chronic obstructive pulmonary disease, gastric dilatation-volvulus, feline hyperthyroidism or renal insufficiency.[64] Several reasons for the presence of falsely increased cTnI have been described in humans such

as the presence of heterophilic antibodies (anti-mouse), rheumatoid factor, fibrin clot, hemolysis and malfunction of the analyzer.[86] To the best of our knowledge the presence of falsely increased cardiac troponins have not been formally described in the equine peer reviewed literature.

The use of cardiac troponins in equine cardiology and emergency and critical care has increased substantially in the last ten years and it is a promising marker of myocardial injury and of severity of disease in severely ill patients.[68, 79-81, 87-91] The cause of myocardial damage in the event of critical illness is multifactorial and often the initiating disease is non-cardiac. The secondary myocardial damage and the rise of cardiac troponins is accepted to be a poor prognostic indicator in humans in a critical care unit regardless of the cause.[77] The presence of myocardial damage in the critically ill horse and its use in the evaluation of horses with different types of cardiac and non-cardiac diseases causing myocardial injury is likely to become more common in equine medicine in the future as more knowledge is gained.

2.5 Coagulation profiles, fibrin components and degradation products

Coagulation is defined as the formation of a blood clot. The coagulation process is complex and can be separated into three stages: primary hemostasis (formation of a platelet plug), secondary hemostasis (formation of a crosslinked fibrin meshwork), and fibrinolysis (removal of excessive fibrin from the platelet-fibrin clot).[92] The secondary hemostasis has been traditionally described as being initiated by two separate pathways: the extrinsic and intrinsic pathways. Factors III and VII are involved in the extrinsic pathway and factors XII, XI, IX and VIII are involved in the

intrinsic pathway. The intrinsic and extrinsic pathways activate the common pathway (mediated by factors X, V and II) to generate thrombin (activated factor II). Thrombin forms fibrin by cleavage of fibrinogen and is cross-linked by factor XIII.[92] Plasmin is the enzyme responsible for breakdown and remodeling of fibrin (fibrinolysis) and circulates as a proenzyme, plasminogen, which is activated by plasminogen activator. The intrinsic/extrinsic/common pathway model is useful for understanding in vitro coagulation and for the instruction of physiology students. However the importance of the intrinsic pathway in the coagulation cascade may be minor. The current understanding is that secondary hemostasis is mainly initiated by tissue factor (Factor III or thromboplastin) in what would be the initiation of the traditionally described extrinsic pathway.[92] A more complex and comprehensive ('cell-based') model has been proposed to describe the coagulation cascade in vivo[93] and is replacing the traditionally described intrinsic/extrinsic common pathway paradigm. As a summary, secondary hemostasis is started by tissue factor expressed by tissue macrophages and vascular smooth muscle cells. The formation of fibrin is activated when endothelium is damaged and tissue factor is exposed to blood. Fibrinolysis is the process that limits and remodels the blood clot during normal coagulation.[92]

Many clinical pathology tests have been reported for the evaluation of coagulation and fibrinolysis in horses. Some of the most commonly used tests in veterinary medicine are: platelet count and function evaluation, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, fibrinogen degradation products (FDPs), D-dimer plasma concentration, antithrombin activity (AT), viscoelastic coagulation analysis and thromboelastography (TEG). The field of coagulation and fibrinolysis evaluation is continuously evolving and some researchers and clinicians have suggested that newer tests could replace these

classically used methods[92, 94] in the future.

An appropriate number of platelets is needed for primary hemostasis. The lower end of the normal reference range for the platelet count in horses is approximately 100,000 platelets/µl although prolongation or spontaneous bleeding do not frequently occur until the platelet counts fall under 30,000 platelets/µl and 10,000 platelets/µl respectively. Platelet function is as important, or more important than platelet numbers and assessing function is indicated when disorders of primary hemostasis are suspected.[92,94] Viscoelastic methods of coagulation evaluation, TEG or other point-of-care analyzers are becoming more popular in the field of coagulation and platelet function evaluation.[94,95]

Prothrombin time evaluates the fibrinogen conversion to fibrin through the extrinsic and common pathways. PT can be prolonged due to hypofibrinogenemia or with degradation or consumption of coagulation factors in the extrinsic or common pathways (factor VII, V, or X or II (prothrombin)). In human patients diagnosed with disseminated intravascular coagulation (DIC), only approximately 50% have prolonged PT, and some believe that measurement of PT is not a sensitive measure of coagulopathy. Prothrombin time measurements may be more useful in the equine patient than in other species and this test is part of the coagulation profile to evaluate and monitor horses with suspected coagulopathies DIC in many institutions.[93]

Activated partial thromboplastin time evaluates the intrinsic and common pathways of fibrin formation. Activated partial thromboplastin time becomes prolonged when factor VIII, IX, or XI

are decreased. Prolonged aPTT was the most frequently observed coagulation abnormality in two studies evaluating hemostatic indices in horses with acute abdominal disease[92, 95] and is part of the routine evaluation of the coagulation system in horses.

Fibrinogen concentration, FDPs and D-dimer plasma concentration are the commonly used tests to evaluate fibrinolysis in horses. Hypofibrinogenemia is found often in humans with DIC but is not a common finding in horses with coagulopathy likely due to the rapid increase of fibrinogen concentration in response to inflammation in this species.[92] Fibrinogen degradation products and D-dimers are products of fibrinolysis. Fibrinogen degradation products may result from the degradation of fibrin or fibrinogen. D-dimer is specifically formed by the plasmin digestion of cross-linked fibrin only and therefore a better marker of clot degradation after increased clot formation.[96] D-dimers are for this reason favored by many clinicians to assessed fibrinolysis. Fibrin and fibrinogen degradation products can be increased as a result of DIC, thrombotic and thromboembolic events or severe inflammation.[95,97,98] D-dimer analysis has been used as a prognostic indicator for horses with colic.[97,98] There are currently many quantitative and semi-quantitative tests available for D-dimer analysis. The reference ranges are specific for the species, methodology and laboratory and this needs to be taken into account when interpreting results for clinical purposes or when reviewing the literature.[95]

Antithrombin is the most commonly measured component of the anticoagulant system in horses. Antithrombin III is the most abundant and powerful physiologic inhibitor of thrombin. Antithrombin activity is typically measured by chromogenic assay in an automated analyzer, by combining the patient's plasma with excess heparin and Factor Xa.[92] A decrease in AT can be

associated with inherited disorders in people, consumption, endotoxin inhibition, liver dysfunction, or protein loss.

Viscoelastic coagulation analysis and TEG use a probe placed within a cup of blood with or without the addition of an activator. As the blood clots in the cup the torque placed on the probe as the cup oscillates changes. The forces obtained give information about the rate of clot formation, the strength of the clot, and the clot degradation by fibrinolysis.[95] These assays are promising as they are stall side and can assess primary and secondary hemostasis. Many reports of the use of TEG have been published in recent years.[99-102] The clinical usefulness of these assays in horses needs to be further defined and some investigators suggest that this technology may substitute the more classic tests that assess coagulation and fibrinolysis.[99-102]

The most relevant and commonly studied coagulation disorder in horses is DIC. Disseminated intravascular coagulation has been defined as an acquired coagulopathy characterized by a marked activation of the coagulation system that overwhelms the inhibitory systems.[96] Disseminated intravascular coagulation may be particularly important in the management of the critically ill horse with gastrointestinal disease[96] but is important in the management of other conditions in the equine intensive care unit.[103,104] Disseminated intravascular coagulation can cause both hyper- and hypo- coagulation. The hypercoagulatory state is more commonly seen in horses although frequently there is overlap between hyper- and hypocoagulable states and the clinical distinction between the two can be difficult. When the activated coagulation overwhelms the inhibitory system, exaggerated fibrin and thrombus (micro- and macro-) formation may lead to ischemia, multiorgan failure and death. If the hypercoagulatory state causes depletion of

platelets, coagulation factors, and coagulation inhibitors, consumption coagulopathy and spontaneous hemorrhage can occur. This clinical presentation is less common in equine practice[96]. Laboratorial diagnosis of DIC is made when horses have 3 or more abnormal parameters in a coagulation profile.[97]

From the standpoint of the cardiac disease, hypercoagulability and the potential for thromboembolism are more relevant than hypocoagulability or DIC. This aspect of equine cardiology has remained unexplored likely due to rare reports of thromboembolic disease in horses with cardiac disease. Atrial fibrillation causes a hypercoagulable state in humans that increases the risk of thromboembolism.[105] Thromboembolic events have been diagnosed in horses with systemic disease[98,106] and plasma D-dimmer concentration has been recently demonstrated to be a sensitive marker of hypercoagulation associated with different diagnoses and prognoses.[97] However, the presence of a hypercoagulable state in horses with atrial fibrillation has not been tested.

3. Myocardial damage during acute hemorrhage

Myocardial damage after severe hemorrhage is a well-recognized phenomenon in humans and an increase in cTnI plasma concentration increase appears to be an early marker of severe hemorrhage.[107-109] The decreased oxygen delivery (DO2), sympathetic activation and reperfusion injury are likely to play a role in the myocardial damage and arrhythmogenesis in the scenario of acute hemorrhage. Adequate DO2 is needed to support metabolism of all tissues.[110] When DO2

is too low to support tissue demandsthe mitochondria cannot sustain aerobic metabolism and oxygen consumption (VO2) decreases.[111] Compensatory mechanisms can maintain oxygenation in cases of mild blood loss. Blood loss greater than 15% of the blood volume requires volume replacement and a 30% to 50% blood loss reduces DO2 to the point of causing circulatory failure, shock and death if this is not immediately treated by fluid resuscitation and/or blood transfusions.[110, 112] If hemorrhage is accompanied by trauma and a systemic inflammatory response the effect of blood loss is compounded by the effects of inflammation on the cardiovascular system and a loss of less than 30% of the volemia can produce the same consequences as a 50% hemorrhage alone.[110]

Oxygen delivery is defined by the product of CO and arterial oxygen content (CaO2) and dictates the amount of oxygen available for tissues. This definition implies that DO2 is dependent on stroke volume (SV), HR, hemoglobin concentration ([Hb]), hemoglobin oxygen saturation (SaO2) and arterial partial pressure of oxygen (PaO2). All of these factors and the local regulation of blood flow are altered in the clinical scenario of a bleeding horse making the pathophysiological process of acute hemorrhage complex.[110] Stroke volume is partially dependent on preload which decreases due to hypovolemia after bleeding. Heart rate will be initially increased due to sympathetic activation although the effects dysoxia on the myocardium and on sympathetic reflexes blunt this response during severe hemorrhage. Oxygen-carrying capacity is obviously reduced during hemorrhage due to a decrease in [Hb] and also due to decreases in PaO2 and SaO2.[112, 113] The cause of the decrease in PaO2 during acute hemorrhage has been proposed to be the reduction in functional CO.[113]

In the event of severe hemorrhage, secondary sympathetic activation attempts to prevent tissue hypoperfusion by causing vasoconstriction, increased cardiac contractility, increased HR and decreased filtration fraction.[112] Acid–base balance, electrolyte disturbances, hypoxemia and hypothermia can blunt baro/chemoreceptors and the vascular response to the sympathetic activation leading to a poor compensatory response.[111] If the decrease in DO2 overwhelms the compensatory mechanisms anaerobic metabolism and production of lactate, cytokines and local tissue factors are started. This leads to further tissue dysoxia due to a loss of local tissue blood flow regulation and poor distribution of the flow.[111]

The sympathetic activation can turn into sympathetic overstimulation[114] and be a factor in myocardial damage and arrhythmogenesis[115,116] during severe hemorrhage. The mechanisms by which sympathetic overstimulation produce cardiac arrhythmias are: increased myocardial automaticity, ectopic pacemaker activity, enhancement of reentrant activity, induction of vagal reflexes and increased cardiac work and oxygen demands.[117]

The myocardium is sensitive to tissue dysoxia and hemorrhage predisposes to myocardial injury due coronary disregulation,[118]acidosis, expression of myocardial depressing factor or formation of inflammatory mediators.[119] Myocardial hypoxia occurs when the decrease in coronary perfusion pressure cannot be balanced by a decrease in coronary vascular resistance.[119,120] Coronary autoregulation is the capacity of the heart to maintain coronary flow relatively constant in response to variations in perfusion pressure. Myocardial flow remains constant during variations of MAP within a range of 70 to 180 mm Hg.[118] Coronary autoregulation responds different to hypotension depending on the mechanism involved and this response has been

shown to the poor during hemorrhage-induced hypotension when compared to arrhythmia-induced hypotension.[118]

Although necessary for recovery after a decrease, or lack, of perfusion the process of reperfusion may be pathologic by causing life-threatening arrhythmias and myocardial stunning.[69, 121] Interestingly, in some models of hemorrhagic shock, blood resuscitation caused a more exaggerated depression of myocardial function than the hemorrhage itself. Possibly due to the provision of neutrophils, which are activated by reperfusion.[69]

The myocardial injury consequence of hypoxic or reperfusion injury after hemorrhage is one of the compounding factors of the complex pathophysiology and clinical presentation of horses with hemorrhage. Arrhythmias and myocardial injury have been reported in the equine literature[e,122] and can have serious clinical consequences. It is our clinical impression, and one of the motivations for this study, that myocardial injury and arrhythmia development are under-recognized in equine medicine.

4. Hypertensive cardiomyopathy

4.1 Background of hypertensive heart disease in humans

Hypertensive heart disease (HHD) is the group of cardiac abnormalities that represent the

accumulation of functional and structural adaptations to increased blood pressure.[123] Elevation of blood pressure leads to changes in the myocardium, coronary vasculature and conduction system such as left ventricular hypertrophy (with potential late dilation) and fibrosis, atherosclerosis and systolic and diastolic dysfunction. These manifest as myocardial infarction, cardiac arrhythmias (especially atrial fibrillation and ventricular ectopy), congestive heart failure or sudden cardiac death. Essential hypertension accounts for 90% of cases of hypertension in human adults and secondary causes of hypertension account for the remaining 10%. Hypertension is estimated to affect one billion people worldwide and HHD is the most common cause of hypertension-associated death. Approximately one quarter of all cases of human heart failure are secondary to hypertension[124] making HHD one of the leading causes of mortality in western societies. The relevance of hypertension as a public health problem cannot be overemphasized.

The pathogenesis of HHD involves the progression from hypertension to left ventricular hypertrophy to diastolic dysfunction and, eventually, to ventricular dilation and cardiac failure.[123] Neuroendocrine activation and possibly insulin resistance are components of this syndrome.[125] The traditional explanation for the development of HHD is that the left ventricular wall thickens in response to elevated afterload.[124] This is likely an oversimplification of the process and neurogenic, humoral, and endocrine factors (mainly the renin-angiotensin-aldosterone system and the sympathetic systems) are thought to play a key role.[125]

Arrhythmias and sudden cardiac death are features of HHD that have been attributed to increased ventricular ectopic activity. Non- homogenous left ventricular hypertrophy, fibrosis, membrane channel alterations and neuroendocrine factors such as sympathetic overload or renin angiotensin

activation[126] increase the risk of reentry, myocardial ischemia, triggered activity and enhanced automaticity setting the stage for arrhythmogenesis. [125,127]

4.2 Hypertension in horses

Systemic hypertension is very rarely cited as a medical concern in equine literature.[53-55] The most commonly reported cause of hypertension in horses is laminitis.[53,54,128,129] Laminitis is regarded as a parallel phenomenon consequence of the same physiopathology that causes the lamellar injury or as a consequence of the foot pain. The pathophysiology of hypertension during laminitis is different depending on the stage and the cause. An increased sympathoadrenal outflow, renin activity and aldosterone concentration caused by pain may be the main factors.[129] Equine metabolic syndrome[54,130] could be a predisposing factor to both laminitis and hypertension. To the best of our knowledge the clinical and echocardiographic features of hypertensive cardiomyopathy (HC) or HHD have not been reported in the equine literature.

5. Atrial fibrillation

5.1. Relevance in equine medicine

Atrial fibrillation is the most common clinically relevant and performance limiting arrhythmia in horses.[131] Its prevalence in racehorses is estimated to be approximately 0.3%.[132] The

pathophysiological mechanism that provides the substrate for atrial fibrillation is reentry. Focal areas of heterogeneity in refractoriness in the atria cause regions of slow conduction or unidirectional block that allow atrial depolarization waves to loop back into the atria, instead of stopping at the AV node, forming a circus (reentry loop). The circus depolarization wave continues for as long as there is excitable tissue (not refractory) where it can propagate. If there is only one large wave of reentry in the atrial tissue turning around in a constant pattern the rhythm is called atrial flutter. Conversely, during atrial fibrillation multiple small chaotic atrial reentry waves occur simultaneously.[3]

Horses are naturally predisposed to atrial fibrillation even in the absence of structural heart disease (lone atrial fibrillation) due to their large atrial mass and their intrinsically high vagal tone. The high vagal tone increases heterogeneity of refractoriness, setting the stage for a higher likelihood of reentry.[3] Risk factors for the development of atrial fibrillation include electrolyte abnormalities (especially hypokalemia and hypomagenesemia), previous episodes of atrial fibrillation, atrial premature contractions or underlying cardiac disease that causes atrial enlargement.[a,132]

In human beings with atrial fibrillation restoration of sinus rhythm, rate control and antithrombotic therapy are the three main potential components of treatment.[133] Antithrombotic therapy is not considered part of atrial fibrillation therapy in horses as thromboembolism is not a described sequela to this arrhythmia in equine species. Horses most often have normal rates while on atrial fibrillation and therefore rate control in less frequently needed when compared with humans or other veterinary species.

The decision to restore sinus rhythm and the treatment method (pharmacological vs. electrical) is multifactorial. Horses do not necessary develope secondary problems to the arrhythmia if this is left untreated. Atrial remodeling that will cause horses with atrial fibrillation of more than three to four months duration to become more susceptible to recurrence after cardioversion may be the only risk in many cases. Many horses will be able to perform low intensity exercise while in atrial fibrillation in the absence of concomitant arrhythmias or severe underlying cardiac disease. Ventricular arrhythmias can however occur in some horses when they are exercised in atrial fibrillation[134] and horses with atrial fibrillation that are to be left untreated should undergo a complete cardiac evaluation that includes an exercising ECG[a]. Horses that participate in sports that require high intensity exercise (racehorses, three day eventers and some high-level jumpers) will not be able to do so while in atrial fibrillation due to the decrease in preload and cardiac output that the absence of atrial contraction implies.

All cardioversion methods have potential adverse effects and the success rate of the therapy ranges from 85-95% in the horse with a short duration of the arrhythmia and without underlying cardiac disease.[131, 135] Factors such as the presence of concomitant arrhythmias, the presence of atrial enlargement or the duration of the arrhythmia can be considered when deciding the cardioversion method.[135] The decision to use pharmacological or electrical cardioversion methods often depends on non medical factors such as the availability of the technology or drugs, the experience of the clinician performing the procedure or economical factors. Quinidine sulfate is the most commonly used drugs for pharmacological cardioversion of atrial fibrillation. Other drugs (amiodorone, bretilium, digoxin etc.) have been used with varying success but the

presence of adverse effects, variable efficacy, availability, cost or familiarity with the drugs make quinidine sulfate the preferred drug for most clinicians.[a] Most recommendations to minimize the risk of atrial fibrillation recurrence are based on human studies or experimental animal models and more information specific to the horse is needed.[a]

5.2. Atrial fibrillation in humans and the thromboembolic risk

Atrial fibrillation is the most common sustained arrhythmia in human beings. One in every four people is expected to develop atrial fibrillation over the course of their lifetime.[133, 136] Lone atrial fibrillation occurs less frequently in humans (30%), when compared to horses, although it is described as a benign occurrence that is more frequent in elite or endurance athletes.[137]

Thromboembolism prevention is one of the main components of the therapy of many humans with atrial fibrillation and some consider evaluation of the risk for stroke the first step after diagnosing a patient with atrial fibrillation.[138] Atrial fibrillation causes a hypercoagulable state[105] that makes stroke and thromboembolism the major cause of morbidity and mortality in patients with this rhythm disturbance.[139, 140] Atrial fibrillation causes approximately one fourth of all the deaths and disabilities associated with stroke.[133]

The pathogenesis of the procoagulant state during atrial fibrillation is multifactorial. It has been proposed that the three legs of the Virchow's triad for thrombogenesis (changes in vessel walls,

blood flow and blood constituents) are altered in the event of atrial fibrillation due to atrial tissue changes, endothelial damage and dysfunction, increased atrial size, decreased atrial motion and inflammation, among other factors.[141, 142] Fibrinolysis and primary hemostasis are also affected during atrial fibrillation and an increase in fibrinolysis had been suggested due to a pathophysiological response to the prothrombotic state. Conflicting results have been reported regarding the importance of platelet activation and fibrinolysis in the thromboembolic risk.[142]

The increased risk of thromboembolism and stroke during atrial fibrillation is not homogeneous and different comorbidities can affect the hypercoagulable state.[140,142] This risk can be estimated using historical, clinical, imaging and laboratory data.[143] Factors such as female gender, the presence of congestive cardiac failure, recent embolism, hypertension, diabetes, underlying heart disease (mainly left-ventricular dysfunction or hypertrophy), permanent atrial fibrillation, duration of the atrial fibrillation, the presence of spontaneous echo contrast in transesophageal echocardiogram,[140, 142, 144] or recent cardioversion[145-148] have been reported to influence the risk for embolism.

Several scoring systems have been developed to try to integrate the different risks factors for thromboembolism. The CHADS2 score is reportedly the most commonly used scoring system.[140] This score is used in the evaluation of humans with atrial fibrillation to determine whether or not treatment with anticoagulants or antiplatelet therapy is needed. The CHADS2 score calculates the risk of thrombogenesis and stroke based on the presence or absence of Congestive heart failure (C), Hypertension (H), Age greater or equal to 75 years (A), Diabetes mellitus (D) and

prior Stroke (S2). The validity of the score has been questioned and the importance of the different factors and the criteria to determine the need for anticoagulation therapy are a constant topic of research and debate. Conflicting results are often found in the literature, possibly due to the differences between study groups or methodologies, and many controversies remain in the field of the antithrombotic therapy in the patient with atrial fibrillation.[140]

The effects of the atrial fibrillation on the results of many clinicopathological tests that evaluate coagulation and fibrinolysis have been studied and support the presence of a hypercoagulable statein the arrhythmic patient and even after sinus rhythm has been susccesfully restored. Some of the laboratorial tests that have been studied are: plasma fibrinogen concentration,[149-]aPTT[149], plasma D-dimer concentration,[153-157] von Willebrand factor,[150-152, 158] tissue-plasminogen activator antigen, tissue plasminogen activator inhibitor, plasmin-antiplasmin complex,[142] markers of platelet activation[142,] prothrombin fragments, thrombin-antithrombin III complex,[159] AT[160, 161] and P selectin.[150,151] Plasma D-dimer concentration appears to be the single most used and potentially the most useful clinicopathological test to assess the risk of thrombogenesis in patients with atrial fibrillation.[153- 157, 162-164]

The frequency of atrial fibrillation and thromboembolic events associated with this arrhythmia is expected to increase in the next decades due to the prolongation of life expectancy and the increase in occurrence of comorbidities such as diabetes, hypertension, obesity or chronic heart disease.[138] Equine atrial fibrillation has been described as an animal model of atrial fibrillation[3] and the comparative aspects of the hypercoagulable state in humans and horses were one of the

motivations for the development of this study.

OBJECTIVES

OBJECTIVES

1- To determine if acute hemorrhage is associated with increased cTnI plasma concentration and/or cardiac arrhythmias.

2- To describe the types and clinical course of arrhythmias present in horses with acute hemorrhage.

3- To determine the ability of classically used clinical or clinicopathological variables to predict an increase in cTnI, the presence of arrhythmias and outcome in horses with acute hemorrhage.

4- To determine the associations of cTnI and cardiac arrhythmias with outcome in horses with acute hemorrhage.

5- To describe the prognosis and clinical, echocardiographic, and pathologic features of HC in horses.

6- To test the hypotheses that a hypercoagulable state is present in horses with atrial fibrillation and that the plasma concentration of D-dimers is the best marker of a procoagulant state in horses with atrial fibrillation.

7- To describe the coagulation profiles of horses with atrial fibrillation and their relationship with the duration of the arrhythmia and the presence of structural heart disease.

STUDIES

STUDIES

To fulfill the objectives of this thesis, the following studies were designed and performed:

1. STUDY #1:

   Navas de Solis C, Dallap-Schaer BL, Boston R, Slack J. Myocardial insult and arrhythmias after acute hemorrhage in horses

2. STUDY #2:

   Navas de Solis C, Slack J, Boston RC, Reef VB. Hypertensive cardiomyopathy in horses: 5 cases (1995-2011). J Am Vet Med Assoc. 2013 Jul 1;243(1):126-30.

3. STUDY # 3:

   Navas de Solis C, Reef BV, Slack J, Jose-Cunilleras E. Evaluation of coagulation and fibrinolysis in horses with atrial fibrillation

STUDY #1

Myocardial insult and arrhythmias after acute hemorrhage in horses

MYOCARDIAL INSULT AND ARRHYTHMIAS AFTER ACUTE HEMORRHAGE IN HORSES

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The data was partially presented at 2010 ACVIM Forum, Anaheim, CA as a poster presentation.

Running title: myocardial insult and arrhythmias after hemorrhage

Keywords: cTnI, continuous ECG, ventricular ectopy, arrhythmia

MYOCARDIAL INSULT AND ARRHYTHMIAS AFTER ACUTE HEMORRHAGE IN HORSES

ABSTRACT

Objective: The objectives of this investigation were to 1) determine if acute hemorrhage is associated with increased cardiac troponin I plasma concentration (cTnI) and/or cardiac arrhythmias, 2) describe the types of arrhythmias and their clinical course in horses with acute hemorrhage, 3) determine the ability of classically used clinical or clinicopathological variables to predict an increase in cTnI, the presence of arrhythmias and outcome 4) determine the associations of cTnI and cardiac arrhythmias with outcome.

Design Prospective study

Setting Large Animal Veterinary Teaching Hospital

Animals: Eleven client-owned adult horses admitted to a referral practice with acute hemorrhage (HG) and four adult horses undergoing controlled blood collection (BDG).

Methods: Serial cTnI and continuous ECGs were obtained from horses with acute hemorrhage and from blood donors. Statistical tests were used to determine associations between the presence of acute hemorrhage in clinical cases, cTnI and cardiac arrhythmias, clinical data commonly used to monitor blood loss [heart rate (HR), PCV, total protein (TP), blood lactate and plasma creatinine concentration)] and outcome.

Results: Cardiac troponin I plasma concentration and ECGs were normal at all time points in the BDG. All horses in the HG had increased cTnI (0.1-29.9 ng/ml). Arrhythmias were detected in eight of these horses. There was an association between acute hemorrhage with increased cTnI ($p=0.004$, $\rho=0.77$), and with the presence of arrhythmias ($p=0.026$, $\rho=0.64$). There was an association between maximal cTnI and presence of arrhythmias ($p=0.005$), treatment for arrhythmia ($p=0.036$), and poor outcome ($p=0.024$).

Conclusions: Acute hemorrhage results in myocardial injury that can be detected by measuring cTnI. Arrhythmias were frequent in hospitalized horses with acute hemorrhage.

ABBREVIATIONS

HG	Hemorrhage group
BDG	Blood donor group
HR	Heart rate
RR	Respiratory rate
Lactate	Blood lactate concentration
CO	Cardiac output
cTnI	Cardiac troponin I plasma concentration
CVP	Central venous pressure
IV	Intravenous
NIBP	Non-invasive blood pressure
Creatinine	Plasma creatinine concentration
TP	Total protein
SVPC	Supraventricular premature complex
VPC	Ventricular premature complex

INTRODUCTION

Myocardial injury secondary to severe hemorrhage, a well-recognized phenomenon in humans,[1-3] is poorly described in horses. An increase in cTnI has been shown to be an early marker of cardiac damage after severe hemorrhage in humans[3] and a marker of myocardial damage in horses independent of the cause.[4-12] However, the effect of hemorrhage on the concentration of this cardiac specific enzyme has not been studied in horses. Cardiac monitoring is not routine practice in hemorrhaging horses and there are few reports of arrhythmias after severe hemorrhage in this species.[a,13] It is our clinical impression that arrhythmias may be more frequent than reported and an under-recognized consequence of hypoxic or reperfusion injury[14,15] to the equine myocardium after hemorrhage.

The specific objectives of this investigation were to 1) determine if acute hemorrhage is associated with increased cTnI and/or cardiac arrhythmias, 2) describe the types of arrhythmias present in horses with acute hemorrhage and their course, 3) determine the ability of classically used clinical or clinicopathological variables to predict an increase in cTnI, the presence of arrhythmias and outcome 4) determine the associations of cTnI and cardiac arrhythmias with outcome.

MATERIAL AND METHODS

Adult horses with acute hemorrhage admitted to a referral practice were studied prospectively. Inclusion criteria in the HG included evidence of internal or external hemorrhage in combination with the presence of any abnormality in clinical or clinicopathological data suggestive of hemodynamic disturbances including: increased HR, RR, lactate[b], creatinine, or decreased PCV, total protein (TP), or non-invasive blood pressure (NIBP).

Cardiac troponin I plasma concentration was determined at the time of diagnosis of the hemorrhage, at 12 and 24 hours after diagnosis, and daily for the first 5 days of hospitalization or until normalized. Cardiac troponin I was measured in heparinized plasma using a two-site chemiluminescent immunoassay with a reportable range of 0-50 μg/L (0-50 ng/ml) and analytical sensitivity of 0.03 μg/L (0.03 ng/ml).[c] The normal range for adult horses was ≤ 0.07 μg/L (0.07 ng/ml) (unpublished laboratory reference range, similar to the range reported for other methods[16]). The time when the maximal cTnI was obtained was recorded.

Continuous ECGs[d] were obtained from all horses in the HG during the first 48 hours after diagnosis and during the last 24 hours of hospitalization. Additional cardiac rhythm monitoring was performed if clinically significant arrhythmias were detected during the initial 48 hours. A follow-up 24-hour continuous ECG was performed if significant arrhythmias were present at the time of discharge. Recordings were considered normal if they consisted of sinus rhythm, sinus arrhythmia, first degree or low grade second degree atrioventricular block and/or there were ≤1

single supraventricular (SVPC) or ventricular (VPC) premature complexes per hour. When assessing rhythm progression, arrythmias were considered to be more severe if there was multifocal vs. unifocal ectopy or there were runs of ectopic beats vs. single ectopic beats. Arrhythmias were divided into simple or complex for statistical analysis. Arrhythmias were defined as complex if therapy or intense monitoring were warranted. Complex arrhythmias included supraventricular tachycardia, multifocal ventricular ectopy and unifocal ventricular tachycardia. Simple arrhythmias included supraventricular premature contractions and ventricular ectopy that did not meet the above-mentioned criteria (single unifocal ectopic beats, couplets, triplets or unifocal idioventricular rhythms).

Variables commonly used to monitor horses with acute hemorrhage i.e. mentation, HR, RR, urination, PCV, TP, lactate, creatinine, NIBP and CVP were obtained from the medical records. Hemorrhage severity was classified (I-IV) according to the amount of blood loss or its physiological consequences following a previously described scale.[17] The clinical management of all horses was at the discretion of the attending clinician. Plasma electrolyte abnormalities at the time the continuous ECGs were performed were obtained from the medical records. Plasma electrolytes were measured in heparinized plasma samples when requested by the attending clinician and using automated analyzers.[e,f] The specific reference range for horses and analyzer were used to determine abnormalities. The laboratory used did not have an internal reference range for ionized magnesium and previously reported reference ranges were used (0.45-0.65 mmol/l) [0.9-1.3 mEq/L]).[18]

A comparative group of adult blood donors (BDG) from which 8-10L of blood was obtained were also evaluated. Continuous ECGs were obtained at the time of blood collection, and again at a time greater than one month from blood collection; the rhythm was recorded for 48 and 24 hours respectively. Cardiac troponin I plasma concentration was measured before blood collection, and 12, 24 and 48 hours afterwards. All blood donors received 10L of intravenous isotonic fluids following blood collection. The pertinent Institutional Animal Care and Use Committee approved the study and owner consent was obtained for animal enrollment.

Statistical analysis Descriptive statistics were calculated for all the variables and results reported as means ±SD and ranges. Medians and interquartile ranges (IQR) were also reported for cTnI results and duration of the arrhythmia. A positive outcome was defined as survival to discharge. Kruskal-Wallis equality-of-populations rank test and Fisher's exact test were used to determine associations between the presence of acute hemorrhage with cTnI and arrhythmias respectively. Associations between cTnI (maximal and at presentation) and arrhythmias (presence of arrhythmias, arrhythmia group and treatment for arrhythmias) was tested using Kruskal-Wallis equality-of-populations rank test. Logistic regression for dichotomous outcomes (presence of arrhythmias, arrhythmia group and treatment for arrhythmia) or linear regression for outcomes described by continuous variables (maximal cTnI and cTnI at presentation) were used to test the associations between these variables and clinical or clinicopathological variables commonly used for monitoring hemorrhaging patients (HR, PCV, TP, lactate and creatinine). Associations between variables were tested at two points: at presentation (in all cases), at maximal values during hospitalization (in the case of HR, lactate and creatinine) and minimal values during hospitalization (for PCV and TP). Non-invasive blood pressure and CVP were

eliminated from the study due to insufficient data. Odds ratios (OR) or Spearman's rho (ρ) were used to quantify significant associations when allowed by the data. The statistical software Stata 11.1®[g] was used for all analyses. A p value of ≤ 0.05 was used to distinguish significant from non-significant associations.

RESULTS

Blood donors were geldings aged 14.5±4.3 years (range 6-22 years) and of weight 586±27 kg (range 547-615 kg). There were two Thoroughbreds, one Standardbred and one Warmblood. Cardiac troponin I plasma concentration was <0.03 μg/L (0.03 ng/ml) at all time points in the BDG. Continuous ECGs showed a predominantly normal sinus rhythm interspersed with occasional periods of sinus arrhythmia and sinus tachycardia. There were no episodes of supraventricular or ventricular ectopy. Maximal HR during blood collection was 56±12/min (range 45-72/min).

Eleven horses met the criteria for inclusion in the HG. There were six mares and five geldings. The mean age was 10.7±5.6 years (range 4-18 years) and the weight 570±72 kg (range 500-725 kg). In 4 cases the weight was estimated. There were nine Thoroughbreds, one Warmblood and one Quarter Horse. All horses were reported to have abnormal mentation (depression) during part of hospitalization. A decrease in urination was not reported in any case. The causes of hemorrhage were periparturient hemorrhage in three horses and in the eight remaining horses, one case each of bleeding during laparotomy, intra-uterine bleeding after cesarean section,

splenic tear, splenic neoplasia, pelvic fracture, neck laceration, guttural pouch mycosis and sinus surgery. The reported duration of the hemorrhage at the time of diagnosis was 7.9±7.2 hours (range 0-24 hours). In the horses with splenic bleeding the duration of hemorrhage was unknown, and therefore the duration of clinical signs was reported instead. Hemorrhage was controlled shortly after diagnosis in two cases of external hemorrhage and no suggestion of repeated bleeding episodes were detected in 4 of the cases of internal hemorrhage. In 5horses repeated hemorrhage was observed (2 cases) or suspected (3 cases). Hemorrhage was classified as class IV in 2 horses, class III in 6 horses, class II in 2 horses and class I in 1 horse.[17] The actual volume of blood loss was known in one horse and the combination of clinical signs was used to determine the hemorrhage class in 10 cases. Six horses received whole blood transfusions (7-10 liters) at the discretion of the attending clinician. Other treatments given during hospitalization to the HG horses were: crystalloid fluids in all cases (variable rate and composition), flunixin meglumine in 7 horses (0.5-1.1 mg/kg IV q 12-24 hrs), phenylbutazone in 2 horses (1.7-3.7 mg/kg PO q 12-24 hrs), gentamicin in 9 horses (6-10 mg/kg IV q 24 hrs), potassium penicillin in 9 horses (20,000-30,000 IU/kg IV q 6 hrs), metronidazole in 4 horses (15-24 mg/kg PO q 8 hrs, chloramphenicol in 2 horses (50 mg/kg PO q 6 hrs), trimethoprim sulfamethoxazole in 1 horse (27 mg/kg PO q 12 hrs), enrofloxacin in 2 horses (7.5 mg/kg IV q 24 hrs), polimyxin B in 4 horses (3,000-4,000 IU/kg IV q 8-12 hrs), aminocaproic acid in 4 horses (20-69 mg/kg IV q 6 hrs), Yunnan Baiyao in 3 horses (approximately 8 mg/kg PO q 6 hrs), furosemide in 1 horse 1 mg/kg IV q 6 hrs, acepromazine in 1 horse (0.016 mg/kg IV q 4 hrs), mannitol in 1 horse (1g/kg IV once), altrenogest in 1 horse 0.044mg/kg PO q 24 hrs, oxytocin in 2 horses (0.02 IU/kg IV q 1hr), domperidone in 1 horse (1.4 mg/kg PO q 24 hrs), sucralfate in 1 horse (20 mg/kg PO q 6 hrs, topical 1% diclofenac cream in 1 horse and vitamin C

in 1 horse (16 mg/kg PO q 24 hrs). Treatments before hospitalization included flunixin in 2 horses (1.1 mg/kg), dypirone in 1 case (unknown dose), aminocaproic acid in 1 horse (40 mg/kg IV once), naloxone in 1 horse (unknown dose) and ceftiofur in 1 horse (2 mg/kg IV once). The details of treatments before hospitalization were not available for 1 horse. Eight horses (72.7%) survived to hospital discharge and 3 (27.3%) were euthanized due to poor prognosis, financial reasons, or a combination of both. Heart rate, RR, PCV, TP, lactate, creatinine, NIBP, and cTnI data are presented in Table 1. Plasma concentrations of sodium, potassium, chloride and calcium were available for 9 horses (6/8 horses with arrhythmias and 3/3 horses without arrhythmias). Electrolyte abnormalities detected during rhythm monitoring were hyponatremia in 4 cases (3/6 horses with arrhythmias and 1/3 without), hypochloremia in 3 cases (3/6 horses with arrhythmias), hypocalcemia in 4 horses (3/6 horses with arrhythmias and 1/3 without). None of the horses were hypokalemic. For the 4 hypocalcemic horses both total and ionized calcium were low in 4 horses and in one horse only total calcium was measured. Ionized magnesium was measured in 6 horses (4 with arrhythmias and 2 without) and hypomagnesemia was detected in 3/4 horses with arrhythmias and 2/2 without). Overall 7/10 horses (4/6 of the horses with arrhythmias and 3/3 of the horses without) had at least one electrolyte abnormality.

The presence of acute hemorrhage was associated with an increased cTnI ($p=0.004$, $\rho=0.77$). All eleven horses with acute hemorrhage had increased cTnI at some point during hospitalization and 7 horses (63.7%) had increased cTnI at the time of diagnosis of the hemorrhage (Table 1). The maximal cTnI was detected at 28.6±32.9 (0-96) hours after diagnosis of hemorrhage.

Acute hemorrhage was associated with the presence of arrhythmias ($p=0.026$, $\rho=0.64$). Arrhythmias were detected in 8 horses (72.7%) enrolled in the HG and in seven cases (63.7%) these arrhythmias were classified as complex. Arrhythmias observed were SVPCs in one case, single multifocal VPCs in three cases, and ventricular ectopy that included runs of ectopic beats in 4 cases. In these latter four cases the arrhythmias were described as: 1- paroxysmal unifocal runs of ventricular ectopy (rate 60/min) accompanied by ectopic ventricular beats from a second focus, 2- sustained unifocal ventricular tachycardia (rate 87/min) accompanied by ectopic ventricular beats from a second focus, 3-paroxysmal multifocal runs of ventricular ectopy with ventricular rates of 80/min and 65/min (for each focus), 4- paroxysmal multifocal runs of ventricular ectopy with ventricular rates of 140/min and 44/min (for each focus). Two horses received antiarrhythmics (3 and 4) and responded to lidocaine (0.5 mg/kg IV bolus and 0.05 mg/kg/min IV at a constant rate infusion) and/or propafenone (2mg/kg PO q 8 hrs). In the other cases with complex arrhythmias treatment for the primary problem was instituted, the rhythm monitored via telemetry and the arrhythmias resolved without antiarrhythmic medication. Two horses had very occasional multifocal VPCs at the time of discharge that were no longer present on 24-hour continuous ECGs performed 6 and 8 months later. In 7 horses of the HG group arrhythmias were detected within the first 24 hours after diagnosis. Arrhythmias were more severe or frequent in the second 24-hour period in all horses in which comparison was possible (n=6). One horse was euthanized during the first 24 hours of hospitalization and therefore comparison was not possible. The three euthanized horses had developed complex arrhythmias and the arrhythmias were not the immediate cause of euthanasia in any case.

There was a higher maximal cTnI in animals with arrhythmias (p=0.005) and in animals treated for arrhythmia (p=0.034). There were no associations between cTnI or arrhythmias with HR or lactate at any time point. Associations and correlations between cTnI and arrhythmias and other clinicopathological variables are shown in Table 2. Maximal cTnI was higher (p=0.024), treatment for arrhythmia more frequent (p=0.037) and minimal TP lower (p=0.014) in non survivors

DISCUSSION

In this group of horses acute hemorrhage was associated with myocardial injury evidenced by increased cTnI. Arrhythmias were frequent in horses treated for acute hemorrhage. The occurrence of arrhythmias was more common [8 horses, (72.7%)] than previously described (10% in a previously reported group of periparturient hemorrhage) [13] and an elevated cTnI was detectable in all cases. The difference in the frequency of arrhythmias could be due to the variation in inclusion criteria between the two studies, but is more likely due to the more intensive cardiac monitoring performed in the present study. The increase in concentration of cardiac troponins is proportional to the extent of myocardial damage in humans with myocardial infarction.[19] The increase in cTnI ranged from very mild to severe in the study group (0.1-29.9 ng/ml) and this different magnitude of elevation perhaps represents varying degrees of myocardial damage in horses with hemorrhage.

We speculate that the cause of the myocardial damage is myocardial hypoxia and/or reperfusion injury. Tissue hypoxia occurs when oxygen delivery is too low to support demands.[17] Oxygen delivery is dependent on stroke volume, HR, hemoglobin concentration, SpO_2, PaO2 and regional tissue perfusion regulation.[17-20] All these factors may be altered in the horse with acute hemorrhage. Sympathetic stimulation is an important component of the physiologic response to hemorrhage[21,22] and is associated with elevated troponin concentration in acute ischemic stroke.[23,24] The autonomic response was likely marked in the hemorrhage group due to the underlying disease, trauma in some cases, comorbidities or stress (due to transport or hospitalization) and possibly a significant contributor to the myocardial injury. Sympathetically mediated activation of myocardial automaticity, ectopic pacemaker activity[25-27] and reperfusion[14,15] can contribute to arrhythmogenesis after hemorrhage. Arrhythmias in this study were more severe or frequent during the 24-48 hour period after hemorrhage diagnosis (when compared to 0-24 hours), supporting the theory that reperfusion injury may be the primary cause of myocardial damage.

Abnormal plasma concentrations of electrolytes are a common cause of arrhythmias and a common consequence of blood loss in horses.[28] Electrolyte abnormalities were frequent in the HG and could have contributed to the arrhythmia development. Electrolyte abnormalities were also frequent in horses in the HG that did not develop arrhythmias and due to the study design it was not possible to effectively study the relationship between electrolyte abnormalities and arrhythmia development. Previous studies have shown a 12-48 hour delay between myocardial insult and cTnI peak concentration, depending on the type of injury and whether there is ongoing damage after the initial insult.[10,15,29-34] In the present study, the time from hemorrhage diagnosis

to detection of the maximal cTnI was highly variable (28.6±32.9, range 0-96 hours) likely due to the variable intervals from onset of hemorrhage to hospital admission, the presence of repeated bleeding episodes in some horses, and the variability in resuscitation efforts.

Associations were found between cTnI, arrhythmias and TP, PCV and creatinine (Table 2). In the present study lactate concentrations (maximal and at presentation) were not associated with myocardial injury. This was unexpected as lactate has been shown to increase early during experimental acute hemorrhage.[22] Clinicians often use blood lactate concentration to guide fluid therapy or trigger blood transfusions and therefore the lack of association between maximal lactate and other variables in this study may reflect a rapid resolution of hyperlactatemia due to successful goal directed therapy in horses in the HG. This lack of association could also be the consequence of timing of lactate measurements or low statistical power of this preliminary study. Creatinine, or the more classic parameters to monitor hemorrhage (PCV and TP) were more informative in the cases reported here. The associations shown between cTnI and creatinine (Table 2) are interesting and perhaps renal and myocardial hypoperfusion/hypoxia are parallel events in the scenario of clinical hemorrhage. Renal failure is a recognized cause of increased cardiac troponins in dogs and humans via incompletely understood mechanisms.[35] One of the horses in the study developed renal failure. However there was no clinical suggestion of renal disease in any of the other ten horses, suggesting that the increase in creatinine and increased cTnI were both consequences of acute hemorrhage and not caused by renal failure.

When analyzing variables at presentation it is interesting that, TP was associated with the development of myocardial injury and the detection of complex arrhythmias (Table 2). There

was a 2.36 fold increase in the odds of developing a complex arrhythmia for every 10 g/L (each g/dL) decrease in the TP at presentation (p=0.049), and a lower TP was associated with a higher maximal cTnI (p=0.041). Maximal cTnI and treatment for arrhythmias were associated with non-survival. The arrhythmias observed in this study were not the primary cause of death, however myocardial damage has been reported to be a marker of disease severity in humans.[36,37] Myocardial injury, and more specifically arrhythmia development, is relevant to the clinical management of critically ill horses. Furthermore, if arrhythmias are undetected in horses and persist beyond discharge from the hospital, they could be a concern for the safety of their riders. In all horses reported herein, the elevated cTnI and cardiac arrhythmias were transient, although persisted up to the time of discharge in two cases. This study was not designed to monitor the long-term effects of acute hemorrhage on myocardial function or the presence of arrhythmias, but suggests such an investigation might be warranted. Other tests such as echocardiograms, exercising ECGs and cardiac histopathology could be necessary to evaluate if the injury has long-term sequelae.

The study group was heterogeneous in severity of hemorrhage, duration, presence of comorbidities and treatment. It is an important limitation of the study that the amount of blood loss in the HG was unknown. Thus the study does not allow for analysis of the isolated effect of hemorrhage and it is likely that some, or all, of the above-mentioned factors play a role in the degree of myocardial injury and development of arrhythmias. Yet the frequent presence of myocardial injury and arrhythmias suggests that horses with acute hemorrhage may benefit from cardiac monitoring including continuous ECGs and cTnI measurements.

In conclusion, horses with hemorrhage secondary to a variety of causes experienced myocardial injury, characterized by elevations in cTnI, and also developed cardiac arrhythmias. Arrhythmias observed included SVPCs, multifocal VPCs and ventricular ectopy that included runs of ectopic beats. In this heterogeneous group a lower TP at the time of hemorrhage diagnosis was associated with detection of complex arrhythmias. A higher maximal creatinine was associated with treatment for arrhythmias and a higher maximal cTnI were associated with detection and treatment of arrhythmias. The TP at presentation and the minimal PCV were associated with a higher maximal cTnI. A lower minimal TP, higher maximal cTnI and treatment for arrhythmia were poor prognostic indicators. Further studies are needed to know whether recognition and treatment of cardiac arrhythmias in horses with severe hemorrhage affects long-term outcome.

FOOTNOTES

a Foreman JF, Navas de Solis C, Benson BM, Tennent Brown BS. Ventricular tachycardia secondary to acute anemia from hemorrhage or hemolysis in horses. J Vet Intern Med 2007 21 (3): 660-661.

b Critical Care Xpress. Stat profile®. Nova Biomedical Corporation, Waltham MA, USA and Accutrend Roche Diagnostics, Mannheim, Germany

c Stratus® CS. Dade Behring Inc., Deerfield, IL, USA

d Spacelabs Healthcare Inc., Issaquah, WA, USA.

e Vitros 350, Ortho Clinical Diagnostics, 100 Indigo Creek Drive, Rochester, NY 14626. USA

f Nova Biomedicals, CCX-1.200 Prospect Street, Waltham, MA 02254-9141. USA

g Stata 9.0, Stata Corp, College Station, TX

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Table 1. Heart rate, RR, NIBP, CVP and clinicopathological variables obtained from horses in the HG (n=11 with exception of RR n=9, NIBP n=5 and CVP n=1). Maximal values (max) are reported for HR, lactate, creatinine and cTnI, minimal (min) for PCV, TP, NIBP and CVP. Values at presentation are also reported. Median and IQR are reported in brackets for cTnI results.

Table 2. Associations between myocardial injury related variables and clinical/clinicopathological variables. The associations reported are positive for creatinine and negative for TP and PCV. Odds ratios (OR) and Spearman's rho (ρ) are reported for data that allowed quantification of the associations. Max= maximal value, min=minimal value, $_0$=value at presentation.

	Max/min Mean±SD	 Range	Presentation Mean±SD	 Range
HR (beats/min)	74±20	40-100	64±24	40-100
RR(breaths/min)*	30±9	15-48	28±12	12-48
PCV (%)	21.9±7.2	12-34	36.5±5.9	28-44
TP (g/L [g/dL])	4.5±1.2 (45±12)	2.8-6.4 (28±64)	5.8±1.1 (58±11)	4-7.7 (40±77)
Lactate (mmol/L)	5.2±3.5	1.4-12.5	4.2±2.7	1.1-8.2
Creatinine (µmol/L [mg/dL])	2.9±3.5 (256.3± 318.2)	1.0-12.2 (88.4± 1,078.5)	2.4±1.6 (212.2 ±141.4)	1.0-6.4 (88.4± 565.8)
cTnI (µg/L=ng/mL)	4.6±9.6 (0.65)	0.1-29.9(0.23-1.34)	2.9±8.9	0.03-29.9

	PCV_{min}	TP_{min}	$Creatinine_{max}$	PCV_0	TP_0	$Creatinine_0$
$CTnI_{max}$	0.008	0.051	0.54	0.94	0.041	0.062
$CTnI_0$	0.17	0.56	<0.001 ρ= 0.81	0.19	0.057	0.001 ρ= 0.54
Presence of arrhythmia	0.25	0.24	0.2	0.33	0.58	0.98
Treatment for arrhythmia	0.12	0.21	0.004 OR= 1.94	0.77	0.9	0.062 OR= 2.21
Complex arrhythmias	0.055, OR= 0.3	0.085	0.56	0.096 OR= 0.82	0.049 OR= 2.36	1

STUDY #2

Hypertensive cardiomyopathy in horses: 5 cases (1995-2011)

HYPERTENSIVE CARDIOMYOPATHY IN HORSES: 5 CASES (1995-2011)

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HYPERTENSIVE CARDIOMYOPATHY IN HORSES: 5 CASES (1995-2011)

ABSTRACT

Objective: To describe the prognosis and clinical, echocardiographic, and pathologic features of hypertensive cardiomyopathy in horses.

Design: Retrospective study.

Animals: 5 horses with cardiac hypertrophy and systemic hypertension.

Procedures: Demographics, history, physical and cardiac examination findings, diagnosis, clinical progression, prognosis, and pathologic findings were obtained from medical records.

Results: The primary diagnosis was chronic laminitis in 3 cases and chronic renal failure in 2. Persistent tachycardia, hypertension, chronic laminitis, or a combination of these prompted the cardiac evaluations. Non-invasive blood pressure values (median [range]) were determined as 190 mmHg (183-261 mmHg) for systolic pressure, 126 mmHg (100-190 mmHg) for diastolic pressure, and 155 mmHg (126-222 mmHg) for mean pressure. No arrhythmias were reported. All horses had increased relative wall thickness, mean wall thickness, and left ventricular mass. The interventricular septum was thickened at end diastole (n=5) and in peak systole (4). The left ventricular internal diameter was small at end diastole (n=4) and in peak systole (3). The left ventricular free wall was thickened at end diastole (n=3) and in peak systole (4). No associations between blood pressure and variables consistent with hypertrophy were detected. All horses were euthanized because of the grave prognosis of the primary diseases. All 3 horses that underwent postmortem evaluation had cardiovascular abnormalities.

Conclusions and Clinical Relevance: Hypertensive cardiomyopathy should be considered as a comorbid diagnosis in horses with laminitis or chronic renal failure. Information about the development, progression, reversibility, importance of early detection, and long-term sequelae of this condition is needed.

ABBREVIATIONS

cTnI	Cardiac troponin I
FS	Fractional shortening
HR	Heart rate
HC	Hypertensive cardiomyopathy
HHD	Hypertensive heart disease
IQR	Interquartile ranges
IVSd	Interventricular septal thickness at end diastole
IVSs	Interventricular septal thickness at peak systole
LVFWd	Left ventricular free wall thickness at end diastole
LVFWs	Left ventricular free wall thickness at peak systole
LVIDd	Left ventricular internal diameter at end diastole
LVIDs	Left ventricular internal diameter at peak systole
LVM	Left ventricular mass
MWT	Mean wall thickness
NIBP	Non-invasive blood pressure
RVd	Right ventricular internal diameter at end diastole
RVs	Right ventricular internal diameter at peak systole

RWT	Relative wall thickness
2D	Two-dimensional

INTRODUCTION

Hypertensive cardiomyopathy is the myocardial component of HHD [1,2] and is characterized by left ventricular hypertrophy and fibrosis. Hypertensive heart disease is the group of functional and structural cardiac abnormalities described in humans and veterinary species as a consequence of the adaptations to increased blood pressure.[a, 3] Hypertensive heart disease involves mechanical, neurohormonal, and cytokine alterations[1] that, in human patients, can lead to diastolic and systolic dysfunction, arrhythmias, thrombotic and ischemic episodes, coronary and small vessel disease, heart failure, or sudden cardiac death.[1,4,5]

Left ventricular hypertrophy (increase in size of the left ventricle) can be a physiologic adaptation to multiple stimuli. However, there is a relationship between the increase in left ventricular mass and the presence of cardiovascular disease with no clear distinction between physiologic and pathologic hypertrophy.[2] The most commonly described cause of cardiac hypertrophy in horses is the adaptation to intense training.[6,7] In humans and cats,[8] HCM is a common cause of concentric left ventricular hypertrophy (increased wall thickness without ventricular dilation). However, to the best of our knowledge, this genetic disease has not been described in horses. Volume depletion has been found in multiple species, including horses, to cause pseudohypertrophy, an echocardiographic pattern that mimics concentric hypertrophy[9,10] and in which the LVM remains within reference range. Equine valvular disease or primary cardiomyopathies[11] most frequently cause eccentric hypertrophy (increase in size with dilation of

the affected chamber as opposed to increase in the wall thickness), easily distinguishable echocardiographically from the pattern seen in HC, HCM, or pseudohypertrophy.[5] Left ventricular hypertrophy secondary to systemic hypertension has been detected at postmortem examination in ponies with laminitis,[13] but the clinical findings and echocardiographic findings of HHD in horses have not been described. Chronic renal disease is a common cause of HHD and myocardial hypertrophy in many species, [a,13,14] however it has not been reported in horses in peer-reviewed literature.

The purpose of the study reported here was to describe the demographics, history, clinical findings, echocardiographic appearance, prognosis, and pathologic findings associated with left ventricular hypertrophy and hypertension in horses. The hypothesis was that myocardial hypertrophy occurs in hypertensive horses associated with chronic renal failure and chronic pain, and that horses with laminitis are overrepresented.

MATERIALS AND METHODS

Case selection

Records of horses presented to a referral practice for cardiac evaluation between 1995 and 2011 with systemic hypertension (systolic and mean non-invasive blood pressure (NIBP) > 144 and 116 mmHg respectively)[15] and echocardiographically evident left ventricular hypertrophy were studied retrospectively.

Medical records review

Information retrieved included demographic variables (age, sex, breed and weight), primary diagnosis, reason for cardiac evaluation, maximal NIBP (mean of 3 consecutive measurements of systolic, diastolic, and mean blood pressures), HR at the time of cardiac examination, presence of arrhythmias, cTnI, presence of clinical dehydration, fluid therapy administration, standard echocardiographic variables measured via transthoracic 2D and M-mode echocardiography, outcome, necropsy results and cardiovascular histopathologic findings. M-mode echocardiographic variables were obtained from standard right parasternal short axis views of the left ventricle under the chordal attachments and included LVIDd, LVIDs, IVSd, IVSs, LVFWd and LVFWs. Two-dimensional measurements included pulmonary artery diameter and aortic root diameter from right parasternal windows and left atrial size from a standard left parasternal window. The mean of the measurements available in the records (multiple measurements were routinely obtained for each variable) was used in the analysis. The presence of substantial regurgitant jets detected with color flow Doppler echocardiography was also recorded. Calculated values included FS, MWT, RWT and LVM obtained from the following formulas.[6]

$$FS = (LVIDd - LVIDs) / LVIDd$$

$$MWT = (LVFWd + IVSd) / 2$$

$$RWT = (LVFWd + IVSd) / LVIDd$$

$$LVM = 1.04 [(LVIDd + LVFWd + IVSd)^3 - LVIDd^3] - 13.6$$

The raw echocardiographic variables were compared to the most appropriate reference range for each individual on the basis of breed and size.[16,17] The calculated values were compared to the reference values reported in a population of horses of mixed breeds and not in training.[10] Descriptive statistics were calculated for age, NIBP, HR, and the echocardiographic variables as described. Results were reported as medians, IQR, and ranges. Associations between blood pressure (systolic and mean) and each echocardiographic variable indicative of cardiac hypertrophy (IVS, LVID, FW, MWT, RWT and LVM) were studied by use of Spearman rho analysis. Statistical software[b] was used for analyses. Values of $P<0.05$ were considered significant.

RESULTS

Five horses met the inclusion criteria, which was 0.26% of horses evaluated for cardiac disease during the study period. There were 3 geldings and 2 mares (3 Thoroughbreds, 1 Thoroughbred cross, and 1 Paso Fino) with a median age of 18 years (IQR, 3 years; range, 13-24 years). Median weight was 386 kg (IQR, 127 kg; range, 327-613 kg). The primary diagnosis was chronic laminitis in 3 cases and chronic renal failure in 2. Persistent tachycardia, hypertension, chronic laminitis, or a combination of these prompted the cardiac evaluations. No horse was clinically dehydrated when echocardiography was performed. Simultaneous ECGs were performed in all horses during the echocardiographic examinations. No arrhythmias were reported; however, continuous or 12-lead ECGs were not performed. Cardiac troponin I plasma

concentration was measured in 1 horse and was high (0.83 ng/mL [reference range, <0.07 ng/mL]). Cardiac hypertrophy was detected in all horses (Figure 1). All horses were euthanized because of the grave prognosis of their primary disease. All 3 horses that underwent necropsy had macroscopic cardiac hypertrophy and 2 had microscopic cardiovascular changes (arteriosclerosis, intimal degeneration, and cardiac lymphagiectasia were found in 1 horse each).

Descriptive statistics of NIBP, HR, and echocardiographic variables were summarized (Table 1). When echocardiographic values for individual horses were compared with appropriate reference ranges, all horses had increased RWT, MWT and LVM. The interventricular septum was thickened at end diastole (n=5) and in peak systole (n=4). The left ventricular internal diameter was small at end diastole (n=4) and in peak systole (n=3). The left ventricular free wall was thickened at end diastole (n=3) and in peak systole (n=4). The remaining echocardiographic measurements were within reference range. Substantial regurgitant jets were not detected in any horse. No significant associations between NIBP measurements (systolic and mean) and each echocardiographic variable indicative of cardiac hypertrophy (IVS, LVID, FW, MWT, RWT and LVM) were detected.

DISCUSSION

Hypertensive cardiomyopathy is uncommon in horses and was uniformly associated with chronic renal failure or chronic laminitis. Renal hypertension is thought to be caused by volume overload, excessive activation of the renin angiotensin aldosterone system, and anemia.[14] The

relationship between hypertension and laminitis in horses has been known for decades.[18,19] The pathophysiology of laminitis-induced hypertension may differ depending on the stage of the laminitis and the mechanism of the lamellar damage. Systemic hypertension has been proposed to occur during the development of laminitis[18] or be the response to hemodynamic changes during acute laminitis.[19] In the acute phases of laminitis, hypertension has been explained by increased sympathoadrenal outflow, renin activity, and aldosterone concentration caused by pain, dehydration and electrolyte changes.[18] The mild hypertension seen in horses during the pre-laminitic stages of equine metabolic syndrome may be caused by endothelial cell dysfunction.[20] The hemodynamic changes associated with chronic pain and sympathetic system over-stimulation may be the main factors in the hypertension seen in chronic laminitis.

Left ventricular hypertrophy is an adaptation or maladaptation to systemic hypertension easily recognizable in 2D echocardiograms[5] when the myocardial hypertrophy is moderate or severe, as seen in the horses reported here. Myocardial hypertrophy and diastolic dysfunction are the main echocardiographic changes reported in other species with HHD.[a,5] Pressure overload does not appear to be the only mechanism that causes ventricular hypertrophy during hypertension. Renin angiotensin aldosterone system activation, insulin resistance, and catecholamine release play a direct role in the development of hypertensive myocardial hypertrophy in humans.[2] The cardiac response to hypertension in humans has high individual variability and has been described as proportionate to the 'area under the lifetime blood pressure curve'.[3] The degree of left ventricular hypertrophy is not associated with blood pressure measurements in human beings with HHD.[2] Therefore it is not surprising that no significant associations were found between the degree of left ventricular hypertrophy and the severity of hypertension in the horses reported here.

Myocardial fibrosis, a common finding in humans with hypertension,[1] was not found echocardiographically or via postmortem examination of any of the horses reported here. Fibrosis could have been missed because of the retrospective nature of the study and the low sensitivity for detecting fibrous tissue on equine echocardiograms or routine necropsies. Subtle myocardial hypertrophy, fibrosis, or dysfunction occur early during HHD and can be detected by use of more sophisticated imaging techniques such as tissue Doppler imaging, 2D speckle tracking, echocardiogragraphy with integrated backscatter,[5] cardiac MRI, molecular imaging,[1] or measurement of pro-collagen derived propeptides.[1] Of all these tests, only tissue Doppler imaging and 2D speckle tracking have been used in horses.[21] Although potentially useful to detect diastolic dysfunction and subtle early changes associated with HHD in horses, its clinical usefulness in horses with HC is unknown.

In the group of horses reported here, the primary disease was cause for euthanasia in all instances suggesting that HC is associated with severe underlying disease. Because of the lack of long term survivors, the sequelae, long term complications, or reversibility of HC in horses could not be determined. Arrhythmias and sudden cardiac death are frequent complications of HHD in several species.[1,2,4,13] Ventricular hypertrophy, fibrosis, increased myocardial oxygen demand, fluctuations in arterial pressure, and impaired coronary perfusion[22] are the proposed arrhythmogenic factors. In the horses reported here, arrhythmias were not recognized, however auscultation and simultaneous electrocardiography are unlikely to detect sporadic arrhythmias. Plasma cardiac troponin I concentration was measured in only 1 horse and was high. Cardiac troponin I is frequently within reference range or mildly increased in other species during

HHD.[a,23] It was not possible to draw conclusions in regards to the presence of myocardial injury in these horses with HC because of the paucity of data available. Continuous electrocardiography and cardiac troponin measurements should be obtained prospectively in a larger group of horses with HC, chronic laminitis, and chronic renal failure to assess the clinical importance of myocardial injury and arrhythmias in HC in horses.

Some degree of overlap in cardiac measurements and echocardiographic appearance exists between humans with an athlete's heart and those with diseases that cause myocardial hypertrophy such as HHD or HCM.[24] Comparison of the data in the present case series with that collected from equine athletes in other studies[6, 7] suggests that confusion caused by such overlap would be uncommon in horses. Relative wall thickness appears to be uniformly increased in horses with HC and normal in equine athletes. Equine athletes develop a more proportionate left ventricular dilation with respect to the myocardial hypertrophy and therefore RWT remains normal. Human athletes engaged in disciplines that require isometric exercises can potentially develop patterns of hypertrophy that resemble HHD[25]. Studies in populations different than Thoroughbred and Standardbred racehorses would be necessary to determine whether other types of equine athletes develop different patterns of hypertrophy. However, the classic belief that the type of sport determines the pattern of hypertrophy has been challenged[25] and it seems unlikely that any common equestrian discipline mimics the isometric exercises that cause severe concentric hypertrophy in humans.

The echocardiographic appearance of the left ventricle of volume-depleted animals can resemble that of left ventricular hypertrophy.[9,10] The clinical findings and blood pressure measurements of

horses with HC should allow their differentiation from those with pseudohypertrophy. The LVM was increased in horses with HC that had true hypertrophy (vs. pseudohypertrophy) and the values of RWT and MWT were higher than those reported in experimentally hypohydrated horses. Moreover, the systolic left ventricular measurements, which were altered in 4 horses in this series but within reference range in volume-depleted horses,[10] would also help in this differentiation. The data reported here suggest that systemic hypertension causes echocardiographically evident left ventricular hypertrophy in horses that can be differentiated from pseudohypertrophy by use of the calculated values of LVM or by detection of increased wall thicknesses during systole in horses in which the clinical appearance is not definitive. Establishing reference ranges would require the study of populations of more homogeneous groups divided by breed, body weight, use, and training status.

There were several limitations of the study because of the retrospective nature of this case series, the small number of cases, and the lack of long term follow up. Information about the development, progression, reversibility, importance of early detection, and long term sequelae of HC could not be obtained. Prospective study of horses with systemic hypertension at risk of developing HHD would be necessary to accomplish this. Blood pressure recordings were obtained from the medical records. These measurements were routinely obtained from the middle coccygeal artery by use of an automatic oscillometric monitor. The cuff-width-to-tail circumference ratio was not controlled and the recordings were not corrected for vertical distance from the tail to the heart base. Therefore the absolute values of the NIBP recordings should be interpreted with caution. However, the blood pressure values obtained were markedly increased, compared with the reported reference range, and horses were classified as being hypertensive on

the basis of ranges established from a large population of horses by use of an analogous method, [15] making the information clinically useful. The echocardiographic measurements were obtained by different echocardiographers, which introduces some inter-observer variability. In some cases additional measurements would have been needed to counteract intra-observer variability but could not be obtained because of the signs of pain observed during the examination. Although the formula used to calculate LVM has been reported to have low repeatability in horses[26], it has been used in equine studies to assess development of hypertrophy in response to training[6] and has been correlated to postmortem measurements[27] and oxygen consumption[28]. The accuracy of this formula for use in horses with heart disease is uncertain and therefore, the results of the LVM calculations in horses with clinical HHD should be interpreted with caution.

It may be that horses develop HC less frequently than other species because of the less common occurrence of hypertension. Conversely, limited awareness of the potential presence of cardiac disease in hypertensive horses may cause it to be under recognized. Because of the poor outcome of horses in this study and the association of HHD in other species with comorbidities that could affect horses' health and riders' safety, clinician attention to the possibility of horses developing this condition is warranted. Monitoring horses with or at risk of developing hypertension (e.g., horses with laminitis, signs of chronic pain, chronic renal failure, or metabolic syndrome) may help determine the clinical relevance of this equine cardiomyopathy and identify interventions that could assist in the management of such cases.

FOOTNOTES

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Table 1. Descriptive statistics of echocardiographic and other variables in 5 horses with HC. NIBP value is the maximal mean value of 3 consecutive measurements obtained during hospitalization. Syst = Systolic. Dias = Diastolic. 0 = Value at the time of diagnosis. PA = Pulmonary artery diameter obtained from a standard right ventricular outflow tract view from a right parasternal window. Ao = Aortic root diameter obtained in a standard left ventricular outflow tract view from a right parasternal window. LA = Left atrial size obtained from a left parasternal window.

Figure 1. Echocardiographic images of the heart of horse with HC. Notice the small left ventricular internal diameter and increased thickness of the interventricular septum and free wall of the left ventricle in diastole and systole. 10, 20, 30 = Centimeter scale.

A- Two-dimensional image of the left ventricle at the level of the papillary muscles at end diastole. The ECG appears on the bottom of the figure..

B- Two-dimensional image of the left ventricle at the level of the papillary muscles at end diastole.

C- M-mode image of the left ventricle at the level of the papillary muscles. Notice the small left ventricular internal diameter and increased thickness of the interventricular septum and free wall of the left ventricle in diastole and systole.

Variable	**Median**	**IQR**	**Min**	**Max**	**Reference range**
Mean NIBP (mmHg)	155	60	126	222	88±14[15]
Syst NIBP (mmHg)	190	55	182	261	112±16[15]
Dias NIBP (mmHg)	126	80	100	190	77±14[15]
Mean $NIBP_0$ (mmHg)	135	51	101	206	88±14[15]
Syst $NIBP_0$ (mmHg)	175	58	132	241	112±161[15]
Dias $NIBP_0$ (mmHg)	135	71	101	180	77±14[15]
HR_0 (beats/min)	64	40	36	90	-
IVSd (cm)	4.7	0.5	3.8	5	2.85±0.27[16] or 2.4±0.2[17]
IVSs (cm)	5.8	0.6	4.3	6.4	4.21±0.46[16] or 3.8±0.5[17]

LVIDd (cm)	8.7	0.8	7.6	10.4	11.92±0.76[16] or 8.9±1.4[17]
LVIDs (cm)	4.9	0.7	3.4	6.9	7.45±0.61[16] or 5.9±0.9[17]
FWd (cm)	4.2	2.2	2.1	5.7	2.32±0.38[16] or 2.2±0.5[17]
FWs (cm)	5.4	0.6	4.4	5.8	3.85±0.41[16] or 2.7±0.8[17]
PA (cm)	7.1	0.5	6.3	7.2	-
Ao (cm)	8.7	1	7.2	9	8.72±0.50[16]
LA (cm)	11.1	0.7	10.8	12.6	12.87±0.78[16]
FS (%)	40.8	8.5	33.6	60.9	37.42±3.86[16]
RWT	0.97	0.46	0.57	1.26	0.47±0.04[10]
MWT (cm)	4.2	1.1	2.96	5.35	2.71±0.32[10]
LVM (kg)	4.387	0.935	3.359	6.722	3530±950[10]

Figure 1A

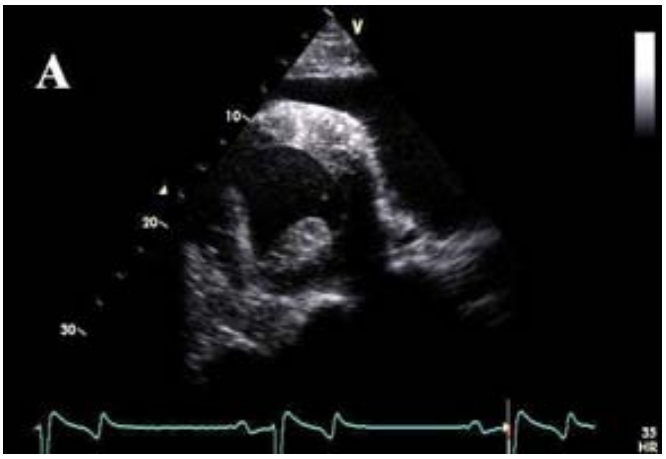


Figure 1B

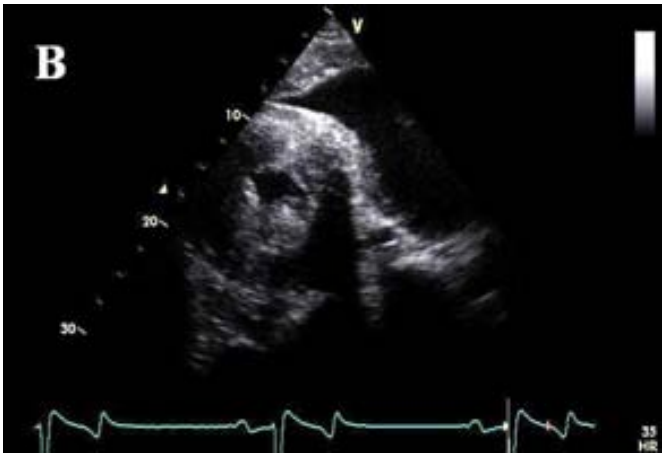
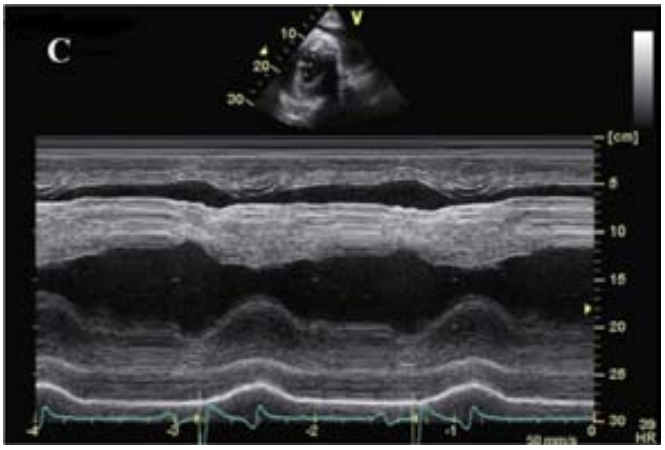


Figure 1C



STUDY #3

Evaluation of coagulation and fibrinolysis in horses with atrial fibrillation

EVALUATION OF COAGULATION AND FIBRINOLYSIS IN HORSES WITH ATRIAL FIBRILLATION

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Running title: coagulation and fibrinolysis atrial fibrillation

Keywords: coagulation, D-dimers, atrial fibrillation

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EVALUATION OF COAGULATION AND FIBRINOLYSIS IN HORSES WITH ATRIAL FIBRILLATION

ABSTRACT

Reasons for performing study: Atrial fibrillation is the most common clinically relevant and performance limiting arrhythmia in horses. Humans in atrial fibrillation are in a hypercoagulable state that makes stroke and thromboembolism (TE) the major cause of morbidity and mortality in patients with this rhythm disturbance. The presence of a hypercoagulable state in horses with atrial fibrillation has not been tested.

Objectives: Test the hypotheses that a hypercoagulable state is present in horses with atrial fibrillation and that the plasma concentration of D-dimers would be the best marker of a procoagulant state in horses with atrial fibrillation. An additional objective was to describe the coagulation profiles of horses with atrial fibrillation and their relation with the duration of the arrhythmia and the presence of structural heart disease.

Results: No clinical signs of hypercoagulation or TE were detected in the study group. Antithrombin activity was different between horses in the atrial fibrillation group (AFG) and control (CG) groups ($p=0.008$) and no significant differences in fibrinogen, D-dimer, prothrombin time and activated partial thromboplastin time were detected. The proportion of horses with abnormal D-dimer concentrations ($p=0.047$), abnormal coagulation profiles ($p=0.018$) and the proportion of abnormal coagulation tests ($p=0.015$) were larger in the AFG than in the CG.

Conclusions and Potential relevance: Horses in atrial fibrillation do not appear to have a clinically evident hypercoagulable state but the presence of subclinical hypercoagulation was proven. This should be borne in mind when assessing horses in atrial fibrillation.

ABBREVIATIONS

TE	Thromboembolic
aPTT	Activated partial thromboplastin time
vWf	von Willebrand factor
AT	Antithrombin activity
AFG	Atrial fibrillation group
CG	Control Group
PT	Prothrombin time
IQR	Interquartile ranges

INTRODUCTION

Atrial fibrillation is the most common clinically relevant and performance limiting arrhythmia in horses.[1] Atrial fibrillation is also the most common sustained arrhythmia in human beings.[2] Humans in atrial fibrillation are in a hypercoagulable state[3] that makes stroke and thromboembolism (TE) the major cause of morbidity and mortality in patients with this rhythm disturbance.[4 5]

The pathogenesis of the procoagulant state during atrial fibrillation is multifactorial. It has been proposed that the three legs of the Virchow's triad for thrombogenesis (changes in vessel walls, blood flow and blood constituents) are altered due to atrial tissue changes, endothelial damage and dysfunction, increased atrial size, decreased atrial motion and inflammation, among other factors.[6,7] Atrial fibrillation is reported to increase the risk of stroke and TE by 5-fold in humans but the increased risk is not homogeneous and several comorbidities can affect the hypercoagulable state.[5, 6] The risk for TE can be estimated using historical, clinical and laboratory data.[7] Historical and clinical factors such as female gender, the presence of congestive cardiac failure, recent embolism, hypertension, diabetes, underlying heart disease (left-ventricular dysfunction or hypertrophy), permanent atrial fibrillation, duration of atrial fibrillation, the presence of spontaneous echo contrast in transesophageal echocardiogram,[5,6,9] or recent cardioversion[10-13] have been reported to influence the risk for TE. The importance of different factors, the predictive ability of scoring systems and the criteria to determine the need

for anticoagulation therapy, have been thoroughly studied in humans; however conflicting results are often found in the literature, possibly due to the differences between study groups or methodologies, and many controversies remain.[5]

Several clinicopathological tests support a hypercoagulable state during and after atrial fibrillation in humans. Some of the laboratorial tests that have been studied are: plasma fibrinogen concentration,[14-17] activated partial thromboplastin time (aPTT),[17] plasma D-dimer concentration,[18-22] von Willebrand factor,[14-16, 23] tissue-plaminogen activator antigen, tissue plasminogen activator inhibitor, plasmin-antiplasmin complex,[6] markers of platelet activation,[6] prothrombin fragments, thrombin-antithrombin III complex,[24] antithrombin (AT)[25,26] and P selectin.[15, 16] Plasma D-dimer concentration appears to be the single most used clinicopathological test to assess the risk of thrombogenesis in patients with atrial fibrillation.[18-22, 27-29]

Thromboembolic events have been diagnosed in horses with severe systemic disease[30-32] but not in horses with atrial fibrillation. Plasma D-dimer concentration is a sensitive marker of hypercoagulation in horses and is associated with different diagnoses and prognoses.[33-34] The hypotheses tested in this study were that a hypercoagulable state is present in horses with atrial fibrillation and that the D-dimers plasma concentration would be the best marker of a procoagulant state in horses with atrial fibrillation. A secondary goal of the study was to describe the coagulation profiles of horses with atrial fibrillation and their relationship to the duration of the arrhythmia and the presence of structural heart disease.

MATERIALS AND METHODS

To test these hypotheses a prospective controlled study was design to compare horses in atrial fibrillation (AFG) to a control group (CG) of horses without cardiovascular or systemic disease. Data from common laboratory tests to assess coagulation and fibrinolysis, as well as historical and clinical variables reported to be associated with a hypercoagulable state in patients with atrial fibrillation in other species were recorded. The AFG was composed of horses 2 years of age and older presented to a Veterinary Teaching Hospital for evaluation of atrial fibrillation or horses that presented to the same facility for evaluation of other problems and in which this arrhythmia was found coincidentally. The CG was composed of horses 2 years of age and older that presented to a Veterinary Teaching Hospital for evaluation of a problem that did not affect systemic health (musculoskeletal, upper respiratory or standardized treadmill evaluation) and in which signs suggestive of systemic disease, cardiovascular disease, systemic inflammation or coagulopathy were not found in the history or general physical examination. All horses in the AFG had a cardiovascular evaluation following hospital standards, that consisted of a physical examination, a 12 lead electrocardiogram (ECG) and echocardiogram. The echocardiogram included B-mode and M-mode studies, color flow evaluation and continuous wave Doppler evaluation of significant regurgitant jets or shunts when present. Horse that were cardioverted had a second echocardiogram performed following hospital protocols. In this second echocardiogram, tissue Doppler measurements of the left atrial wall velocity were also performed. A general physical examination was performed in horses in the CG and all had normal cardiac auscultation.

Historical, signalment and clinical variables recorded on each horse included: age, breed, sex, suspected duration of the arrhythmia, presence of underlying cardiac disease (versus lone atrial fibrillation), pharmacological versus electrical cardioversion (if applicable) or signs of TE (peripheral thrombosis, dyspnea, cough, epistaxis, abdominal pain, neurologic signs or post-mortem findings when available). Underlying cardiac disease was defined as the presence of a cardiac abnormality reported to predispose horses to the development of atrial fibrillation such as valvular or congenital heart disease causing atrial enlargement or areas of abnormal myocardial echogenicity. The suspected duration of the arrhythmia was defined as the time from a sudden decrease in performance or time to the last successful performance to presentation. If this did not exist (sport horses) the last normal cardiac auscultation was used to determine the suspected duration of the arrhythmia. The duration was classified as short if suspected to be less or equal than 2 weeks and long if suspected to be more than 2 weeks.

Blood was obtained by direct jugular venipuncture and placed in blood collection tubes containing sodium citrate. The plasma was separated shortly after collection and stored at -80ºF until the time of analysis. Fibrinogen plasma concentration, D-dimer plasma concentration, prothrombin time (PT), aPTT and AT were measured using a compact hemostasis testing equipment[a] and commercial reagents and controls. Fibrinogen and PT were measured using a nephlometry method.[b] D-Dimers were measured using an automated latex enhanced immunoassay.[c] Activated partial thromboplastin time was measured by adding a contact activator and calcium to a citrated plasma and using the previously mentioned automated hemostasis equipment.[d] Antithrombin was measured using an automated chromogenic assay.[e]

Reference ranges used were those established by the laboratory for healthy horses (Table 1). Activated coagulation was defined as the presence of ≥1 of the following: abnormal fibrinogen or D-dimers, longer PT or aPTT or decreased AT and DIC as the presence of ≥3 abnormal results in the coagulation profiles as previously described.[33]

Descriptive statistics were calculated for all continuous variables. Results were reported as median and interquartile ranges (IQR). Laboratory variables were treated as non-parametric due to the sample size and variability. The results of the different laboratorial tests in horses with and without atrial fibrillation were compared using a Wilcoxon (Mann-Whitney) rank-sum test. The proportion of horses with abnormal D-dimer concentration, abnormal coagulation profiles and abnormal coagulation parameters in the AFG and CG were compared using a Chi-Square test. Results of laboratory tests in horses with underlying cardiac disease versus lone atrial fibrillation; in horses with short versus long duration of the arrhythmia and before and after cardioversion were described but formal statistics were not performed due to the small group size. The statistical software SPSS 15.0 statistics package was used for the analysis. A p value of 0.05 was used to distinguish significant from non-significant associations.

RESULTS

Samples from 42 horses, 25 in the AFG and 17 in CG were obtained. Horses in the AFG group were of age [median (IQR)] 10 (5-14) years. There were 9 Standardbreds, 5 Thoroughbreds, 4 Warmbloods, 3 draft horses, 1 Arabian, 1 Tennessee Walking Horse, 1 Quarter Horse and 1

Warmblood-Standardbred cross. There were 3 stallions/colts, 17 geldings and 5 mares/fillies. None of the horses had clinical signs of TE in their histories, evaluation or during hospitalization. Seven horses in the AFG had underlying heart disease and 18 did not. The underlying heart disease was described as: moderate or severe valvular disease in 4 cases, focal abnormal myocardial echogenicity in 1 case, congenital heart disease (Tetralogy of Fallot) in 1 case and myocardial hypertrophy and pulmonary hypertension 1 case. Cardioversion was attempted in 16 horses and was successful in 15. Two horses were converted using transvenous electrical cardioversion and 13 using quinidine sulfate via a nasogastric tube. One horse failed to respond to both electrical and pharmacological cardioversion. In the 9 horses where cardioversion was not attempted this was due to the presence of underlying heart disease, owners' concerns about cost of the procedure, risks associated with cardioversion or concerns about recurrence. Twelve horses were classified as having a short duration and 13 horses were classified as having a suspected long duration of the arrhythmia.

Horses in the CG were of age [median (IQR)] 6 (3-11) years. There were 5 Standardbreds, 5 Thoroughbreds, and 7 Warmbloods. Three were stallions/colts, 12 geldings and 2 were mares/fillies. Thirteen horses presented for elective musculoskeletal procedures, 4 for upper airway evaluation and 1 for a standardized treadmill evaluation due to poor performance.

Summary statistics for the laboratory tests are summarized in Table 1. In the AFG 4 individual results were not obtained due to laboratory problems with samples or reagents. Antithrombin was different between horses in the AFG and CG ($p=0.008$) and no significant differences in fibrinogen, D-dimer, PT and aPTT were found between groups. The distribution of horses with

abnormal laboratorial tests results, coagulation profiles (≥1 abnormal result in the profile) and D-dimer plasma concentration is listed in Table 2. The proportion of horses with abnormal D-dimer concentrations (p=0.047), with abnormal coagulation profiles (p=0.018) and the proportion of abnormal coagulation tests (p=0.015) were larger in the AFG than in the CG. Two horses in the AFG and no horses in the CG were classified as having DIC.

DISCUSSION

Results suggest that there is subclinical hypercoagulation in horses with atrial fibrillation. The results of this study also suggest that the hypercoagulable state is subclinical. No clinical signs of hypercoagulation or TE were detected in the study group and to the best of our knowledge these have not been reported in the literature. The AT activity was lower (p=0.008) and the proportion of horses with abnormal laboratory tests (p=0.015), abnormal coagulation profiles (p=0.018) and high D-dimers was larger in the AFG. Therefore we consider the hypothesis that a hypercoagulable state is present in horses with atrial fibrillation proven. The proportion of horses with abnormal D-dimers (40%) was higher (p=0.047) in the AFG than in the CG (11.8%). However there was not a statistically or clinically significant difference in results of this marker of fibrinolysis between the AFG and the CG and therefore the hypothesis that plasma D-dimers concentration would be the best marker of a procoagulant state in patients with atrial fibrillation was not proven. The conclusions regarding differences in D-dimer concentrations should be interpreted with caution as the size of the study group coupled with the high variability of the D-dimers test results may have resulted in the study being underpowered. Taking into account the variability obtained, a sample size of 376 horses in each group would have been needed to make

calculations with a power of 80% using a Wilcoxon (Mann-Whitney) rank-sum test with a 0.05 two-sided significance level.

The reason for why the subclinical hypercoagulatory state in horses with atrial fibrillation does not cause clinically relevant thrombogenesis is uncertain. Perhaps the degree of the hypercoagulability is less than in other species and the magnitude of the changes are not sufficient to cause thrombogenesis. The magnitude of the changes in coagulation test results is smaller in the study reported here when compared to humans with cardiac disease in which hypercoagulation has been demonstrated[7, 35-37] or in groups of horses in which a hypercoagulable state was considered clinically relevant.[33,38] In Table 3 results of laboratory tests obtained in studies of horses with hypercoagulation and humans with atrial fibrillation or TE are displayed. When compared to these studies the magnitude (or lack) of changes in the study reported here suggests that hypercoagulability is less likely to be a relevant problem in horses with atrial fibrillation in the absence of concomitant systemic disease. It would be interesting to assess a larger group of horses and horses with atrial fibrillation and comorbidities that would predispose to a hypercoagulable state such as systemic inflammation/sepsis, gastrointestinal disease, post-operative period, neonatal sepsis, or EHV-1 infection.[30-33,38-43] Coagulation and fibrinolysis can also be affected by exercise and it would also be interesting to assess this in exercising horses with atrial fibrillation.[16,44-46]

We chose to study the laboratory tests most often included in the clinical evaluation of coagulation in horses. Prothrombin time measures the extrinsic and common pathways of the traditionally described coagulation cascade while aPTT measures the intrinsic and common

pathways.[47] There were no differences between groups in the results of these classically used coagulation tests. D-dimer is a marker of activated coagulation, fibrinolysis and turnover of cross-linked fibrin associated with a procoagulant state in horses.[33,47] D-dimers is also the most commonly cited test as being useful in monitoring the risk of thromboembolism in humans with atrial fibrillation.[18-22, 27-29] Results for D-dimers measurements were frequently outside the reference range in horses in the AFG (40% versus 11.8% in the CG, p=0.047) but the magnitude of the change was overall small and under the threshold that has been suggested as clinically relevant in horses.[33,47] Antithrombin activity was significantly lower (p=0.008) in the AFG although only low in 2/25 horses. A decrease in AT can be associated with inherited disorders, consumption, endotoxin inhibition, liver dysfunction, or protein loss[47] and is decreased due to consumption in humans with atrial fibrillation.[25,26] The specific cause of the lower AT was not evaluated in the study reported herein but it is plausible that the difference between groups was due to consumption, similar to that reported in humans.

The presence of atrial fibrillation is in and of itself a source of a hypercoagulable state[35] in humans and the presence of structural heart disease[48] or signs of congestive heart failure[9] have been shown to increase the risk of stroke in patients with atrial fibrillation. Other studies have been unable to prove that organic heart disease was associated with abnormalities in D-dimer concentration[35] and for some authors the effect of structural heart disease on the hypercoagulable state remains controversial.[5] In the study we present here 28.6% of the laboratory test results and 28.6% of D-dimers results were abnormal in the group of horses with underlying heart disease vs. 17.4% and 44% (respectively) in horses with lone AF. Only seven horses had underlying

heart disease and the difference between groups was not tested statistically due to the small group size.

The risk for TE is increased in human patients with non-paroxysmal atrial fibrillation and is associated with arrhythmia duration.[9] Furthermore some laboratory markers of a hypercoagulable state are affected by atrial fibrillation duration.[16,18] Interestingly the duration of atrial fibrillation is not a factor in the recommendations to implement antithrombotic therapy in patients with atrial fibrillation[49] and paroxysmal atrial fibrillation is seen more frequently in patients with stroke.[50] In the study we present here 22.9 and 53.8% of the laboratory test results and D-dimers results (respectively) were abnormal in the group with atrial fibrillation of long duration vs. 17.4 and 25% (respectively) in patients with short term atrial fibrillation. Differences between groups were not be tested statistically due to the small size of the group.

The hypercoagulable state and risk of TE remains, and can increase, in humans in the immediate post-cardioversion period (6-72 hours post-cardioversion) due to atrial stunning, mobilization of thrombi in the left atrium and because comorbidities are still affecting the hypercoagulable state.[10-13] Different coagulation parameters may be altered for different periods of time.[10-13,16] There was insufficient data (n=6) to statistically analyze changes before and after cardioversion but no remarkable differences were subjectively appreciated in the six horses in which pre- and post-cardioversion samples were obtained (data not presented).

The study has several limitations. The main limitation is the small size of study group that, coupled with the intrinsic high variability of D-dimers measurements, could make differences to remain unproven. The heterogeneity of the group regarding the presence of underlying heart disease, estimated duration of the arrhythmia in many cases and lack of long term follow up are other limitations.

In conclusion horses in atrial fibrillation present subclinical hypercoagulation that does not appear to have thromboembolic consequences. This should be borne in mind when assessing horses in atrial fibrillation.

Table 1. Coagulation tests results in horses with atrial fibrillation group (AFG) and control group (CG). PT = prothrombin time. aPTT= activated partial thromboplastin time. AT=antithrombin activity. Ref=reference. Results are reported as median (Interquartile Range) for all variables.* indicates differences between groups ($p<0.05$). In the AFG 4 individual results were not obtained due to laboratory problems with samples or reagents.

Table 2. Number of abnormal results for D-dimers, coagulation profiles (≥1 abnormal result) and all coagulation tests in the different diagnostic groups. Results are given as total number and (%). Atrial fibrillation group (AFG) and control group (CG).* denotes different ($p<0.05$) in proportions when compared to the control group.

Table 3. Comparison between coagulation parameters in this study and studies of horses with enteritis, peritonitis (Cesarini et al. 2010,) or neonatal septicemia (Armengou et al. 2008) and groups of people with chronic atrial fibrillation (Kumagai et al. 1990, Lip et al. 1995), non-valvular atrial fibrillation, (Inoue et al. 2004), and people without a history of TE that eventually developed thromboembolic problems (Wannamethee et al. 2012). Results are described in median (IQR) or mean±SD.

FOOTNOTES

a. ACL™ 7000, Instrumentation Laboratory, 180 Hartwell Road Bedford, MA 01730.
b. PT-Fibrinogen- 0009756710. HemosIL™, Instrumentation Laboratory Company- Bedford MA 0730-2443 (USA).
c. D-Dimer- 0020008500. HemosIL™, Instrumentation Laboratory Company- Bedford MA 0730-2443 (USA).
d. APTT-SP (liquid)– 0020006300. HemosIL™, Instrumentation Laboratory Company- Bedford MA 0730-2443 (USA).
e. Antihrombin- 0020008900. HemosIL™, Instrumentation Laboratory Company- Bedford MA 0730-2443 (USA).
f. SPSS ver.15, Chicago, IL, USA.

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Table 1

	Fibrinogen(mg/dL)	D-Dimer(ng/mL)	PT(secs)	aPTT(secs)	AT(%)
AFG (n=25)	250 (212-311.5)	254 (206-601)	11.3 (10.9-12.2)	45.5 (43.1-49.7)	198 (184-222)*
CG (n=17)	256 (219-279)	233 (208-293)	11.2 (10.7-11.7)	47.7 (45.6-49.7)	232 (202-256)
Ref range	150-375	39-409	9.0-12.1	34.3-55.3	157-261

Table 2

	CG (n=17)	AFG (n=25)	Underlying disease (n=7)	Lone AF (n=18)	Short term (n=12)	Long term (n=13)
Abnormal D-dimers	2/17 (11.8%)	10/25 (40%)*	2/7 (28.6%)	8/18 (44%)	3/12 (25%)	7/13 (53.8%)
Abnormal coagulation profile	6/17 (35%)	18/25 (72%)*	6/7 (85.7%)	12/18 (66.7%)	8/12 (66.7%)	10/13 (76.9)
Abnormal tests	7/85 (8.2%)	25/121 (20.7%)*	10/35 (28.6%)	15/86 (17.4%)	9/51 (17.4%)	16/70 (22.9%)

Table 3

		Fibrinogen	D-Dimers	PT	aPTT	AT
Navas de Solís *et al*.	AF Control	250 (212-311.5) 256 (219-279)	254 (206-601) 233 (208-293)	11.3 (10.9-12.2) 11.2 (10.7-11.7)	45.5 (43.1-49.7) 47.7 (45.6-49.7)	198 (184-222) 232 (202-256)
Cesarini *et al.*	Peritonitis Enteritis Control		4204 (1652-5928) 1048 (435-2711) 695 (586-742)	13.9 (13-15.1) 13.6 (12.1-15.6) 11.05 (10.85-11.4)	54.7 (46-61.5) 55.3 (64.1-192) 39 (36.9-41.2)	164 (144-204) 192 (166-214) 188 (172- 211)
Armengou *et al.*	Septic foal Control	205 (179-308) 204 (167–237)	567 (244-2012) 313 (151–495)	17.1 (14.8-20.4) 12.7 (11.9–13.3)	71.6 (63.4-93) 39.1 (32.2–47.6)	113.5 (94-132) 158 (148–184)
Kumagai *et al.*	AF Control		150±19 61±3			
Inoue *et al.*	AF Control		92±6.7 31± 7.4			
Lip *et at.*	AF Control	378 (300-470) 260 (224-299)	158 (81-292) 76 (53-103)			
Wannamethee *et al.*	TE Control		103 (61-161) 79 (47-77)			

DISCUSSION

DISCUSSION

The first part of the presented thesis showed that acute hemorrhage was associated with myocardial injury, evidenced by increased cTnI, and that cardiac arrhythmias were frequent in these horses. To the best of our knowledge the frequency of myocardial injury after hemorrhage in horses had not been previously studied formally although arrhythmias had been reported in cases series and abstracts.[e,122] In the group of horses studied the occurrence of arrhythmias was more common than previously described[122] and myocardial injury uniformly detected. Arrhythmias in this study were more severe or frequent during the 24-48 hour period after hemorrhage diagnosis (when compared to 0-24 hours), supporting the theory that reperfusion injury may be a relevant and perhaps the primary cause of myocardial damage in the event of acute hemorrhage in horses.

The study group was heterogeneous in the cause and severity of the hemorrhage, duration of the disease, presence of comorbidities and therapy. Thus the study does not allow for analysis of the isolated effect of hemorrhage and it is likely that some, or all, of the above-mentioned factors play a role in the degree of myocardial injury and development of arrhythmias. Yet the frequent presence of myocardial injury and arrhythmias suggests that horses with acute hemorrhage may benefit from cardiac monitoring including continuous ECGs and cTnI measurements. Further studies are needed to know whether recognition and treatment of myocardial injury and cardiac arrhythmias in horses with

severe hemorrhage affects various outcomes. A larger population is needed to precisely quantify risks, recommend monitoring protocols or suggest specific interventions.

The primary motivation of the study that corresponds to the first part of this thesis was to prove the clinical impression that myocardial injury and arrhythmias are underdiagnosed and to raise awareness about this clinical problem. We consider that this has been proven and hope that this initial study will help clinicians design monitoring protocols and interventions for horses with acute hemorrhage. Another less formal motivation was to obtain preliminary information about the possibility of using markers of myocardial injury (cTnI) as a trigger for blood transfusions.

Many variables are reported to estimate the severity of hemorrhage and guide the decision to provide a blood transfusion. In horses, suggested guidelines for transfusion triggers include a PCV less than 12%, an acute blood volume loss of 30% to 40%, a lactate concentration greater than 4 mmol/L (or >2 mmol/L in the presence of fluid resuscitation), persistent hypotension despite adequate fluid resuscitation and uncontrolled hemorrhage.[112]

Hemorrhage can lead to changes in physical examination findings such as pale mucous membranes, prolonged capillary refill time, deterioration of mental status, tachypnea, hypothermia, poor pulse quality and cool extremities. However physical examination

findings can be misleading as to the degree of hemorrhage[112,113,166,167] and the amount of blood lost is rarely known in the hemorrhaging equine.

The packed cell volume (PCV) is an easy to obtain clinicopathological parameter to be monitored during hemorrhage. A PCV under 12% is below the minimum amount of oxygen-carrying capacity and warrants blood administration. However, PCV (and total protein [TP] concentration) are poorly correlated with blood loss in acute hemorrhage and may remain constant for several hours after bleeding has occurred.[112,113] Furthermore, acidemia, hypoxemia, and circulating cytokines decrease red blood cell affinity for oxygen and decrease red blood cell deformability decreasing DO2. For these reasons many guidelines[111,120] consider a higher PCV (or [Hb]) as the threshold for transfusion. For example, 7–8 g/dL of hemoglobin in humans with no clinical evidence of tissue hypoxia and 10 g/dL in actively bleeding patients or in groups with an already compromised health status (elderly patients or patients with preexisting myocardial infarction)[120] have been suggested as transfusion triggers. It is interesting that when analyzing variables at presentation in our study group TP was associated with the development of myocardial injury and the detection of complex arrhythmias and seemed to be the best early predictor of myocardial injury. This is difficult to reconcile with the delayed decrease in TP that follows acute hemorrhage and may represent delayed recognition of the hemorrhage in many cases in the study group.

An increase in blood lactate concentration is a good marker of poor tissue oxygenation.[111] However, tissue hypoxia is not necessarily the only cause of hyperlactatemia during

hemorrhage[167] and inhibition of pyruvate dehydrogenase, lactate production by leukocytes, increased sodium potassium ATPase activity in response to inflammatory mediators and impaired hepatic clearance[168] may also play a role. In a model of blood loss, blood lactate concentration increased (and CVP decreased) significantly compared with baseline values while HR and results of central venous blood gas analysis did not change significantly. This elegant study concluded that blood lactate concentration and CVP should be monitored to assess the potential need for blood transfusions.[113] In the present study, lactate concentrations (maximal and at presentation) were unexpectedly not associated with myocardial injury. Clinicians often use blood lactate concentration to guide fluid therapy or trigger blood transfusions and therefore the lack of association of maximal lactate with other variables in this study may reflect a rapid resolution of hyperlactatemia due to successful goal directed therapy of the clinicians responsible of the treatment of horses in the hemorrhage group. This lack of association could also be the consequence of low statistical power of this preliminary study.

Arterio-venous differences in oxygen content and carbon dioxide or oxygen extraction ratio (O2ER) have been suggested to be useful parameters in the assessment of tissue oxygenation.[169,170] As mentioned above, in an experimental model of moderate blood loss[113] results of central venous blood gas analysis did not change significantly. A decreased oxygen consumption (VO2) or an O2ER of 45-50% (25% being normal) have also been suggested as a useful transfusion trigger because it represents the maximum extraction compensation for a decrease in DO2 and marks the onset of tissue dysoxia.[111] Performing blood gas analyses in our group of horses would have been interesting.

During the design of the experiment we considered that obtaining these samples would have decreased the willingness of owners or clinicians to participate in the study and it was decided to not measure this clinicopathological variables.

Other parameters described as being relevant to the decision of administering a blood transfusion are gastric tonometry results, CVP, urine output, coagulopathies with increased PT and aPTT, systolic blood pressure, the rate of bleeding, CO or pulmonary artery wedge pressure.[120] We attempted the collection of data regarding blood pressure and CVP but clinicians had the perception that obtaining these measurements could upset horses and potentially affect the recovery. A horse remaining calm and quiet was considered a priority and an important part of the therapy to avoid disturbance of forming blood clots. This caused CVP and blood pressure data to be unavailable for most cases.

In the study presented in this thesis analysis of the relations between myocardial injury related variables (cTnI and arrhythmias), markers of local blood flow (creatinine and cTnI) and variables classically used to monitor hemorrhaging animals showed significant associations. It is interesting that markers of local regional blood flow such as creatinine, or the more classic parameters to monitor hemorrhage (TP and PCV) were more informative in the cases reported here. The associations shown between cTnI and creatinine are interesting and perhaps renal and myocardial hypoperfusion/hypoxia are parallel events in the scenario of clinical hemorrhage. Renal failure is a recognized cause of increased cardiac troponins in dogs and humans via incompletely understood mechanisms and[114] one of the horses in the study developed renal failure. However there

was no clinical suggestion of renal disease in any of the other ten horses supporting that azotemia and increased cTnI were parallel phenomena and not caused by renal failure.

The study design did not allow for evaluation of the use of myocardial injury (increased cTnI plasma concentration) or arrhythmias as transfusion triggers. However the fact that a higher maximal cTnI and treatment for arrhythmias were associated with a poor outcome suggests that these variables related to myocardial injury could be markers of severity of disease. Using myocardial injury and arrhythmias as transfusion triggers would be an interesting topic for future investigations.

The arrhythmias observed in this study were not the primary cause of death in any horse but treatment for arrhythmia was associated with a poor prognosis suggesting that arrhythmia development may be a marker of disease severity. Myocardial injury, and more specifically arrhythmia development, is relevant to the clinical management of critically ill horses but more importantly, and as already mentioned several times in this document, persistent arrhythmias could be a concern for the safety of their riders. Although elevated cTnI and cardiac arrhythmias were transient in the horses reported in this thesis, this study was not designed to monitor the long-term effects of acute hemorrhage. The results suggest that such an investigation might be warranted. Other tests such as echocardiograms, exercising ECGs and cardiac histopathology could be necessary to evaluate if the injury has long-term sequelae and would be an interesting corollary to this experiment.

In conclusion, the first part of the thesis presented here demonstrated that horses with hemorrhage secondary to a variety of causes experience myocardial injury, characterized by elevations in cTnI, and also developed cardiac arrhythmias. Arrhythmias observed included supraventricular premature contractions, multifocal ventricular premature contractions and ventricular ectopy that included runs of ectopic beats. In this heterogeneous group a lower TP at the time of hemorrhage diagnosis was associated with detection of complex arrhythmias. A higher maximal creatinine was associated with treatment for arrhythmias and a higher maximal cTnI was associated with detection and treatment of arrhythmias. The TP at presentation and the minimal PCV were associated with a higher maximal cTnI. A lower minimal TP, higher maximal cTnI and treatment for arrhythmia were poor prognostic indicators. Further studies are needed to know whether recognition and treatment of cardiac arrhythmias in horses with severe hemorrhage affects long-term outcome and to suggest specific interventions to prevent and treat this myocardial injury and arrhythmias.

The second study of this thesis investigated the occurrence of HC in horses. The motivation of the study was the clinical impression that horses with systemic hypertension due to chronic pain or chronic renal disease develop myocardial hypertrophy. To the best of our knowledge this is the first clinical and echocardiographic description of the appearance of HHD or HC in horses. A retrospective study was designed to gain knowledge about the prognosis and clinical, echocardiographic, and pathologic features of this clinical problem. Hypertensive cardiomyopathy was uncommonly found in the medical records database of the horses presented to the large

animal cardiology and ultrasound department of the University of Pennsylvania. Five cases were found in a 16 year period; this was 0.26% of all horses evaluated for cardiac disease at this institution during the study period. Hypertensive cardiomyopathy was uniformly associated with chronic renal failure or chronic laminitis.

The presence of hypertension in individuals with renal disease is caused by volume overload, excessive activation of the renin angiotensin aldosterone system, and anemia.[171] Despite the fact that hypertension is commonly recognized and managed as a sequela to chronic renal failure in horses, to the best of our knowledge HHD in the event of renal disease had not been previously reported in this species.

The relationship between hypertension and laminitis in horses has been known for decades.[128,129] The pathophysiology of laminitis-induced hypertension may differ depending on the stage of the laminitis and the mechanism behind the lamellar damage. Systemic hypertension has been proposed to occur during the development of laminitis[128] or to be the response to hemodynamic changes during acute laminitis.[129] In the acute phases of laminitis, hypertension has been explained by increased sympathoadrenal outflow, renin activity, and aldosterone concentration caused by pain, dehydration and electrolyte changes.[128] The hemodynamic changes associated with chronic pain and sympathetic system over-stimulation may be the main factors in the hypertension seen in chronic laminitis. The recognition of the presence of hypertension and development of HHD may be useful to equine practitioners as laminitis is a very common life threatening

complication of many equine conditions that frequently needs prolonged care.[172] Furthermore the monitoring of blood pressures in horses with laminitis may be useful to assess the degree of pain and response to analgesics or mechanical interventions.[173]

The mild hypertension seen in horses during the pre-laminitic stages of Equine Metabolic Syndrome (EMS) may be caused by endothelial cell dysfunction.[54] It is uncertain if horses with EMS develop cardiovascular problems as humans with metabolic syndrome do. It is likely that the type and severity of cardiac disease, if present, will be somewhat different. The national library of medicine describes human metabolic syndrome as a group of risk factors that occur together and increase the risk for coronary artery disease, stroke, and type 2 diabetes. Equine metabolic syndrome shares some of the features of human metabolic syndrome, including increased adiposity, hyperinsulinemia and insulin resistance, but differs in that laminitis is the primary disease of interest.[130] Atherosclerosis and coronary heart disease are not reportedly detected in horses with EMS and the presence of these conditions in horses is indeed very rare. Frank in a recent review of the current knowledge on EMS suggested that this can be explained by the herbivorous diet of horses causing different lipoprotein composition of equine blood.[130] Most circulating cholesterol is carried within high-density lipoproteins in horses, rather than the atherogenic low-density lipoproteins.[130] None of the horses in our study were formally diagnosed with EMS but it is interesting that three of them presented with laminitis, cardiac disease and hypertension. One of the horses also had atherosclerosis detected during the postmortem examination. The three horses with laminitis presented

with chronic foot pain that is in and of itself a cause for systemic hypertension and an explanation for the consequential cardiac changes. Nevertheless the potential association of EMS, hypertension, laminitis and cardiac disease in horses deserves further attention. Human metabolic syndrome is a pandemia of modern western societies due to changes in diet and lifestyle. The study of cardiovascular disease in horses with EMS is especially interesting from the perspective of the comparative physiopathology of EMS and human metabolic syndrome and the potential for translational cardiovascular research.

Left ventricular hypertrophy is an adaptation or maladaptation to systemic hypertension easily recognizable in two-dimensional echocardiograms[174] when the myocardial hypertrophy is moderate or severe. Pressure overload does not appear to be the only mechanism that causes ventricular hypertrophy during hypertension. Renin angiotensin aldosterone system activation, insulin resistance, and catecholamine release play a direct role in the development of hypertensive myocardial hypertrophy in humans.[125] The cardiac response to hypertension has high individual variability and has been described as proportionate to the 'area under the lifetime blood pressure curve'.[123] The degree of left ventricular hypertrophy is not associated with blood pressure measurements in human beings with HHD [125] and therefore it is not surprising that no significant associations were found between the degree of left ventricular hypertrophy and the severity of hypertension in the horses reported in our study. Many questions remain in the assessment of hypertension in horses and even in the assessment of blood pressures in normal horses. The best method, the effect of the procedure itself on the pressure

measurements (referred as white coat hypertension) and the results that should prompt therapy are to be determined.

Pseudohypertrophy, caused by dehydration or endotoxemia, and HC have now been added to the spectrum or conditions that create the echocardiographic appearance of hypertrophy in horses.[44,b] There are clinical and echocardiographic differences between these recently reported conditions in the horse[44,b] and other previously reported conditions such as athlete's heart, valvular disease or primary cardiomyopathies that also cause left ventricular hypertrophy. In humans and cats[175] hypertrophic cardiomyopathy (HCM) is a common cause of concentric left ventricular hypertrophy but to the best of our knowledge this genetic disease has not been described in horses. Valvular disease or primary cardiomyopathies in horses will cause almost uniformly eccentric hypertrophy while the rest of the above-mentioned conditions cause concentric hypertrophy. Some degree of overlap in cardiac measurements and echocardiographic appearance exists between humans with an athlete's heart and those with diseases that cause myocardial hypertrophy such as HHD or HCM.[176] Comparison of the data in the case series part of this doctoral thesis with that collected from equine athletes in other studies[48,49] suggests that confusion caused by such overlap would be uncommon in horses. Relative wall thickness (RWT) appears to be uniformly increased in horses with HC and normal in equine athletes. Equine athletes develop a more proportionate left ventricular dilation with respect to the myocardial hypertrophy and therefore RWT remains normal. Human athletes engaged in disciplines that require isometric exercises can potentially develop patterns of hypertrophy that resemble the ones observed with HC.[177] Studies in

populations different than Thoroughbred and Standardbred racehorses would be necessary to determine whether other types of equine athletes develop different patterns of hypertrophy. However, the classic belief that the type of sport determines the pattern of hypertrophy has been challenged[177] and it seems unlikely that any common equestrian discipline mimics the isometric exercises that cause severe concentric hypertrophy in humans. Perhaps quarter horse racing or some non-FEI disciplines that involve pulling may be situations that could resemble sports that involve isometric exercises and in which athletes are reported to develop concentric hypertrophy but this remains speculative.

The clinical findings and blood pressure measurements of horses with HC should allow their differentiation from those with pseudohypertrophy due to dehydration or endotoxemia.[44,178] The left ventricular mass (LVM) was increased in horses with HC that had true hypertrophy and was reportedly normal in the study by Underwood et al. using an experimental model of de(hypo)hydration. The values of RWT and mean wall thickness (MWT) in horses with HC were higher than those reported in experimentally hypohydrated horses. Moreover, the systolic left ventricular measurements, which were altered in 4 horses in this series but within reference range in volume-depleted horses,[44] would also help in this differentiation. The data reported here suggests that systemic hypertension causes echocardiographically evident left ventricular hypertrophy in horses that can be differentiated from pseudohypertrophy by the use of the calculated values of LVM or by detection of increased wall thicknesses during systole in horses in which the clinical appearance is not definitive.

The echocardiographic appearance of horses after an experimental endotoxin challenge has been recently described.[b] Left ventricular internal diameter in diastole was small during the endotoxin infusion similarly to most horses with HC. Left atrial diameter was significantly decreased during endotoxin infusion and mildly so in some of the horses with HC. Left ventricular free wall thickness during systole was significantly decreased at hours 3, 4, and 5 after endotoxin infusion while it is increased (n=4) or normal (n=1) in horses with HC. The appearance of the LVFW in systole may be one of the keys to differentiate the echocardiographic appearance of horses with HC from endotoxemic horses. The endotoxic challenge also decreased fractional shortening (FS) to less than 30% in some horses (normal or increased in horses with HC) and created a marked spontaneous contrast within the left ventricle which was interpreted by the investigators as evidence of myocardial dysfunction. In summary HC and myocardial dysfunction caused by an endotoxemic challenge could be differentiated by the increase in left ventricular mass in cases of HC. Also the decrease in the LVFW in systole or FS in horses with experimentally created endotoxemia when compared to an increase, or normal, LVFW and normal to increased FS in HC can aid the echocardiographic differentiation in cases in which the clinical presentation is not definitive.

In the group of horses with HC reported here, the primary disease and the poor prognosis for recovery were the cause for euthanasia in all instances suggesting that HC is associated with severe underlying disease. Because of the lack of long term survivors, the sequelae, long term complications, or reversibility of HC in horses could not be

determined. Arrhythmias and sudden cardiac death are frequent complications of HHD in several species.[125, 127,179, 180] Ventricular hypertrophy, fibrosis, increased myocardial oxygen demand, fluctuations in arterial pressure, and impaired coronary perfusion[181] are the proposed arrhythmogenic factors. In the horses we reported, arrhythmias were not recognized, however auscultation and simultaneous electrocardiography are unlikely to detect sporadic arrhythmias. Similarly, it was not possible to draw conclusions with regards to the presence of myocardial injury in these horses with HC because of the paucity of data available. As stated for the first study of this series, the presence of myocardial injury and arrhythmias are of great relevance to horses' health but most importantly to human safety due to the serious consequences that collapse and or sudden cardiac death can have to horses handlers and riders. Continuous electrocardiography and cardiac troponin measurements should be obtained prospectively in a larger group of horses with HC, chronic laminitis, and chronic renal failure to assess the clinical importance of myocardial injury and arrhythmias in horses with this condition. The effect of subclinical hypertension on exercise tolerance and arrhythmogenesis is another topic that is relevant to horse owners and riders.

Only 0.26% of cases presented to the University's of Pennsylvania large animal cardiology department during the study period met the inclusion criteria to be classified as having HC. It may be that horses develop HC less frequently than other species because of the less common occurrence of hypertension. Conversely, limited awareness of the potential presence of cardiac disease in hypertensive horses may cause it to be under recognized. Because of the poor outcome of horses in this study and the association

of HHD in other species with comorbidities that could affect horses' health and riders' safety, clinicians' attention to the possibility of horses developing this condition is warranted. Monitoring horses with, or at risk of developing, hypertension (e.g., horses with laminitis, signs of chronic pain, chronic renal failure, or EMS) may help determine the clinical relevance of this equine cardiomyopathy and identify interventions that could assist in the management of such cases. We hope that the publication and communications of this study and future research will make the veterinary community aware of the potential presence of hypertension as comorbidity in horses with chronic laminitis or renal disease. We also hope that more knowledge will be gained in the near future about this disease and in the field of cardiac associated complications of systemic hypertension.

The third study of this thesis was motivated by the great relevance of atrial fibrillation in humans and performance horses. Humans in atrial fibrillation are in a hypercoagulable state that makes stroke and thromboembolism the major cause of morbidity and mortality in patients with this rhythm disturbance.[139,140] The presence of thromboembolic complications in horses with atrial fibrillation has not been reported but to the best of our knowledge the presence of a hypercoagulable state in horses with atrial fibrillation had not been prospectively or formally tested. Despite the lack of formal testing the potential for thromboembolism in horses with atrial fibrillation is often, and understandably, disregarded due to the lack of clinical evidence of thromboembolic disease in horses with this arrhythmia.

The study proved that a subclinical hypercoagulable state is present in horses with atrial fibrillation. The results suggest that horses in atrial fibrillation are not in a clinical hypercoagulable state as no clinical signs of hypercoagulation or thromboembolism were detected in the study group and to the best of our knowledge have not been reported in the literature.

Antithrombin activity was lower ($p=0.008$) and the proportion of horses with abnormal laboratory tests ($p=0.015$), abnormal coagulation profiles ($p=0.018$) and high D-dimers was larger in the group of horses with atrial fibrillation. There was not a statistical or clinically significant difference in results of D-dimers between the group of horses in atrial fibrillation and the control group when tested using a Wilcoxon (MannWhitney) rank-sum test and therefore the hypothesis that plasma D-dimers concentration would be the best marker of a procoagulant state in horses with atrial fibrillation was not proven. The conclusions regarding differences in D-dimer concentrations should be interpreted with caution as the size of the study group coupled with the high variability of the D-dimers test results may have resulted in the study being underpowered. Taking into account the variability obtained, a sample size of 376 horses in each group would have been needed to make calculations with a power of 80% using the statistical method mentioned and with a 0.05 two-sided significance level.

The reason for why the subclinical hypercoagulatory state in horses with atrial fibrillation does not cause clinically relevant thrombogenesis is uncertain. We compared the magnitude of the changes in coagulation tests results in our group of horses with the

results of studies of humans with cardiac disease and[141,182-184] in groups of horses in which a hypercoagulable state was considered clinically relevant.[97, 185] The magnitude (or lack) of changes in our study group was less than in the above-mentioned studies.

The presence of atrial fibrillation is in and of itself a source of a hypercoagulable state[182] in humans and the presence of structural heart disease,[186] signs of congestive heart failure,[144] recent cardioversion[145-148] or a prolonged duration of the arrhythmia[144,153,150] have been shown to increase the risk of stroke. In our study the coagulation profiles of horses with and without underlying heart disease and with long and short duration of the arrhythmia were presented. However, due to the small sizes of the groups formal statistical analysis was not attempted. The small size of the study group coupled with the intrinsic high variability of D-dimers measurements was the main limitation of the study.

In conclusion some horses in atrial fibrillation present subclinical hypercoagulation that does not appear to have thromboembolic consequences. The subclinical hypercoagulation should be borne in mind when assessing these patients.

Overall, the three studies have contributed to the body of knowledge in equine cardiology and have answered relevant, previously unanswered questions. Acute hemorrhage causes myocardial injury and arrhythmias during acute hemorrhage in horses. Horses develop HC in the event of laminitis or chronic renal failure and some horses in atrial fibrillation present subclinical hypercoagulation without thromboembolic consequences.

CONCLUSIONS

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1. Horses with acute hemorrhage secondary to a variety of causes experienced myocardial injury, characterized by elevations in cTnI, and also developed cardiac arrhythmias. A higher maximal cTnI was associated with detection and treatment of arrhythmias.
2. Arrhythmias in horses with acute hemorrhage included supraventricular premature contractions, multifocal ventricular premature contractions and ventricular ectopy with runs of ectopic beats. Arrhythmias were transient and still present at the time of discharge from the hospital in two cases.
3. In horses with acute hemorrhage a lower TP at the time of diagnosis was associated with a higher maximal cTnI and detection of complex arrhythmias. The minimal PCV was associated with a higher maximal cTnI and a higher maximal creatinine was associated with treatment for arrhythmias.
4. In horses with acute hemorrhage a lower minimal TP, higher maximal cTnI and treatment for arrhythmia were poor prognostic indicators.
5. Hypertensive cardiomyopathy should be considered as a comorbid diagnosis in horses with laminitis or chronic renal failure. Persistent tachycardia, hypertension, chronic laminitis, or a combination of these prompted the cardiac evaluations. No arrhythmias were reported in these

horses. No associations between blood pressure measurements and variables consistent with hypertrophy were detected.

6. Horses with HC had increased RWT, MWT and LVM. The IVS was thickened at end diastole (n=5) and in peak systole (n=4). The LVID was small at end diastole (n=4) and in peak systole (3). The LVFW was thickened at end diastole (n=3) and in peak systole (n=4).
7. All horses with HC were euthanized because of the grave prognosis of the primary diseases.
8. All horses with HC that underwent postmortem evaluation had cardiovascular abnormalities.
9. Horses in atrial fibrillation are in a state of subclinical hypercoagulation but no signs of thromboembolic disease were detected.
10. Antithrombin activity was lower in horses in the atrial fibrillation than in a control group and no significant differences in fibrinogen, D-dimer, PT and aPTT were detected.
11. The proportion of horses with abnormal D-dimer concentrations, abnormal coagulation profiles and abnormal coagulation tests were larger in a group of horses in atrial fibrillation than in a control group.
12. Coagulation profiles in horses with different atrial fibrillation duration and horses in atrial fibrillation with and without underlying cardiac disease were described but differences between groups could not be tested due to the paucity of the data.

SUMMARY

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The presence of cardiac disease is a common complaint in equine practice. Cardiac disease can present as a primary problem or a secondary event due to severe or critical illness. The presence of cardiac disease can have serious repercussions to horses' health and wellbeing but the risks for humans associated with equine cardiovascular collapse or sudden cardiac death (particularly the risks for riders and drivers during exercise) make equine cardiology a discipline that has implications that go beyond animal health. The thesis presented here attempted to gain further knowledge in three equine cardiac problems that had received little or no attention before: 'Myocardial insult and arrhythmias after acute hemorrhage', 'Hypertensive cardiomyopathy' and the 'Evaluation of coagulation and fibrinolysis in horses with atrial fibrillation'. To investigate these three conditions we designed three separate studies.

The first part of this thesis studied the effects of acute hemorrhage on the plasma concentration of a marker of myocardial injury (cTnI) and in the development of cardiac arrhythmias. We designed a prospective controlled study in which a group of horses presented to a large animal veterinary teaching hospital with acute hemorrhage were compared to a control group. Serial measurements of cTnI plasma concentration and continuous ECGs were performed in both groups. The associations between physical and

clinicopathological variables classically used to monitor horses with acute hemorrhage and myocardial injury, arrhythmias and outcome were determined.

We concluded that acute hemorrhage results in myocardial injury that can be detected by measuring cTnI and that arrhythmias were frequent in hospitalized horses with acute hemorrhage. Intense cardiac monitoring by means of serial cTnI plasma concentration measurements and continuous ECGs allowed the detection of these sequelae at a higher frequency than previously reported in the literature when these methods were not used. This proves that the myocardium of horses is similar to other species in the susceptibility to the effects of acute hemorrhage. Hypoxia, reperfusion injury and the sympathetic response associated to the acute hemorrhage are plausible mechanisms behind the myocardial injury and the arrhythmias.

Both atrial and ventricular arrhythmias were observed in the study group. Some of the arrhythmias met classic criteria for emergency therapy and horses were successfully treated for them. All arrhythmias resolved and none of the horses died due to the direct effects of the myocardial injury or arrhythmias. In addition cTnI, TP, PCV and creatinine plasma concentration were useful parameters in the monitoring of horses with acute hemorrhage that correlated with the development of myocardial injury and/or arrhythmias. The study design did not allow for analysis of the effect or necessity of different therapies or the benefits of intense monitoring in outcome. The cTnI plasma concentration, presence of arrhythmias and treatment for arrhythmias were correlated

with a poor prognosis and seems intuitive that being informed of the development of these complications would help in the clinical management of these cases. The study of the potential use of cTnI as a transfusion trigger seems warranted.

The goal of the second part of this thesis was to study the clinical, echocardiographic and pathological appearance of HC in horses. For this we designed a retrospective study in which the medical records of the University's of Pennsylvania New Bolton Center (between 1995 and 2011) were searched for the presence of horses with myocardial hypertrophy and hypertension. Information regarding demographics, history, physical and cardiac examination findings, diagnosis, clinical progression, prognosis, and pathologic findings were obtained. We described the presence of HC as a comorbid diagnosis in horses with laminitis or chronic renal failure. This clinical entity had not been previously diagnosed in horses and should be borne in mind when evaluating horses with hypertension.

Hypertensive cardiomyopathy should be added to the list of differentials diagnoses for horses that present with left ventricular hypertrophy. The clinical presentation (increased blood pressure and an underlying condition causing renal disease or chronic pain) should raise the suspicion of the presence of HC versus other etiologies of equine left ventricular hypertrophy such as athlete's heart, valvular disease, cardiomyopathies or causes of pseudohypertrophy such as de(hypo)hydration or endotoxemia. In cases in which the clinical presentation is questionable, the increase in echocardiographically calculated

values of LVM or the frequent presence of increased wall thickness in systole for horses with HC should allow for echocardiographic differentiation.

The presence of HHD in the group of horses that we studied uniformly resulted in euthanasia. The cardiac disease was not the cause or death or the main factor for performing humane euthanasia in any of the cases. Despite this it seems that clinicians should be aware that HC could be associated with hypertension caused by chronic laminitis or severe renal disease in horses. Information about the development, progression, reversibility, importance of early detection, and long-term sequelae of this condition would help clinicians to provide better medical care to horses.

The third part of this thesis had the motivation of investigating the presence of a hypercoagulable state in horses with atrial fibrillation. Atrial fibrillation is the most common clinically relevant arrhythmia in horses and in humans. Humans in atrial fibrillation are in a hypercoagulable state that makes stroke and thromboembolism the major cause of morbidity and mortality in patients with this rhythm disturbance. We consider that testing the presence of a hypercoagulable state in horses with atrial fibrillation answered a relevant question to the veterinary community. Due to the fact that D-dimers plasma concentration seems to be the single most useful clinicopathological parameter to estimate the thromboembolic risk in humans beings and because D-dimers have been shown to be a clinically useful marker of fibrinolysis in horses with diagnostic and prognostic importance in many conditions we hypothesized that D-dimers would be the best marker of hypercoagulability in horses with atrial fibrillation. An additional

objective was to describe the coagulation profiles of horses with atrial fibrillation and their relation with the duration of the arrhythmia and the presence of structural heart disease as these two factors have been reported in humans to affect the hypercoagulable state and the risk of thrombogenesis.

To test the two above mentioned hypotheses we designed a prospective controlled study in which the most frequently used tests to evaluate coagulation and fibrinolysis in horses were performed on citrated blood samples obtained from equine patients with atrial fibrillation and in a control group. The results of PT, aPTT, plasma fibrinogen concentration, AT and D-dimers in the atrial fibrillation and control groups were compared. The presence of a difference in the proportion of abnormal results, abnormal coagulation profiles (one or more abnormal results per horse) or abnormal D-dimers concentrations between groups was also tested.

The proportion of horses with abnormal D-dimer concentrations, abnormal coagulation profiles and the proportion of abnormal coagulation tests was larger in the atrial fibrillation group than in the control group. Only AT was different between groups when results to individual tests were compared using a Wilcoxon (Mann-Whitney) sum rank test (and no significant differences in fibrinogen, D-dimer, PT and aPTT were detected. No signs of hypercoagulability or thrombogenesis were detected in any of the horses. This study demonstrated that atrial fibrillation causes a state of hypercoagulability in horses that did not have clinical thromboembolic consequences. The reason for why the hypercoagulability does not translate in thromboembolism is uncertain. Comparison of

the results of the coagulation profiles in our study group with the results in studies in humans with atrial fibrillation and in humans and horses with propensity to thromboembolism supported that the hypercoagulability in horses with atrial fibrillation may be quantitatively less than in these groups and under a threshold that creates thromboembolic complications.

In conclusion, the three studies have contributed to the body of knowledge in equine cardiology and have answered relevant questions that had not been previously investigated. Acute hemorrhage causes myocardial injury and arrhythmias during acute hemorrhage in horses. Horses with laminitis or chronic renal failure can develop hypertensive cardiomyopathy and horses in atrial fibrillation present subclinical hypercoagulation without thromboembolic consequences.

RESUMEN

RESUMEN

La presencia de enfermedades cardiacas es un problema común en medicina equina. Los problemas cardiacos pueden ser primarios o complicaciones secundarias a enfermedades sistémicas severas o críticas. La presencia de enfermedad cardiaca puede tener repercusiones serias sobre la salud y el bienestar de los equinos pero el riesgo a las personas, asociado con el desarrollo de colapso cardiovascular o de muerte súbita cardiaca (en particular el riesgo a jinetes o conductores durante la práctica de disciplinas ecuestres) hacen de la cardiología equina una disciplina con implicaciones que van más allá de la salud animal. La tesis aquí presentada trata de aportar conocimientos sobre tres problemas cardiacos equinos que habían recibido poca o nula atención previamente: "el daño miocárdico y las arritmias después de hemorragias agudas', la "cardiomiopatía hipertensiva" y la "evaluación de la coagulación y fibrinolisis en caballos con fibrilación atrial". Para estudiar estos tres problemas diseñamos tres estudios.

La primera parte de esta tesis estudió los efectos de la hemorragia aguda en la concentración plasmática de un marcador específico de daño miocárdico, como es la troponina cardiaca I, y en el desarrollo de arritmias cardiacas. Para esto diseñamos un estudio prospectivo en el que un grupo de caballos presentados a un hospital veterinario universitario con hemorragia aguda se compararon a un grupo control. Se realizaron

mediciones seriadas de troponina I plasmática y electrocardiogramas continuos en ambos grupos. También se estudiaron las asociaciones entre las variables del examen físico y valores clinicopatológicos usados tradicionalmente para monitorizar caballos con hemorragia aguda con el daño miocárdico, la presencia de arritmias y el pronóstico.

El estudio determinó que la hemorragia aguda causa daño miocárdico que puede ser detectado midiendo la concentración plasmática de troponina cardiaca I y que la presencia de arritmias es frecuente en caballos hospitalizados debido a la presencia de hemorragia aguda. La monitorización cardiaca intensiva por medio de la medición de troponina cardiaca I y la monitorización de electrocardiogramas continuos permitió la detección de estas secuelas cardiacas a una frecuencia mucho mayor de la citada en la literatura veterinaria. En las referencias previas sobre daño miocárdico y arritmias durante o después de hemorragia severa los métodos de monitorización cardiaca intensiva que utilizamos en nuestro proyecto no fueron utilizados. Esto prueba que el daño miocárdico en caballos después de hemorragia severa es similar al que se produce en otras especies La hipoxia, el daño por reperfusión y la respuesta simpática asociada a la hemorragia aguda son, posiblemente, los mecanismos involucrados en el daño miocárdico y el desarrollo de arritmias.

Arritmias atriales y ventriculares fueron detectadas en el grupo de caballos con hemorragia aguda. Algunas de las arritmias cumplieron los criterios citados clásicamente para ser clasificadas como arritmias en las que tratamiento antiarrítmico farmacológico es

necesario y fueron tratadas con éxito. Todas las arritmias desaparecieron pero en algunos casos permanecieron hasta el alta hospitalaria. Ninguno de los caballos murió o fue eutanasiado debido a los efectos directos de las arritmias o el daño miocárdico. Además de la concentración plasmática de troponina I la proteína total, el hematocrito y la concentración plasmática de creatinina fueron parámetros útiles en la monitorización de estos caballos con hemorragia aguda al estar asociados con el desarrollo de daño miocárdico y/o arritmias. El diseño experimental no permitió el análisis del efecto o la necesidad de distintas terapias o de las ventajas de la monitorización intensiva. La concentración plasmática de troponina I, la presencia de arritmias y el tratamiento antiarrítmico estuvieron asociados con un pronóstico desfavorable y parece intuitivo que el estar informado del desarrollo de estas complicaciones podría ayudar a los clínicos veterinarios a tratar los caballos con hemorragia aguda. El estudio del potencial de la medición de la concentración plasmática de troponina I para guiar la necesidad de administrar transfusiones sanguíneas parece indicado.

La segunda parte de esta tesis se diseñó para estudiar la apariencia clínica, ecocardiográfica y anatomopatológica de la cardiomiopatía hipertensiva en caballos. Diseñamos un estudio retrospectivo en el cual se revisaron los archivos médicos de la Universidad de Pensilvania desde 1995 a 2011 para encontrar casos en los que hipertrofia del miocardio del ventrículo izquierdo e hipertensión hubiesen sido diagnosticados. Información sobre los aspectos demográficos, históricos, examen físico y cardiaco, diagnóstico, progresión clínica y hallazgos anatomopatológicos se obtuvieron de los informes médicos. A partir de esta información se describió la presencia de

cardiomiopatía hipertensiva como una comorbilidad en caballos con laminitis y fallo renal crónico. Este problema clínico no había sido descrito previamente en equinos y debería de considerarse en caballos que son evaluados por problemas clínicos que pudieses provocar hipertensión.

La cardiomiopatía hipertensiva se debe añadir a la lista de diagnósticos diferenciales en los caballos que presentan hipertrofia del ventrículo izquierdo. La presentación clínica (aumento de la presión sanguínea y enfermedad subyacente que cause fallo renal crónico o laminitis) deben de aumentar las sospechas sobre la presencia de enfermedad cardiaca hipertensiva. En este escenario clínico este diagnóstico parece más probable que otras causas de hipertrofia del ventrículo izquierdo como el 'corazón de atleta', enfermedad valvular, cardiomiopatía primaria o causas de pseudohipertrofia como deshidratación o endotoxemia. En casos en los que la presentación clínica aporta dudas sobre el diagnóstico definitivo los valores ecocardiográficos calculados (principalmente la masa del ventrículo izquierdo y también el 'grosor relativo de la pared') o la presencia de engrosamiento de la pared ventricular en sístole permiten la diferenciación ecocardiográfica.

Todo los caballos en los que se diagnóstico la presencia de enfermedad hipertensiva cardiaca acabaron por ser eutanasiados debido a su enfermedad primaria. Sin embargo, es importante recalcar que la enfermedad cardiaca no fue la causa de la muerte o el motivo por el cual se eligió la eutanasia en ninguno de los casos. A pesar de esto el reconocer que la cardiomiopatía hipertensiva puede estar asociada con la presencia de hipertensión

en caballos y causada por enfermedad renal crónica o laminitis puede ser útil para los clínicos veterinarios de equinos. Información sobre el desarrollo, la progresión, la reversibilidad, la importancia del diagnóstico temprano y las secuelas a largo plazo de esta enfermedad podría ayudar a los clínicos veterinarios a proveer mejor atención médica a sus pacientes equinos.

La tercera parte de estas tesis surgió con la motivación de investigar si existe la presencia de un estado de hipercoagulación en caballos con fibrilación atrial. Las consideraciones previas a esta pregunta son los hechos de que la fibrilación atrial es la arritmia clínicamente mas relevante en caballos y humanos y que la fibrilación atrial causa en humanos un estado de hipercoagulación que provoca que las complicaciones tromboembólicas sean la mayor causa de morbilidad y mortalidad en estos pacientes. Por estas razones creímos que probar que los caballos en fibrilación atrial presentan un estado de hipercoagulacion contestaría una pregunta relevante a la comunidad veterinaria. Puesto que la concentración plasmática de D-dímeros es el parámetro clinicopatológico más útil en la estimación del riesgo tromboembólico en humanos y ya que ha sido comprobado que los D-dímeros tienen importancia en el diagnóstico y pronóstico de caballos con alteraciones de la coagulación y la fibrinolisis también creímos conveniente postular la hipótesis de que los D-dímeros son el mejor marcador de hipercoagulación en caballos con fibrilación atrial. Un objetivo adicional en este tercer proyecto fue el describir los paneles de coagulación en caballos con fibrilación atrial y su relación con la duración de la arritmia y con la presencia de enfermedad cardiaca estructural. Estos dos

factores han sido descritos como determinantes del estado hipercoagulable y el riesgo tromboembólico en humanos con fibrilación atrial.

Para cumplir los objetivos mencionados diseñamos un estudio prospectivo control en el cual los tests de coagulación (y fibrinolisis) más frecuentemente utilizados en medicina equina se realizaron en muestras de sangre citratada obtenidas de caballos con fibrilación atrial y de un grupo control. Los resultados de los tiempos de protrombina y tromboplastina parcial activada, la concentración plasmática de fibrinógeno, la actividad de antitrombina y la concentración de D-dímeros fueron comparados entre los dos grupos. La proporción de resultados anormales, de paneles de coagulación anormales (definidos como la presencia de uno o más resultados anormales) y la proporción de resultados de D-dímeros anormales también fueron comparados.

La proporción de caballos con D-dímeros anormales, paneles de coagulación anormales y la proporción de resultados a los tests de coagulación anormales fue mayor en el grupo de caballos con fibrilación atrial que en el grupo control. Solo la actividad de la antitrombina mostró una diferencia significativa entre los grupos cuando los resultados fueron comparados usando un test de Wilcoxon (Mann-Whitney). No se encontraron diferencias estadísticamente significativas en la concentración plasmática de fibrinógeno, D-dímeros, tiempo de protrombina o tiempo de tromboplastina parcial activada. Ninguno de los caballos mostró signos clínicos de hipercoagulación o tromboembolismo. Este estudio demostró que los caballos en fibrilación atrial presentan un estado subclínico de hipercoagulación sin sintomatología clínica de tromboembolismo. La razón por la cual la

hipercoagulabilidad no se refleja en complicaciones tromboembólicas en estos caballos no es aparente. La comparación de los resultados de los paneles de coagulación en los caballos que formaron parte de este estudio con humanos predispuestos a complicaciones tromboembólicas debido a enfermedad cardiaca y con caballos en los que un estado de hipercoagulabilidad se consideró clínicamente relevante muestra que los caballos en fibrilación atrial presentan alteraciones que son cuantitativamente menores que en estos grupos.

Como conclusión, los tres estudios que forman parte de esta tesis doctoral han contribuido a ampliar los conocimientos en cardiología equina y han contestado cuestiones relevantes que no habían sido investigadas previamente. La hemorragia aguda causa daño miocárdico y arritmias en caballos. Caballos con laminitis y enfermedad renal crónica pueden desarrollar cardiomiopatía hipertensiva y los caballos con fibrilación atrial permanecen en un estado de hipercoagulabilidad sin consecuencias tromboembólicas.

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FOOTNOTES

a. Reef VB, Bonagura J, Buhl R, McGurrin K, Schwarzwald C, van Loon G, Young LE. Recommendations for Equine Athletes with Cardiovascular Abnormalities ACVIM/ECEIM Consensus Statement 2013

b. Slack J, Nolen-Walston RD, Dallap Shaer BL. Echocardiographic and ecg findings in horses with experimentally induced endotoxemia. Research Abstract Program of the 2013 ACVIM Forum. J Vet Intern Med. 2013 27 (3):649

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FUTURE RESEARCH

FUTURE RESEARCH

The completion of the three projects that are part of this thesis has raised several questions that should be answered as part of future studies. We proved that hemorrhage causes myocardial injury and arrhythmias but further studies are needed to know whether recognition and treatment of this myocardial injury and cardiac arrhythmias in horses with severe hemorrhage affects short and long-term outcomes. We have proven that horses developed myocardial injury uniformly in the event of acute hemorrhage and that the concentration of cTnI is associated with an adverse outcome. The use of cardiac troponin I plasma concentration as a transfusion trigger would be the next step to give further clinical relevance to our studies. The effect of different interventions that could prevent or treat the development of myocardial damage or arrhythmias in the event of acute hemorrhage or the preferred antiarrhythmic strategy for these horses would be other interesting topics for further investigations.

The retrospective nature of the study on HC caused several limitations. Information about the development, progression, reversibility, importance of early detection, and long-term sequelae of this condition is needed. Monitoring horses with or at risk of developing hypertension (e.g., horses with laminitis, signs of chronic pain, chronic renal failure, or metabolic syndrome) may help determine the clinical relevance of this equine

cardiomyopathy and identify interventions that could assist in the management of such cases. Particularly the development of arrhythmias, or sudden cardiac death appears important due to the frequent description of these in humans with HHD and the potential consequences of these sequelae on safety of riders and horse handlers.

The association of hypertension and HHD with EMS should be investigated. This could be helpful to the understanding of this disease in horses and the potential for translational research in such a prevalent and remarkably important human condition makes it stand out as a target for future investigations. We are hopeful that prospective studies in this area will be designed in the near future.

Due to the controversy about the accuracy of the current methods to measure blood pressure, validating the available blood pressure monitors against IBP measurements also seems necessary. The validation of ambulatory blood pressure monitors that would be easily adaptable to horses and would eliminated the factor of horse handling in the pressure readings would be interesting. These studies would benefit investigators in cardiovascular medicine and also investigators in pain management and animal welfare, as blood pressure is a good marker of pain or sympathetic activation.

The absence of thromboembolic complications in horses with atrial fibrillation despite the presence of a clinicopathologically demonstrable hypercoagulable state is intriguing. It would be interesting to assess a larger group of horses and horses with atrial fibrillation

and comorbidities that would predispose to thromboembolism such as systemic inflammation/sepsis, gastrointestinal disease, postoperative period, neonatal sepsis, or EHV-1 infection. Coagulation and fibrinolysis can also be affected by exercise and it would also be interesting to assess this in exercising horses with atrial fibrillation.

The studies presented in this thesis have answered some relevant questions and have raised many others. Questions that could benefit the quality of medical care that we provide to horses should be answered. Due to horse-human interactions, horses' health affects the safety of many people. Moreover many equine cardiovascular diseases are shared with humans and provide opportunities for translation research.

APPENDICES

APPENDIX A- ABBREVIATIONS

TDI	Tissue Doppler Imaging
2DST	Two-dimensional speckle tracking
ECG	Electrocardiogram
CO	Cardiac output
MAP	Mean arterial pressure
SVR	Systemic vascular resistance
IBP	Invasive blood pressure
NIBP	Non-invasive blood pressure
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CK-MB	Myocardial bound creatine kinase
PT	Prothrombin time
aPTT	Activated partial thromboplastin time
FDPs	Fibrinogen degradation products
TEG	Thromboelastography

DIC	Disseminated intravascular coagulation
AT	Antithrombin activity
DO2	Oxygen delivery
VO2	Oxygen consumption
SV	Stroke volume
HR	Heart rate
[Hb]	Hemoglobin concentration
CaO2	Arterial content of oxygen
CvO2	Venous content of oxygen
SaO2	Arterial hemoglobin saturation of oxygen
PaO2	Arterial partial pressure of oxygen
HHD	Hypertensive heart disease
HC	Hypertensive cardiomyopathy
FS	Fractional shortening
IVSd	Interventricular septal thickness at end diastole
IVSs	Interventricular septal thickness at peak systole
LVFWd	Left ventricular free wall thickness at end diastole
LVFWs	Left ventricular free wall thickness at peak systole

LVIDd	Left ventricular internal diameter at end diastole
LVIDs	Left ventricular internal diameter at peak systole
LVM	Left ventricular mass
MWT	Mean wall thickness
RWT	Relative wall thickness
PCV	Packed cell volume
CVP	Central venous pressure
O2ER	Oxygen extraction ratio
EMS	Equine metabolic syndrome

APPENDIX B – FORMULAS

$CO = MAP \times SVR$

$CO = SV \times HR$

$CaO_2 = 1.39 \times [Hb] \times SaO_2 + 0.003\ PaO_2$

$DO_2 = CO \times CaO_2$

$VO_2 = CO \times (CaO_2 - CvO_2)]$

$O_2\ ER = VO_2/DO_2 = [CO \times (CaO_2 - CvO_2)] / CO \times CaO_2 = CaO_2 - CvO_2 / CaO_2$

$FS = (LVIDd - LVIDs) / LVIDd$

$MWT = (LVFWd + IVSd)/ 2$

$RWT = (LVFWd + IVSd) / LVIDd$

$LVM = 1.04\ [(LVIDd + LVFWd + IVSd)^3 - LVIDd^3] - 13.6$