Morphological evidence of a potential arrhythmogenic substrate in the caudal and cranial vena cava in horses

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Abstract

Background: Three-dimensional electro-anatomical mapping, previously performed in horses with atrial arrhythmias, has demonstrated the medial region of the caudal vena cava (CaVC), 1-8 cm caudal to the fossa ovalis, as an anatomical predilection site for atrial tachycardia associated with areas of slow conduction and conduction block. Slow conduction has also been recorded in the cranial vena cava (CrVC).

Objectives: To investigate the morphological characteristics of the myocardial sleeves (MS) in the CaVC and CrVC, in order to identify a potential substrate of right sided atrial arrhythmias.

Study design: Cross sectional

Methods: Postmortem dissection of 37 hearts from adult warmblood horses without known cardiovascular disease. Macroscopic examination of the myocardial distribution, evaluated the MS area, length, width, and shape in the CaVC and the CrVC. At least in 2 samples from each vena cava MS were histologically examined using Masson's trichrome staining.

Results: Myocardial sleeves into the medial CaVC and into the CrVC were observed in all horses and showed variations in distribution, shape, and size between horses. Their mean ± standard deviation length from the limbus into the CaVC reached 5.7 ± 1.0 cm (maximum 8.3 cm), and from the azygos vein into the CrVC 5.3 ± 1.6 cm (maximum 8.6 cm). Myocardium-free islands were observed in the

CaVC and CrVC in 30% and 6% of horses, respectively. Histologically, MS showed a non-uniform myocardial fibre arrangement, with presence of fibroadipose tissue, features known to result in slow conduction and pro-arrhythmia.

Main limitations: Study only included horses without history of atrial arrhythmia.

Conclusions: Myocardial sleeves are present in both CaVC and CrVC, showing anatomical variations between horses. Tissue characteristics known to favour re-entry were identified indicating that these venae cavae MS are a potential substrate for atrial tachyarrhythmias and a target for treatment by ablation.

Introduction

Atrial fibrillation (AF) is the most common atrial tachyarrhythmia in horses affecting their performance, characterized by a fast and chaotic, self-sustaining electrical activation of the atria. The electrocardiogram shows irregular baseline fibrillations, absence of P waves, normal QRS morphology and irregular RR intervals.[1; 2] Local atrial fibrillation cycle lengths are typically around 150-175 ms in the right atrium[3; 4] but slower (190-242 ms) in the caudal vena cava,[5] which could be related to slower conduction in this region.

Atrial fibrillation requires a suitable substrate (atrial myocardium) to maintain AF and a trigger to start it. Although AF in horses has often been reported as 'lone AF,' which means AF without apparent detectable underlying disease, it becomes increasingly clear that deviant electrophysiological and structural characteristics are often present[6; 7] but remain undetected with routine diagnostics. Fibrosis, linked to obesity, metabolic syndrome, athlete's heart, and systemic inflammation, is often present in human cases and results in increased AF susceptibility.[8-10] Fatty infiltration of the atrial myocardium has also been described as a substrate for AF in older dogs[8] and in obese humans.[11] On electro-anatomical mapping, these areas appear as low voltage areas, often presenting conduction delay, pivotal in the mechanism of re-entry circuits. Factors that trigger AF include atrial ectopic rhythms such as atrial premature depolarizations or runs of atrial tachycardia caused by a rapidly firing focus or local re-entry mechanisms. In human patients, rapid atrial ectopic rhythms very often originate from myocardial sleeves (MS) extending into the vein wall, especially in the pulmonary veins but also the superior (cranial) vena cava.[12-14] Little is known about the specific triggers for AF in horses, but similar mechanisms have been suggested.[1; 2] In fact, atrial tachycardia (AT) from the caudal vena cava region has been shown to be able to trigger AF in horses.[15; 16] Atrial tachycardia represents a more organized atrial tachyarrhythmia compared to AF and is characterized by a rapid and regular activation of the atria, usually due to a local re-entry mechanism. In horses, the electrocardiogram at rest shows identical P' waves with stable P'P' intervals at a rate of around 140-220 per minute, while the

conduction to the ventricles is usually irregular with normal QRS morphologies.[1] Three-dimensional electro-anatomical mapping in horses with AT showed that in most cases a local re-entry circuit was situated in the medial wall of the caudal vena cava, caudal to the fossa ovalis (FO),[17; 18] characterized by an area of slow conduction around a line of conduction block. While AT can deteriorate spontaneously into persistent AF,[15; 16] it is also known that treatment of AF by quinidine sulphate or transvenous electrical cardioversion can change AF into AT. It therefore seems that, at least in some horses, MS from the vena cava could be the underlying trigger of AF and might be a treatment target for AF.

The aim of this study is to investigate the gross anatomical and histological characteristics of the MS in the CaVC and CrVC, to identify a potential substrate for atrial tachyarrhythmias and provide data that might be useful when performing radiofrequency ablation of this area.

Material and Methods

Study population

The study was performed following the guidelines of the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium. Thirty-seven hearts from adult warmblood horses without known history of cardiovascular disease were used (11 from a slaughterhouse, and 26 from horses euthanized for non-cardiac reasons after owner consent was obtained). Thorough cardiac exams prior to death or euthanasia were not performed. The horse population consisted of 20 mares and 17 stallions or geldings and varied in age from 1 to 26 years (mean ± standard deviation: 14 ± 6 years). In the 26 horses donated for research, the body weight was measured post-mortem and was between 470 to 620 kg (550 ± 43 kg), and the body condition score BCS, using the 5-point scale method,[19] was between 3/5 and 5/5 (4 ± 1).

Gross anatomy

Collection and examination of the hearts and great vessels was performed within 24 hours after death. The right atrium was exposed after cutting through the sinus venarum cavarum (facies atrialis), starting at the lateral wall of the CrVC towards the right atrium and further towards the lateral wall of the CaVC (Figure 1). In 27 horses both veins were studied, while in 7 horses only the CaVC and in 3 horses only the CrVC could be examined because the vein was sectioned too short during removal of the heart from the thorax. In order to standardize the stretching on the CaVC and CrVC, the veins were secured with a clamp and stretched using a newton meter at an arbitrary force of 3 Newton (Figure 2). Using a Leica 48MP camera, standardized images with a scalebar at the same level as the vein were taken and measurements were made using the software ImageJ (version 1.53c by the national institute of health). Based on macroscopic observation, the delineation of the edges of the MS area (sharply demarcated, blurred edges), as well as the appearance of the endocardial surface (homogenous colour, patchy, pale areas) were noted. Measurements of the MS were made in relation to structures that are identifiable on ultrasound, such as the tuberculum intervenosum, the limbus of the FO, the coronary sinus and the crista terminalis (Figure 1; Figure S1). In the CaVC, the length of the MS was defined as the distance between the centre of the limbus of the FO and the most caudal extent of the MS. The width was measured as the widest dorsoventral distance. The length-to-width ratio was used to evaluate the shape of the MS area, whereby a ratio of 1 indicated a circular shape, a ratio >1 an oval shape in craniocaudal direction and a ratio <1 an oval shape in dorsoventral direction. The presence of a ventral extension was recorded and its length measured. The surface area occupied by the MS was also determined on the images of the stretched caudal vena cava using the 'freehand selection' tool of the ImageJ software by manually tracing the macroscopically visible border of the myocardial sleeves. The distance from the caudal edge of the MS area to the tuberculum intervenosum was measured by a straight line passing through the middle of the limbus, while the distance to the middle of the coronary sinus opening was measured in a straight line between the two anatomical locations. On the CrVC, the length of the MS was measured dorsally, from the outlet of the azygos vein, and ventrally, from the

crista terminalis. The depth of the myocardial indentation, separating the narrow dorsal extension from the broader ventral extension of the sleeve, was also determined. Finally, the distance between the centre of the azygos vein and the tuberculum intervenosum was measured.

Histology

Two to three 3-5 cm samples of the edge of the MS area from the CaVC (dorsal, medial and most caudal edge) (Figure 3A) and two samples from the CrVC (dorsal and ventral) were histologically examined. After formalin fixation and paraffin embedding, 8 µm thick slides were stained using Masson's trichrome technique. On light microscopy, longitudinal sections of the venous wall were analysed and the location of the MS within the layers of the vein (tunica intima, media, adventitia) were determined. The thickness of the vein was evaluated at three positions on both CaVC and CrVC. The first measurement was made at the edge of the MS area and including the MS, the next measurement was at 1 cm from the edge of the MS towards the right atrium and the third measurement was of the vein without MS (Figure 3B). Finally, the depth of the MS was measured as the distance from the endocardial surface to the start of the MS.

Data analysis

Normality Assessment:

Quantile-Quantile (Q-Q) plots were utilized for normality evaluation using Microsoft® Excel® 2019 (Version 2309). Data that was normally distributed was presented as mean ± standard deviation.

Statistical Tests:

To explore potential differences between males and females and across different age groups, t-tests were performed on the following variable: length, width, length to width ratio, surface area, and the presence of ventral myocardial extension on the MS of the CaVC, the dorsal and ventral length of the MS in the CrVC and the wall thickness of the CaVC and CrVC. The resulting p-values were subsequently assessed to determine whether they were greater or less than 0.05.

Results

Myocardial sleeves were observed in both caudal and cranial vena cava of all horses. The results of measurements performed on both veins are presented in Table 1. Q-Q plots verified normal distribution in our data; therefore, our findings are presented as mean ± standard deviation. Macroscopically, the MS area in the caudal vena cava was craniocaudally oval shaped in 53% of horses, dorsoventrally oval shaped in 26%, and circular in 21% of horses (Figure 4). A “tongue-like” ventral myocardial extension was present in 65% of horses although it was sometimes not well defined. The caudal delineation in the CaVC appeared obscured, often with blurred edges. The endocardial surface of the MS in the CaVC was heterogenous, with pale regions. Within this area, a greyish-white zone, representing the floor of the FO, could be identified in all horses medial and just caudal to the limbus. In 30% of horses, an additional, well-described pale area, with a similar appearance as the CaVC wall, was found in the caudal part of the MS area. This pale area appeared as an island on the medial aspect of the vein, surrounded by a border of MS.

In the CrVC, the endocardial surface of the MS had a homogenous and more reddish colour. The cranial border of the MS appeared macroscopically sharply delineated, perpendicular to the longitudinal axis of the vein. It had a dorsal indentation in 80% of horses. A pale area, surrounded by myocardium, was visible in the CrVC in 6% of the horses and was located ventromedially to the dorsal myocardial indentation (Fig. 5).

On light microscopy, in both CaVC and CrVC, the MS were localized in the tunica adventitia, often adjacent to the tunica media (layer with smooth muscles). The myocardial fibres displayed a non-uniform and disorganized arrangement, evident under light microscopy as fibres intertwining in longitudinal, oblique, and cross-sections (Figure 3C). Fibroadipose tissue was identified surrounding and between the myocardial fibres of the CaVC (Figure 3D) and to a lesser degree in the CrVC. Cardiac myocytes occupied one to two thirds of the wall thickness. The wall thickness at various levels of the MS in the dorsal, medial and caudal edge of the CaVC were similar. Also the dorsal and ventral samples

of the CrVC showed a similar thickness. At the edge of the MS, the wall thickness of the vein including the MS was 1.1 ± 1.0 mm and 1.2 ± 0.9 mm in the CaVC and CrVC, respectively. The total wall thickness including the MS at 1 cm from the edge of the MS was 1.6 ± 0.1 mm in the CaVC and 2.0 ± 0.1 mm in the CrVC, while the wall thickness of the vein (without MS) was 0.7 ± 0.4 mm, and 0.6 ± 0.3 mm in the CaVC and CrVC, respectively. The MS was found at a depth of 0.4 ± 0.1 mm and 0.3 ± 0.1 mm from the endocardial surface, in the CaVC and CrVC, respectively. At the level of the pale "island" that could be observed macroscopically in both veins, the three vein layers (tunica intima, media and adventitia) were present but there was an absence of MS (Figure 6).

Analysis of the size (length, width, length-to-width ratio and surface area) and presence of ventral myocardial extension of the CaVC MS, the size (dorsal and ventral length) of the CrVC MS, and the wall thicknesses of the CaVC and CrVC, revealed no statistically significant differences between males and females or between age groups.

Discussion:

In this study we describe the presence of MS in the CaVC and CrVC of warmblood horses. We show that these MS have typical macroscopic and microscopic changes that have been associated with arrhythmogenesis in other species.

Important variations between horses were found in the shape and length of the MS in both veins, especially in the CaVC. In human patients, the shape and size of the MS in the venae cavae are known to affect susceptibility to arrhythmias. The long MS in the superior (cranial) vena cava promotes the development of AF in human patients,[20; 21] but the MS of the inferior (caudal) vena cava, which is usually small and regularly delineated, is rarely an origin of arrhythmias.[22-26] In horses, however, the MS area in the CaVC has more tissue characteristics that are associated with arrhythmogenesis compared to that of the CrVC, considering it's irregular and poor delineation. This corresponds to 3D electro-anatomical mapping results from horses with AT as in almost all of them the arrhythmia was based upon a local re-entry circuit located caudal to the FO.[27] These re-entry circuits typically showed

a central line of block surrounded by a circular zone with slow conduction.[15] Our microscopic data showed disorganized myofiber orientation and fibroadipose tissue at the level of the MS. In humans, these histological characteristics are well known to result in slow conduction, an important feature for re-entry.[27; 28]

In addition, islands without myocardium were found in the MS area, surrounded by myocardial fibres in different orientations. This results in an area without conductive properties, which would appear on mapping as an area of conduction block, surrounded by slow conduction as a result of the presence of disorganized fibres. The combination of these characteristics creates an ideal substrate for a re-entry circuit. These myocardium-free islands were located in the same area as where conduction block was found on the electro-anatomical maps of horses with AT.[15; 16] However, as our horses had not been screened for the presence of atrial arrhythmias and no electrophysiological studies had been performed, it is unknow whether these myocardium-free islands increase the risk for developing atrial arrhythmias. Previous studies in horses with sudden cardiac death have shown areas of fibrosis near the sinoatrial node, which is not the same location as the myocardium-free islands observed in our study.[29-31] Myocardium-free islands with similar features to those in the CaVC, were observed in the CrVC in only a few horses, indicating a possible risk of re-entry in this area. However, on mapping and ablation studies performed so far, none of the horses showed an AT originating from the CrVC.[15; 16]

As horses with AT can deteriorate into AF,[15; 16] and because AT shows a high recurrence rate, ablation of a potential arrhythmogenic area of the CaVC might be beneficial in some horses that experience AF recurrence. A 3D electro-anatomical map would first need to be made because of the variation in MS shape and size, and in order to identify areas with slow conduction or conduction block. However, activation mapping cannot be performed during AF, only during a more organized rhythm such as sinus rhythm or AT. This means that cardioversion would first be required. Alternatively, mapping during AF can be performed based upon voltage maps. These voltage maps display local voltages of depolarizing

myocardium, which are lower in MS areas with scar and adipose tissue, compared to healthy myocardium.[32]

Myocardial sleeves in both veins were present adjacent to the endocardial surface, which should allow a good signal registration during intracardiac electrophysiological measurements as well as an easy accessibility for ablation. The thickness of both veins gradually decreases, from the atrial wall into the venous wall, suggesting that when performing radiofrequency catheter ablation, the energy delivery should be reduced when applied further away from the heart, to avoid perforation of the vein.[33; 34] The thickness of both veins, where no MS were present, was similar, approximately 0.6 mm thick. However, considering the average of 34% tissue shrinkage during histological processing, the thickness values in this study are an underestimation.[35]

This study has some limitations. None of the examined hearts came from horses with a history of AT or AF. However, even in the normal horses in this study, typical characteristics of a substrate for arrhythmias were found in the CrVC and the CaVC, being more pronounced in the latter. Whether or not additional pro-arrhythmic properties are present in horses with AT or AF remains to be determined. The presence of specialized cells with conductive characteristics was not evaluated in this study. In humans and dogs, Purkinje-like cells have been reported in the MS of the pulmonary veins and the superior (cranial) vena cava using light microscopy (Masson's trichrome staining and glycogen staining), immunolabeling of the cardiac pacemaker antigen HCN4 and connexins.[36-38] In horses, immunolabeling of Cx43, Cx45, TH, and S100 on the MS of pulmonary veins has revealed adrenergic and non-adrenergic vegetative fibres as well as gap junction proteins, demonstrating a potential role of these MS in the induction and maintenance of atrial arrythmias.[39] An investigation of the immunohistochemical properties of the MS in the venae cavae of horses would be interesting to further evaluate the role of these cells in impulse conduction and conduction disturbance.

Conclusion

Myocardial sleeves are present in both CaVC and CrVC and show anatomical variations between horses. Characteristics known to favour re-entry and slow conduction were identified indicating that MS in both veins of horses are a potential substrate for atrial tachyarrhythmias and a potential target for treatment. Further studies on horses with atrial arrhythmias are required to identify characteristics that predispose to arrhythmias.

Abbreviations

AT: atrial tachycardia

AF: atrial fibrillation

CaVC: caudal vena cava

CrVC : cranial vena cava

FO: fossa ovalis

MS : Myocardial sleeves

Tables

Table 1: Results of the dimensions of the myocardial sleeves (MS) of the caudal (CaVC) and cranial vena cava (CrVC) and distances between MS and important anatomical landmarks in the right atrium. CT, crista terminalis; CS, coronary sinus opening; FO, fossa ovalis; SD, standard deviation; TIV, tuberculum intervenosum.

	Measurements	Mean ± SD	Minimum - Maximum
CaVC	Length (mm)	57 ± 10	36 – 83
	Width (mm)	52 ± 12	26 – 76
	Surface area (mm^2)	258 ± 92	1000 – 4600
	MS to the TIV (mm)	90 ± 13	68 – 125
	MS to the CS (mm)	70 ± 14	42 – 100
	Limbus of FO to CS (mm)	38 ± 8	25 – 61
	Limbus of FO to TIV (mm)	31 ± 6	22 – 46
	CS to TIV (mm)	50 ± 10	34 – 76
	Ventral extension length (mm)	7 ± 8	2 – 35
CrVC	Dorsal length: MS to azygos vein (mm)	53 ± 16	0 – 86
	Depth of the dorsal indentation (mm)	12 ± 10	0 – 35
	Ventral length: MS to CT (mm)	53 ± 9	35 – 70
	Azygos vein to TIV (mm)	68 ± 11	51 – 100

List of legends for figures

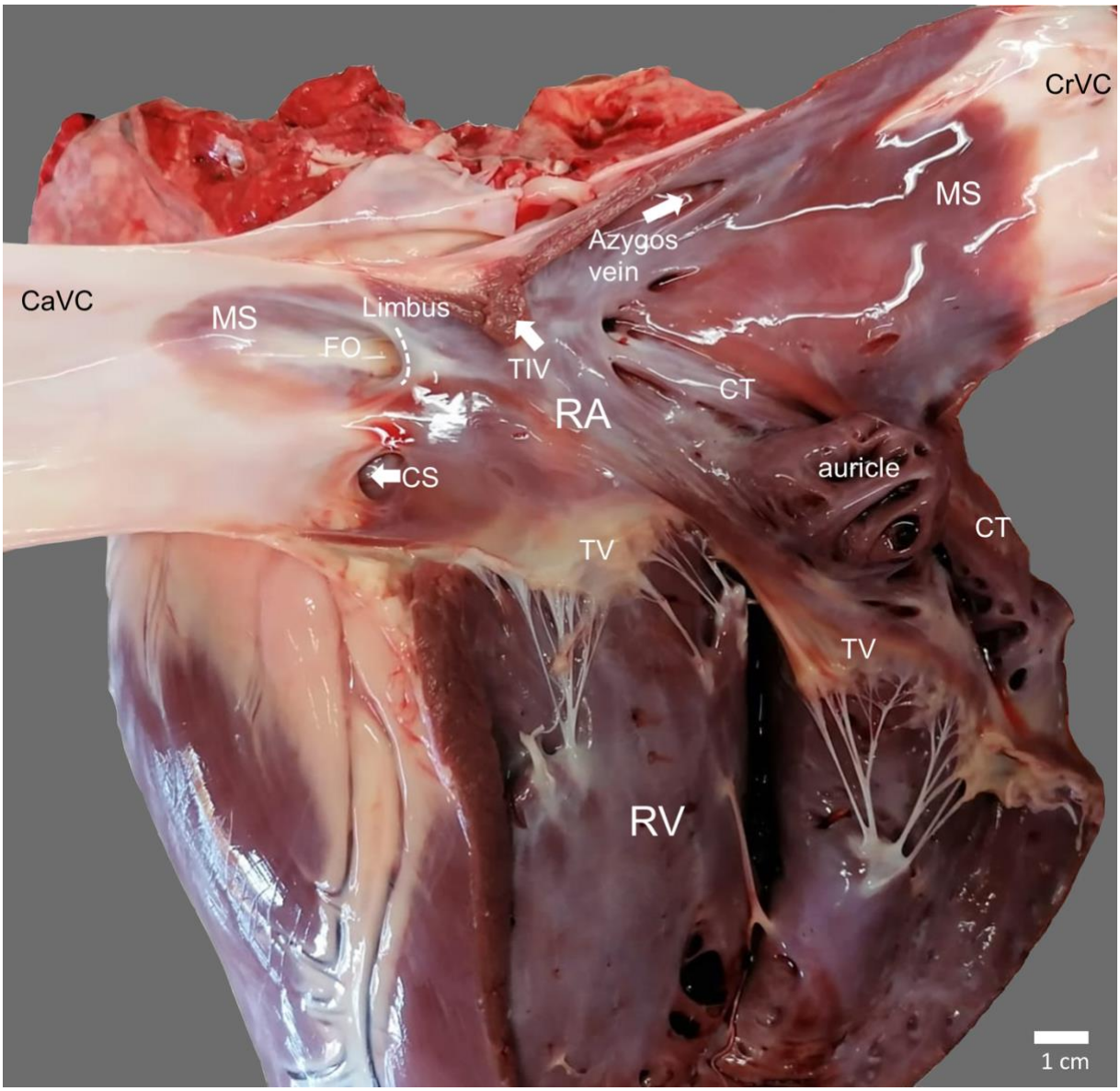


Figure 1: Overview of the right atrium (RA) and ventricle (RV) after opening the right atrial free wall and the ventricular wall along the interventricular septum (facies atrialis view). The dotted line indicates the limbus of the fossa ovalis. CaVC, caudal vena cava; CrVC, cranial vena cava ; CS, coronary sinus opening; CT, crista terminalis; FO, fossa ovalis; MS, myocardial

sleeves; TIV, tuberculum intervenosum; TV, tricuspid valve.

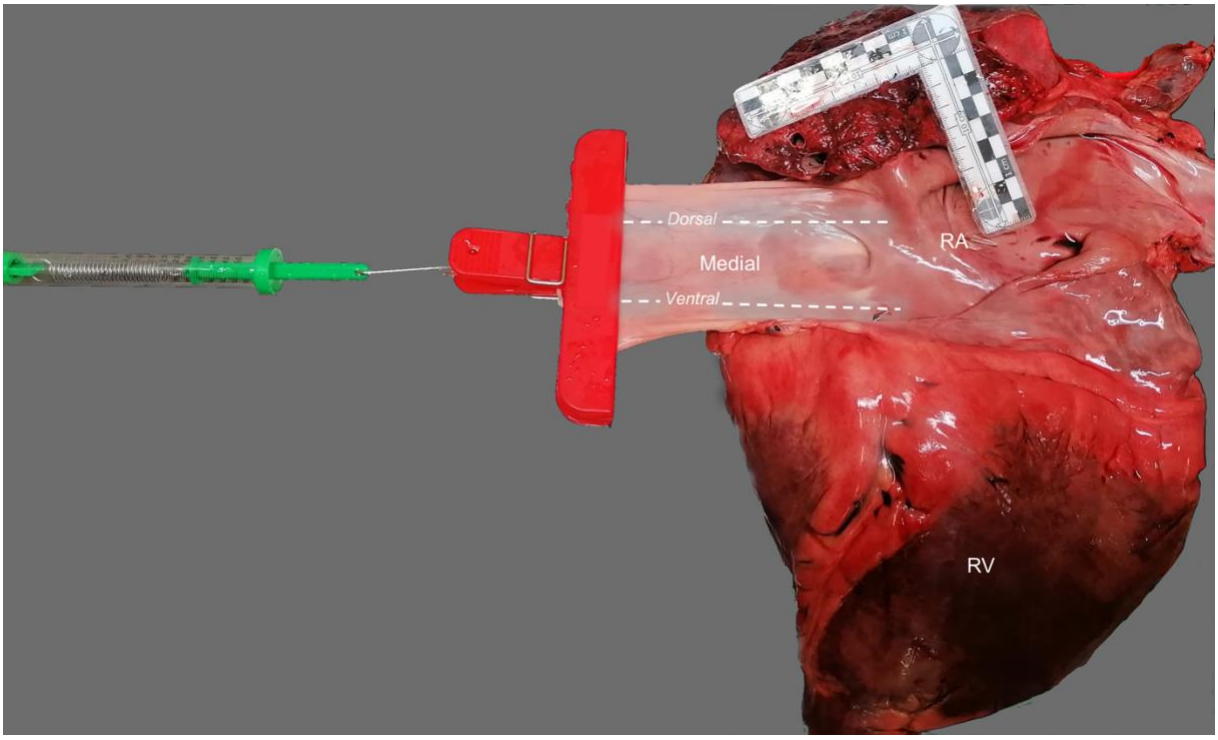


Figure 2: View on the opened sinus venarum cavarum (facies atrialis). The caudal vena cava is stretched by a force of 3 Newton using a clamp (red) and a Newton meter (green). The dorsal, medial and ventral aspects of the caudal vena cava are indicated. RA, right atrium; RV, right ventricle.

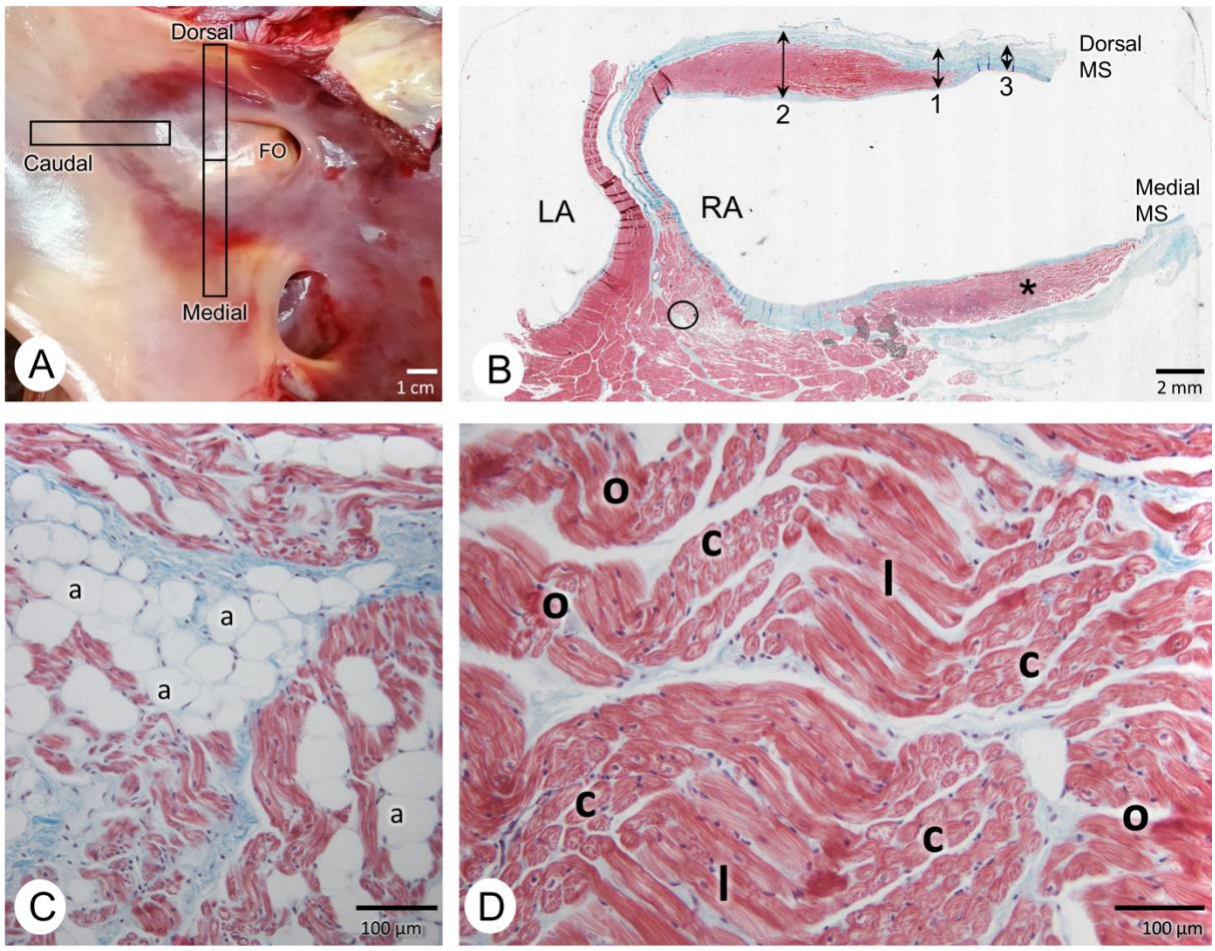


Figure 3: Detailed view from the fossa ovalis (FO) and its surrounding tissue after opening the sinus venarum cavarum from the facies atrialis (A) and indication of the sites where samples for histology were collected (black rectangles: dorsal, medial, caudal edge). Histological images using Masson's trichrome staining (B-D) of the dorsal and medial myocardial sleeves (MS). B: The wall thickness was measured at (1) the edge of the MS area and including the MS, (2) 1 cm from the edge of the MS towards the right atrium, and (3) the vein without MS. The black circle indicates the location where image C was taken and the asterisk (*) the location where image D was taken. C: Abundance of connective tissue (stained in blue) and adipose tissue (a) is seen. D: Fibers in cross-section (c), longitudinal (l) and oblique sections (o) are visible.

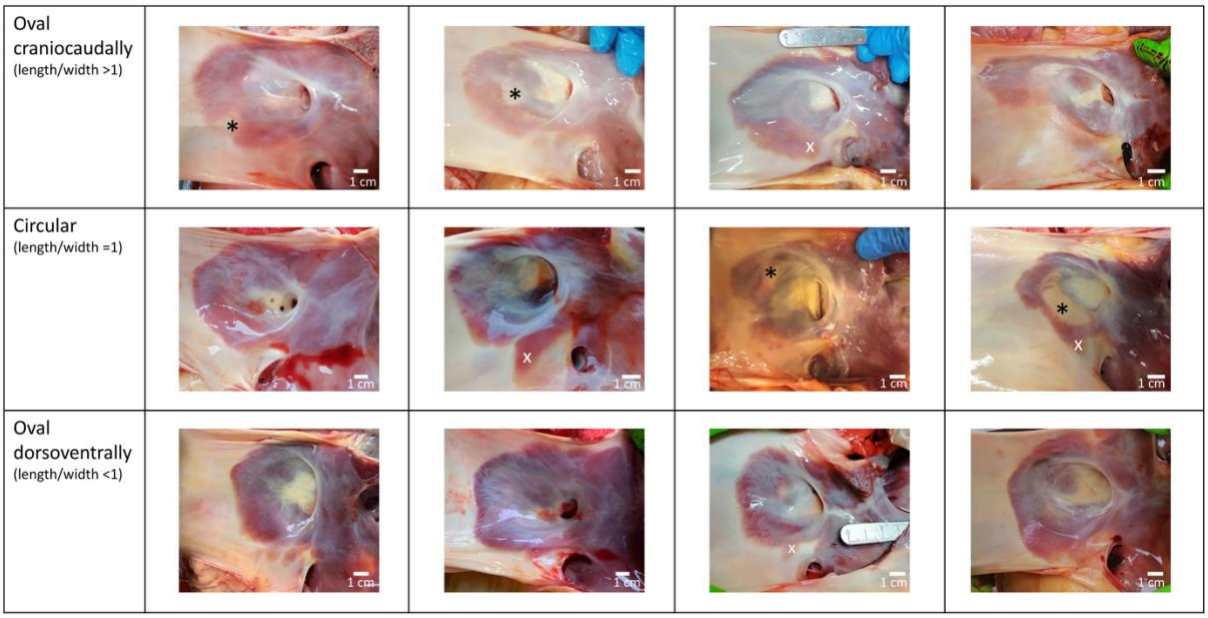


Figure 4: Overview of the different shapes of myocardial sleeve arrangement in the caudal vena cava: oval craniocaudally, circular and oval dorsoventrally. All pictures are taken from the facies atrialis after opening the sinus venarum cavarum. “Tongue-like” ventral myocardial extensions (white X) are often present. Myocardium-free islands (black *) are visible and are a possible region for re-entry.

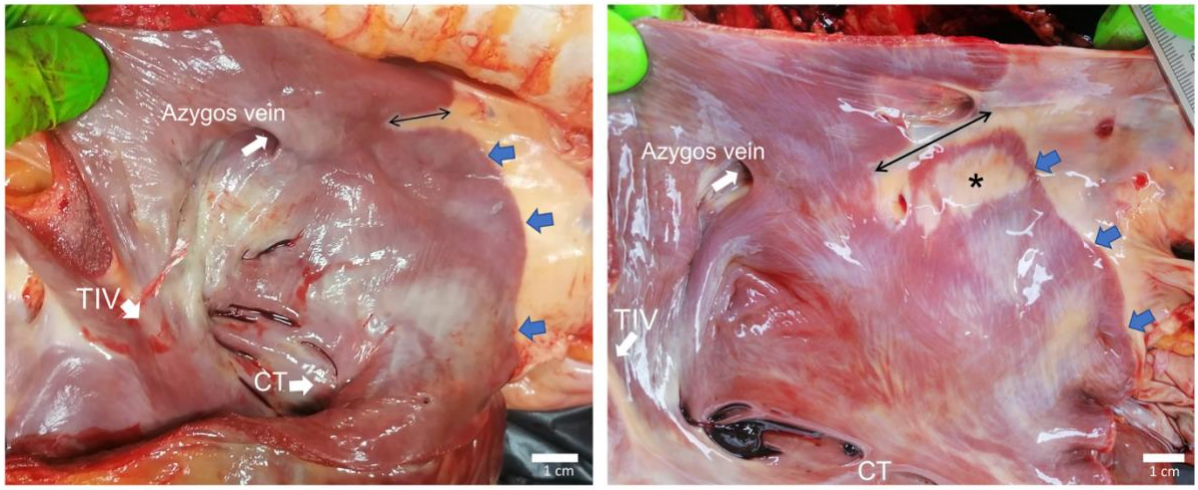
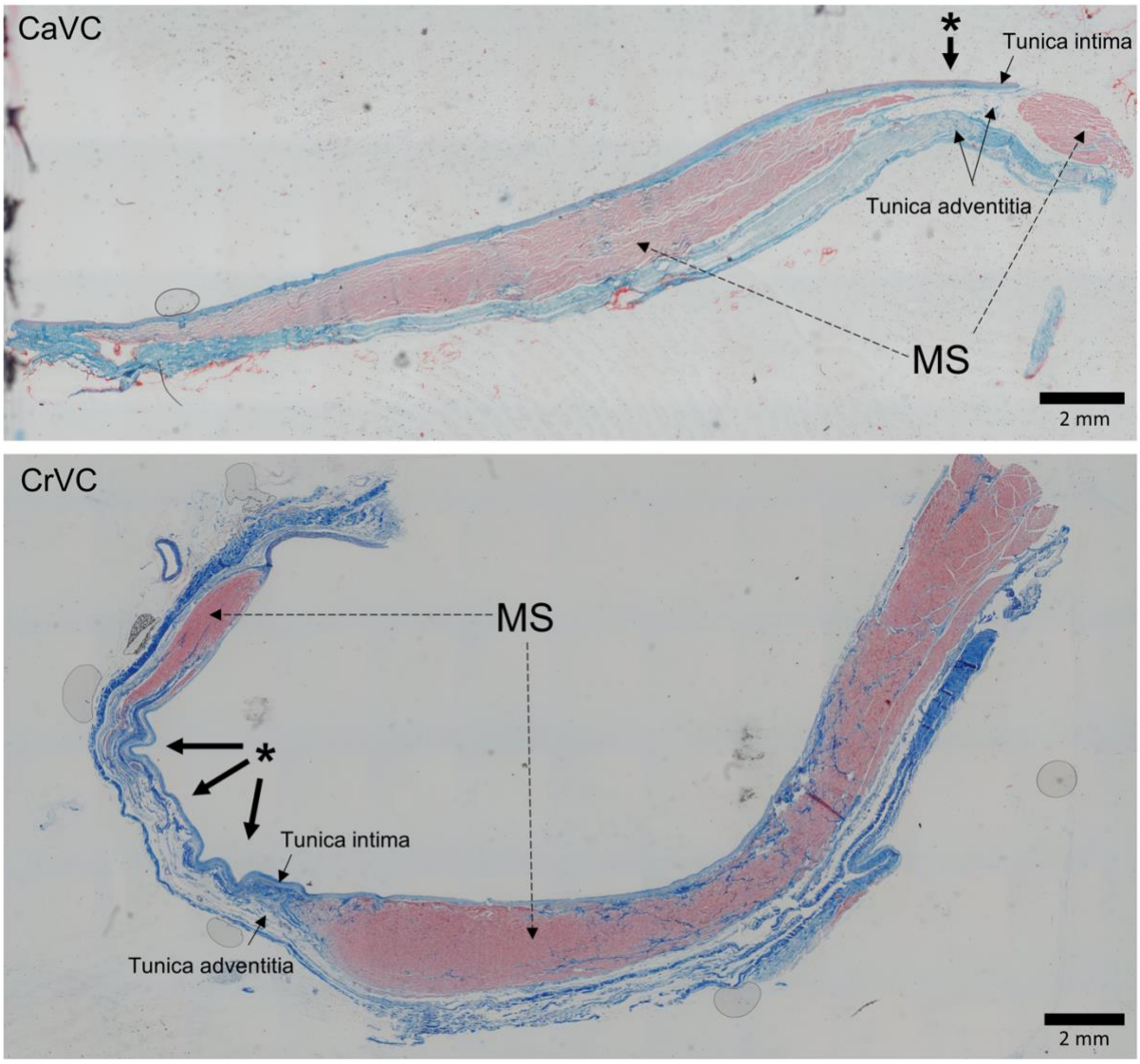


Figure 5: View on the opened cranial vena cava in 2 different hearts. Right is cranial and top is dorsal. On the left image, a homogenous, reddish endocardial surface and a sharply delineated cranial edge of the myocardial sleeves (MS), seen in the majority of horses, is visualized. The

281 right image shows the pale area (*) surrounded by the myocardial sleeves, found in 6% of
282 horses. The black arrow represents the dorsal MS indentation, and the blue arrows represent
283 the cranial edge of the MS. TIV, tuberculum intervenosum; CT, crista terminalis.



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285 **Figure 6:** Histology of the caudal vena cava (CaVC) and cranial vena cava (CrVC) at the level
286 where a pale island (*) was observed in the myocardial sleeves (MS). In that area, myocardial
287 fibres were absent. Masson's trichrome staining was used. Dorsal is inside the vein lumen,
288 ventral is outside the vein, and right is closest to the heart.

List of legends for Supplementary items

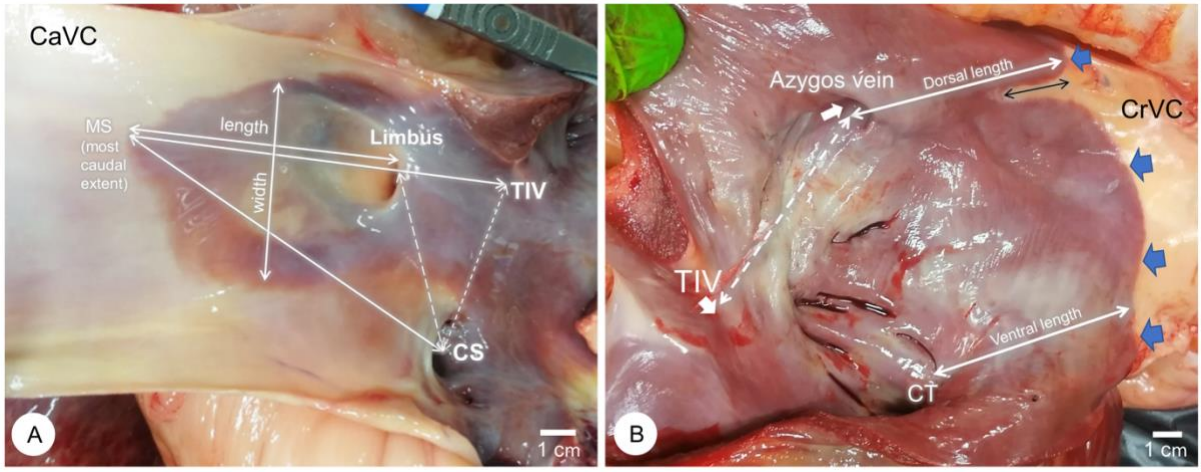

Figure S1: Measurements on the stretched caudal vena cava (CaVC) (A), after opening the sinus venarum cavarum along the entire length of its free wall (view from the facies atrialis). Length is the distance from most caudal extent of the myocardial sleeves (MS) to the center of the limbus of the fossa ovalis, and width is the widest dorsoventral measurement. Dashed lines indicate measurements taken between identifiable landmarks on ultrasound. Measurements on the stretched cranial vena cava (CrVC) (B) after opening the lateral wall of the right atrium. The dashed line indicates the measurement taken between identifiable landmarks on ultrasound. The black arrow represents the dorsal MS indentation, and the blue arrows represent the cranial edge of the MS. Right is cranial and top is dorsal. CS, coronary sinus opening; CT, crista terminalis; TIV, tuberculum intervenosum.

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