J. Comp. Path. 2016, Vol. 154, 225–230

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SPONTANEOUSLY ARISING DISEASE

# Morphometric Properties of the Thoracic Aorta of Warmblood and Friesian Horses with and without Aortic Rupture

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## Summary

Rupture of the aorta is much more common in Friesians compared with other breeds of horse. Rupture always occurs adjacent to the scar of the ligamentum arteriosum. Previous histological examination of ruptured aortic walls suggested the presence of an underlying connective tissue disorder. Therefore, the aim of the present study was to compare the structural characteristics of the tunica media of the mid-thoracic aorta, distant to the lesion, in warmblood and Friesian horses with and without thoracic aortic rupture. In unaffected Friesian horses, the thickness of the tunica media, as well as the percentage area comprised of collagen type I, were significantly higher compared with the warmblood horses, supporting the hypothesis of a primary collagen disorder in the Friesian horse breed. However, in the tunica media of the affected Friesian horses there was no significant wall thickening. Moreover, the percentage area comprised of elastin was significantly lower, while the percentage area comprised of smooth muscle was higher, compared with unaffected Friesian and warmblood horses. These lesions are suggestive of an additional mild elastin deficiency with compensatory smooth muscle cell hypertrophy in affected Friesians.



*Keywords:* aorta; horse; morphometry; rupture

## Introduction

The aorta is a crucial dynamic functional unit in the cardiovascular system (Watanabe *et al.*, 1993). It is composed of multiple constituents that ensure the proper structure and function of the wall. The tunica media plays a major role in aortic stability (Dingemans *et al.*, 2000) and is characterized by lamellar units, consisting of elastin, smooth muscle cells and collagen (Clark and Glagov, 1985). Elastin and collagen impart the aortic elastic properties and tensile strength, respectively (Holzapfel *et al.*, 2000). Elastin is the main component of the thoracic aorta (McCloskey and Gleary, 1974). The aortic collagen consists predominantly of types I and III collagen, which account for 80–90% of the total collagen in the aortic media (Dingemans *et al.*, 2000; Silver *et al.*, 2001). Type I collagen is the major structural component of the vessel wall and type III collagen is mainly a reparative component (Raman *et al.*, 2011).

Rupture of the aorta is extremely rare in horses. When it occurs, it is typically located near the junction with the heart (Sleeper *et al.*, 2001). In Friesian horses, aortic rupture is much more common and occurs as a transverse tear near the ligamentum

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2016.02.001

arteriosum. The rupture is often associated with a dissection, periaortic haematoma or pseudoaneurysm that ruptures into the pulmonary artery (Ploeg *et al.*, 2013). Abnormalities in both elastin and collagen amount and structure could cause a weakening of the aortic wall, resulting in rupture (Tsamis *et al.*, 2013). Details of the composition and structure of the aortic wall in different breeds of horses have not been reported.

The aim of the present study was to analyze the morphological characteristics of the tunica media of the equine thoracic aorta in order to gain insight into the possible role of different structural components in the pathogenesis of aortic rupture in Friesian horses. Therefore, the tunica media structure of the mid-thoracic aorta was compared in Friesian horses with aortic rupture, healthy Friesian horses and warmblood horses.

## Materials and Methods

### *Animals*

The animals investigated were divided into three groups. Group WB comprised of 17 warmblood (WB) horses (0–10 years old, median age 4 years). Twelve of these horses were presented for post-mortem examination for reasons unrelated to the cardiovascular system at either Utrecht University ($n = 5$) or Ghent University ($n = 7$). The other horses were sampled at a Belgian slaughterhouse ($n = 5$).

Group NAF consisted of 18 non-affected Friesian (NAF) horses (0–10 years old, median age 4 years). All but one of these horses were presented for post-mortem examination for reasons unrelated to the cardiovascular system at either Utrecht University ($n = 3$) or Ghent University ($n = 14$). One horse was sampled at a Dutch slaughterhouse.

Group AF consisted of 20 Friesian horses with an aortic rupture (affected Friesian horses, AF; 1–10 years old, median age 5 years). All were diagnosed with aortic rupture during post-mortem examination at either Utrecht University ($n = 8$) or Ghent University ($n = 12$).

All horses that were admitted alive to the University hospital were treated following the institutional guidelines. Formal ethical approval was waived by the chairperson of the ethical committee, based on Belgian and European legislation (EU directive 2010/63/EU), as all tissues were derived *post mortem*.

### *Sampling and Sample Preparation*

The complete thoracic aorta was removed from the heart base to the diaphragm. The surrounding connective tissue was removed. Samples were taken from the middle of the thoracic aorta and fixed in 4% neutral buffered formalin for at least 24 h and then processed routinely and embedded in paraffin wax. Sections were stained with haematoxylin and eosin (HE). For demonstration of elastin and smooth muscle, sections (5 μm) were labelled with monoclonal mouse anti-human elastin antibody BA-4 (Leica Biosystems, Diegem, Belgium; 1 in 600 dilution) or monoclonal mouse anti-human smooth muscle actin (Dako, Brussels, Belgium; dilution 1 in 200), respectively. Immunolabelling was achieved with a highly sensitive horseradish peroxidase diaminobenzidine kit (Envision DAB + kit, Dako) in an automated immunostainer (Dako).

For collagen I and III labelling, sections (3 μm) were pretreated with normal horse serum (1 in 10 dilution) and then incubated with monoclonal mouse anti-collagen type I (Sigma, St. Louis, Missouri, USA) or monoclonal mouse anti-collagen type III (Abcam, Cambridge, UK). 'Visualization' was with biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, California, USA), ABC/PO complex solution elite (Vector) and diaminobenzidine chromogen.

### *Morphometry*

Tissue sections were randomized and examined in blinded fashion. HE-stained sections were used to evaluate lesions of the aortic media. The thickness of the media (defined as the perpendicular distance between the innermost and outermost elastic lamella of the aortic media) was measured on sections labelled to show elastin under low-power magnification (×25). Three digital image frames were taken for each specimen. One measurement was performed for each image and the averages of the three images were calculated.

The percentage areas comprised of elastin, collagens type I and III and smooth muscle actin were determined by image analysis. The measurements were made with a light microscope to visualize the tunica media at a magnification of ×400 using a Leica DFC320 camera (Leica Microsystems, Wetzlar, Germany) coupled to a computer-based image analysis system LAS v.3.8. (Leica Microsystems). Three (collagen) or four (elastin and smooth muscle actin) image frames were taken per slide.

Fragmentation of the elastic fibres in the tunica media was scored from 0 (no fragmentation) to 4 (severe fragmentation) according to the system described by Carr-White *et al.* (2000). Fragmentation of collagen I and III fibres was scored using a scale from 0 (no fragmentation) to 3 (severe fragmentation).