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Spontaneously arising disease

Corrugated intimal surface of the ovine aorta: when physiology resembles pathology

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ABSTRACT

The aortic lumen in healthy animals is characterized by a smooth, whitish surface, but sheep have macroscopic corrugation of the intimal surface in the thoracic aorta (TA). Our aim was to determine if this finding was pathological or physiological. Thirteen sheep aortas were included in this work together with aortas from cattle (n = 3), a goat (n = 1), horses (n = 4), dogs (n = 2), rabbits (n = 2) and a pig (n = 1). A corrugated intimal surface in the TA was seen in all the sheep and the goat but was less evident in the cattle. Histologically, in sheep the TA intimal surface was seen to have multifocal bulging areas that protruded into the lumen. The outer half of the tunica media had numerous, randomly distributed muscle islands that disrupted the arrangement of the elastic lamella, displacing them towards the lumen. We conclude that the intimal corrugation of the TA in sheep is physiological and must not be misinterpreted as pathological.

The aorta is the largest blood vessel of the body. It originates in the left ventricle of the heart and pumps oxygenated blood at high pressure to the ascending aorta. It subsequently forms the aortic arch and continues caudally through the thorax as the descending thoracic aorta (TA) [1]. Microscopically, the aorta is arranged in three concentric layers: the inner tunica intima, the intermediate tunica media and the outer tunica adventitia. According to size and histological structure, the arterial system is classified into large elastic arteries, muscular arteries of medium and small calibre, and arterioles. The aorta is an elastic conducting artery, since its tunica media is composed of a variable number of medial lamellar units, structures consisting of an elastic lamella associated with smooth muscle cells and collagen fibres. These structures constitute the functional unit of the vessel wall, determining the viscoelastic properties of the aorta and, consequently, its haemodynamic behaviour [2,3].

Macroscopically, the aortic lumen in healthy animals is characterized by a smooth, whitish surface, which can be affected by several conditions including arterial degenerative disease (eg, arteriosclerosis, atherosclerosis, mineralization and other pathological deposits), hypertrophy (eg, high-altitude disease of cattle, pulmonary arterial hypertension or medial hypertrophy of the pulmonary arteries in cats) and inflammatory processes (eg, polyarteritis nodosa, viral or rickettsial vasculitides or verminous arteritis) [4–10].

In recent years, our research and diagnostic group has studied a grossly evident corrugation of the intimal surface of the TA of healthy sheep. This finding was repetitive and present in sheep free of clinicopathological cardiovascular processes, and was independent of breed, age or sex. The aim of this study was to determine if this intimal corrugation of the TA in sheep corresponded to a pathological change or to a physiological anatomical characteristic.

Sheep included in this investigation came from two different sources: (1) seven 18-month-old Rasa Aragonesa breed lambs belonging to the control group of a previous study [11], which had been injected only with phosphate buffered saline; (2) six female sheep from 4 days to 3.5 years old selected after referral for post-mortem examination by veterinary clinicians (with no ethical approval therefore required). To complete this study, samples of TA from several other animal species were also collected: cattle (n = 3), a goat (n = 1), horses (n = 4), dogs (n = 2), rabbits (n = 2) and a pig (n = 1). As with the sheep, these other individuals had

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been submitted for post-mortem examination. All animals included in the present study were free of any cardiovascular disease.

At necropsy, the endothelial surface of the ascending TA and the aortic arch was visually examined in longitudinal sections. Sections of TA (approximately 3 cm in length) were collected, fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections (4 μm) were stained with haematoxylin and eosin (HE). Additional stains included Masson's trichrome to visualize type I collagen fibres, Orcein van Gieson and elastic van Gieson to detect elastic fibres and methenamine silver for reticular fibres [12]. Von Kossa staining was used for detecting mineral deposits. Finally, a comparative evaluation of pathologies characterized by a similar macroscopic appearance of the intimal surface of the TA of ruminants was performed.

Gross examination of the ovine TA in both groups revealed a corrugated intimal surface in the ascending segment and the aortic arch, in the absence of any other lesion (Figs. 1 and 2). Corrugation intensity varied among sheep and within individual animals. Similar findings were observed in the goat and cattle, although they were less evident in the latter. In contrast, aortas from the other domestic species studied had a diffusely smooth, whitish, endothelial surface in the TA (Supplementary Fig. 1A and B). In other ruminant species, two lesions are characterized by morphological changes similar to the intimal corrugation found in sheep. In cattle, *Solanum glaucophyllum* intoxication (hypervitaminosis D) was characterized by mineralization of the TA wall, resulting in marked irregularity at the intimal surface (Supplementary Fig. 2A). In the goat, dietary lipid imbalances caused vascular atheromatous plaques that were prominent in the TA (Supplementary Fig. 2B).

Fig. 1. Normal thoracic aorta, sheep. Longitudinal section. Diffuse, marked, corrugation of intimal surface.

Fig. 2. Normal thoracic aorta, sheep. Longitudinal section. Intense corrugation of intimal surface at higher magnification.

Histologically, in sheep the TA had marked irregularities at the inner surface, with multifocal bulging areas that protruded into the lumen in the absence of endothelial alterations (Fig. 3; Supplementary Fig. 3A). The outer half of the tunica media of the cranial TA had numerous, randomly distributed muscle islands. These muscle islands disrupted the arrangement of the elastic lamella, causing a discontinuous and wavy appearance of these elements (Fig. 4; Supplementary Fig. 3B–D). Only a few lamellae were seen traversing the muscle islands, while most surrounded the islands (Fig. 4; Supplementary Fig. 3C and D). Collagen fibres were homogeneously arranged between the elastic lamellae of the luminal half, while collagen was more abundant and irregularly distributed at the outer half of the TA (Fig. 3; Supplementary Fig. 3B–D). As in the case of elastic fibres, collagen mostly surrounded muscle islands, while a few fibres traversed them (Fig. 3; Supplementary Fig. 3B and C).

Histological examination of the TA of other species revealed a regular intimal surface and the presence of concentrically arranged elastic lamellae, collagen fibres and smooth muscle cells. No muscle islands were found in any of these species (Supplementary Fig. 4A–D). Lesions reviewed here were characterized by

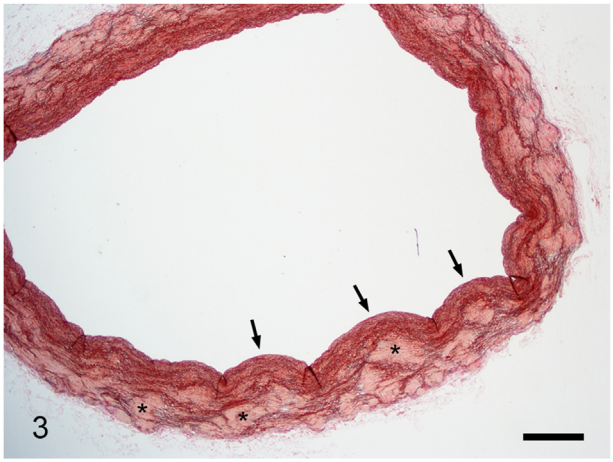

Fig. 3. Normal thoracic aorta, sheep. Intraluminal protrusions (arrows) associated with pale brownish muscle island (*) in adventitial half of thoracic aorta. Orcein van Gieson. Bar, 1,000 μm.

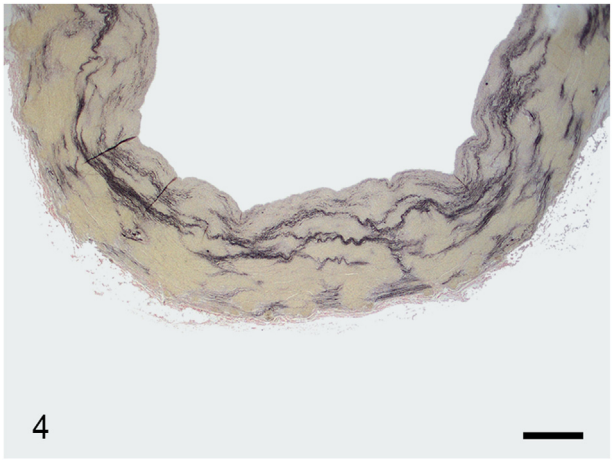

Fig. 4. Normal thoracic aorta, sheep. Pale yellow muscle island altering concentric arrangement of elastic fibres (black) and causing corrugation of intimal surface of thoracic aorta. Elastic van Gieson. Bar, 1,000 μm.

multifocal areas of mineralization, fibrosis and, in the case of the goat, cartilaginous metaplasia (Supplementary Fig. 4E and F).

This investigation demonstrates that macroscopic corrugation of the ovine cranial TA is a physiological feature linked to the concurrent presence of muscle islands. This corrugation may resemble incipient vascular pathology and can be misinterpreted during diagnostic procedures.

To the best of our knowledge, physiological macroscopic endothelial corrugation at the proximal TA of sheep has not been previously described. Two publications in the early 1970s described similar changes in goats as physiological features without clinical significance, but neither report associated the corrugation with the presence of muscle islands [13,14]. Muscle islands are normally conspicuous histological structures in small ruminants, while in cattle and calves they are less noticeable. They are exclusively seen in the outer half of the tunica media of the ascending aorta and the aortic arch in these three ruminant species [15–17].

The intimal corrugation observed in all examined sheep was probably linked to the presence of muscle islands in the outer part of the tunica media. These muscle islands disrupt the parallel arrangement of elastic lamella and fibres, causing an intimal protrusion towards the lumen. It is possible that this corrugation is evident at post-mortem examination partially due to spontaneous contraction of muscle islands and the aortic tunica media. Indeed, many of the animals studied in this work had been euthanized a few minutes before necropsy. In any case, this description is important, as normal gross morphology of the endothelium of the cranial TA in sheep can be misdiagnosed as incipient pathological vascular changes. Indeed, pathological macroscopic findings in the sheep, goat and cows reviewed in our study show a similar aspect to the normal intimal corrugation in sheep, at least at the initial stages [18,19]. With regard to goats and cattle, despite only a few cases having been included in our work, a similar macroscopic corrugation was observed, especially in the goat. As in sheep, this finding is probably related to the microscopic presence of muscle islands, as described in cattle and goats [16,17].

The function of muscle islands is unknown but vascular smooth muscle cells have an important role in vascular physiology and pathology [20]. The ascending aorta receives a turbulent blood flow from the systolic output of the heart and the aortic elastic tissue expands considerably. During diastole, the aorta releases this energy to create a laminar flow, maintaining arterial blood pressure and propelling blood towards the periphery [2]. Muscle islands of sheep and other ruminants may have a function in ensuring this laminar flow but, paradoxically, they create a mildly irregular endothelial surface, which is grossly visible. Muscle islands have been hypothesized to strengthen and regulate the aortic wall elasticity, transforming cardiac pulsatile blood flow to a laminar one and functioning as an auxiliary cardiac pump [15]. However, in other species, similar functions are also achieved by aortas that lack muscle islands.

We conclude that the intimal corrugation of the TA in sheep is a physiological feature linked to the presence of muscle islands in the outer half of the tunica media. The origin and function of these muscular structures are unknown but they do not seem to interfere with normal laminar blood flow. Similar intimal corrugation is seen in other ruminant species, being prominent in goats and more subtle in cattle, but further studies in these two species are required. The presence of intimal corrugation of the TA in sheep should not be misinterpreted as vascular pathology.

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Statement of author contributions

A. Rodríguez-Largo, R. de Miguel, E. Pérez, L. Puzol: Necropsies; Gross examination and description; Sample collection; Some histopathological procedures – resources, methodology. **Á. Gómez, L. Luján, N. Calvo-Sánchez:** Histological evaluations – methodology, investigation, visualization. **J.F. Micheloud:** Cattle and sheep necropsies; Sample collection; Digital material – resources, methodology. **N. Calvo-Sánchez, L. Luján:** Writing – supervision, original draft, review and editing. The other authors contributed to the writing of the manuscript.

Declaration of competing interests

The authors declared no conflicts of interest with respect to the research, authorship and/or publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcpa.2024.03.207.

References

[1] Collins JA, Munoz JV, Patel TR, Loukas M, Tubbs RS. The anatomy of the aging aorta. Clin Anat 2014;27:463–6.
[2] Mitchell RN, Halushka MK. Blood vessels. In: Kumar V, Abbas AK, Aster JC, Turner JR, editors. Pathologic basis of disease. 10th ed. Philadelphia: Elsevier; 2021. p. 485–7.
[3] Robinson WF, Robinson NA. Cardiovascular system. In: Maxie GM, editor. Pathology of domestic animals. 6th ed. St. Louis: Elsevier; 2016. p. 54–6.
[4] Wu M, Rementer C, Giachelli CM. Vascular calcification: an update on mechanisms and challenges in treatment. Calcif Tissue Int 2013;93:365–73.
[5] Williams KJ. Coronary arteriosclerosis with myocardial atrophy in a 13-year-old dog. Vet Pathol 2003;40:695–7.
[6] Brenner OJ, Botero-Anug AM, Rojas A, Hahn S, Baneth G. Aberrant mesenteric migration of *Spirocerca lupi* larvae causing necrotizing eosinophilic arteritis, thrombosis, and intestinal infarction in dogs. Vet Pathol 2020;57:281–5.
[7] Browne LE, Carter TD, Levy JK, Snyder PS, Johnson CM. Pulmonary arterial disease in cats seropositive for *Dirofilaria immitis* but lacking adult heartworms in the heart and lungs. Am J Vet Res 2005;66:1544–9.
[8] Lim S, Park S. Role of vascular smooth muscle cell in the inflammation of atherosclerosis. BMB Rep 2014;47:1–7.
[9] Tang PK, Geddes RF, Jepson RE, Elliott J. A feline-focused review of chronic kidney disease - mineral and bone disorders - Part 2: pathophysiology of calcium disorder and extraosseous calcification. Vet J 2021;275:105718.
[10] Viallard JF, Vergier B, Lazaro E, Greib C, Pellegrin JL. Cutaneous necrotizing small-vessel vasculitis induced by acute hepatitis E. Clin Case Rep 2019;7:1539–41.
[11] Asín J, Molín J, Pérez M, Pinczowski P, Gimeno M, Navascués N, et al. Granulomas following subcutaneous injection with aluminum adjuvant-containing products in sheep. Vet Pathol 2019;56:418–28.
[12] Kiernan JA. Methods for connective tissue. In: Histological and histochemical methods: theory and practice. 5th ed. Banbury: Scion Publishing Ltd; 2015. p. 184–205.
[13] Majeed S, Goudswaard J. Aortic lesions in goats infected with *Mycobacterium johnei*. J Comp Pathol 1971;81:571–4.
[14] Prasad MC, Rajya BS, Mojxanty GC. Caprine arterial diseases I. Spontaneous aortic lesions. Exp Mol Pathol 1972;17:14–28.

[15] Csibi D, Gal AF, Ratiu C, Miclâus V. Structural features in tunica media of the aorta in lamb. Bull Univ Agric Sci Vet Med Cluj Napoca 2017;74:111–7.
[16] Knieriem HJ. Electron-microscopic study of bovine arteriosclerotic lesions. Am J Pathol 1967;50:1035–65.
[17] Ogeng'o JA, Malek AAK, Kiama SG, Olabu BO. Muscle 'islands' in the tunica media of the goat thoracic aorta. Braz J Morphol Sci 2009;26:171–5.
[18] Machado M, Castro MB, Gimeno EJ, Barros SS, Riet-Correa F. Enzootic calcinosis in ruminants: a review. Toxicon 2020;187:1–9.
[19] Tsang HG, Rashdan NA, Whitelaw CBA, Corcoran BM, Summers KM, MacRae VE. Large animal models of cardiovascular disease. Cell Biochem Funct 2016;34:113–32.
[20] Hu D, Yin C, Luo S, Habenicht AJR, Mohanta SK. Vascular smooth muscle cells contribute to atherosclerosis immunity. Front Immunol 2019;10:1101.