Presence of heterotopic bone in the aortic valve in the mare – a case report

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Abstract

Calcific aortic valve disease (CAVD) is the most common reason for valve replacement surgery in elderly people. CAVD is a slowly progressive disorder ranging from a mild aortic sclerosis to a more severe aortic stenosis. This article describes a case of heterotopic ossification in the aortic valve accidentally found at necropsy of presumably healthy 21-year-old mare euthanized for reasons unrelated to the cardiovascular system. Histopathological examination confirmed mature lamellar bone in the aortic valve area. To our best knowledge, heterotopic ossification of the aortic valve is a rare condition in horses and it has been reported in only two cases.

Equine, heart, cardiology, calcification, histology

Calcific aortic valve disease (CAVD) is the most common reason for valve replacement surgery in elderly people (Parolari et al. 2009). It is a slowly progressive disorder ranging from a mild valve thickening without blood flow obstruction, termed aortic sclerosis, to a severe calcification causing stiffness of the leaflets and obstruction of blood flow, termed aortic stenosis (Freeman and Otto 2005).

Aortic valve calcification is a degenerative change that develops due to repetitive mechanical stress during heart contractions. This leads to substantial tissue deformations during each contraction, and transvalvular pressure gradients in the closed phase of each contraction of approximately 120 mm Hg for the mitral and 80 mm Hg for the aortic valve (Kumar et al. 2015).

Osseous metaplasia, or heterotopic ossification, is a late histological finding in the progression from aortic sclerosis to aortic stenosis (Torre et al. 2006). Aortic calcific stenosis is usually the consequence of age-associated "wear and tear" of either anatomically normal valves or congenitally bicuspid valves (in approximately 1% of the population). It is likely a consequence of recurrent chronic injury due to hyperlipidaemia, hypertension, inflammation, and other factors similar to those implicated in atherosclerosis. However, the valve injury of calcific aortic stenosis differs in some important aspects from atherosclerosis. Most notably, the abnormal valves contain cells resembling osteoblasts that synthesize bone matrix proteins and promote the deposition of calcium salts. Moreover, interventions that improve atherosclerotic risk (e.g., statins), do not appear to significantly impact valvular calcific degeneration (Kumar et al. 2015).

Calcification of the aortic valve has been described in dogs as a rare radiographic finding with no clinical significance in most of the affected animals (Schwarz et al. 2002). In cats, aortic mineralization has been described in association with hypertension (Lefbom et al. 1996).

Calcification of cardiac valves with osseous metaplasia is a rare condition in horses and it has been reported in the mitral valve (Matsuda et al. 2010), tricuspid valve (Marr 2019) and aortic valve (Hammond et al. 2022).

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Case presentation

A 21-year-old Slovak Warmblood horse was brought to The University of Veterinary Medicine and Pharmacy in Košice, Slovakia, for routine dissection. The mare had been euthanized in field the same day for reasons unrelated to the cardiovascular system. At macroscopic examination the heart showed firm, sharp, palpable areas at the level of the aortic valve. No other cardiac abnormalities were observed macroscopically. Abnormal areas were cut and samples were washed in saline and fixed in 4% neutral buffered formaldehyde (Mikrochem Trade spol. s.r.o. Pezinok, Slovakia). The hard excisions were placed into ethylenediaminetetraacetic acid disodium salt dihydrate solution (Chelaton III, Gavax s.r.o., Vranov nad Topľov, Slovakia) for decalcification. Tissues were processed using the standard paraffin method, cut into 5–7 μm sections, and stained with haematoxylin-eosin (Fisher Scientific PTE LTD, Singapore). Non-specific collagen was stained with Masson Trichrome (Agilent Technologies, Inc., Santa Clara, California, United States). Samples were observed under a light microscope Olympus CX43 (Olympus, Tokio, Japan), pictures were taken with Promicam 3-5CP+ camera (Promicra s.r.o., Prague, Czech Republic) and processed by QuickPhoto Micro 3.2 software (Promicra s.r.o.). Histopathological examination confirmed metaplastic cartilage and bone in the aortic valve. A lipomatous medulla was present in the metaplastic lamellar bone (Plate III, Fig. 1). Osteocytes were organized in the lacunae within bone trabeculae. Remnants of bone marrow with lipid droplets were found between the trabeculae (Plate IV, Fig. 2). Hypertrophic chondrocytes and chondrocytes organized in isogenetic groups were present as well. Ischaemic changes (myofibrillar degeneration), undulated and corrugated cardiomyocytes with eosinophilic cytoplasm, were shown within the cardiac tissue. Subendocardial fibrosis was confirmed too. Accumulation of non-specific collagen was observed both in the myocardium and under the abnormal areas (Plate IV, Fig. 3).

Discussion

Heterotopic ossification is defined as ectopic, new bone formation in soft tissues where typically no bone is present. The bone tissue has a typical trabecular shape and contains bone marrow (Pape et al. 2004; Anthonissen et al. 2014). The aetiology of heterotopic ossification is still not clear (Anthonissen et al. 2014). Heterotopic bone in the heart valves is associated with the age-associated "wear and tear" mechanism, atherosclerosis, blood flow disorders, diabetes mellitus, hypercholesterolaemia, inflammation, hypertension, metabolic syndrome, and rheumatic fever in humans (Mohler et al. 2001; Butany et al. 2005; Steiner et al. 2007; Kumar et al. 2015; Kostyunin et al. 2019). In horses, ectopic bone formation was observed in different organs such as the colon, conjunctiva, heart valves and uterus (Rottman et al. 1991; Matsuda et al. 2010; Donaldson et al. 2012; Marr 2019; Savage 2019; Hammond et al. 2022). In healthy equine heart, the area around the origin of the aorta – trigonum fibrosum sinistrum is formed by triangular shaped cartilage – cartilago cordis (Popesko 1992). Some authors have stated that heterotopic ossification of heart valves, mainly the aortic valve, is a physiological process associated with advanced age (Ghoshal 1975a,b; Sandusky et al. 1979). To the authors' knowledge, only two cases of left heart valve ossification have been reported in horses. Matsuda et al. (2010) describes a case of heterotopic ossification of the mitral and aortic valve in a 4-year-old mare, whereas Hammond et al. (2022) describe a case of heterotopic bone formation in a 20-year-old mare. In both cases the horses presented clinical signs of cardiac disease. A different situation was present in our case since the mare was observed to be healthy and without cardiac related clinical signs. Histopathological examination showed results very similar to the previous two cases (Matsuda et al. 2010; Hammond et al. 2022). Mature

lamellar bone tissue, haematopoietic and fat tissue was found in the area of the aortic valve and osteons with Harversian canals were detected. We found areas of ischaemic changes in our patient. Ischaemic changes can be associated with coronary insufficiency as a result of myocardial hypertrophy (Mohan 2010). In dogs, mineralization or ectopic bone formation within heart structures occur in older animals and larger breeds and it mainly affects the aortic annulus (Douglass et al. 2003). In humans, heterotopic ossification is considered an active process of repair and affects mainly the aortic valve and the mitral valve (Mohler et al. 2001). One of the possible theories of valve calcification is a change in blood flow and rheology, which causes abnormal mechanical stress on the valve wall leading to its damage (Thubrikar et al. 1987). Endothelial dysfunction and damage lead to the deposition of low-density lipoprotein (LDL) and cholesterol in the tissue of the valves. It triggers an inflammatory process that is typical for the presence of macrophages, B and T cells and the expression of pro-inflammatory cytokines. Bone formation is induced by osteogenic transformations of valvular interstitial cells. Bone matrix protein 2 and 4 are important proteins which are involved with osteogenic transformation of valvular interstitial cells and were measured in humans with CAVD (Hulin et al. 2018; Kostyunin et al. 2019). We can suppose that the changes found in the aortic valve in the case described were age-related and the mare did not show clinical signs since the aortic valve was not enlarged or stenotic.

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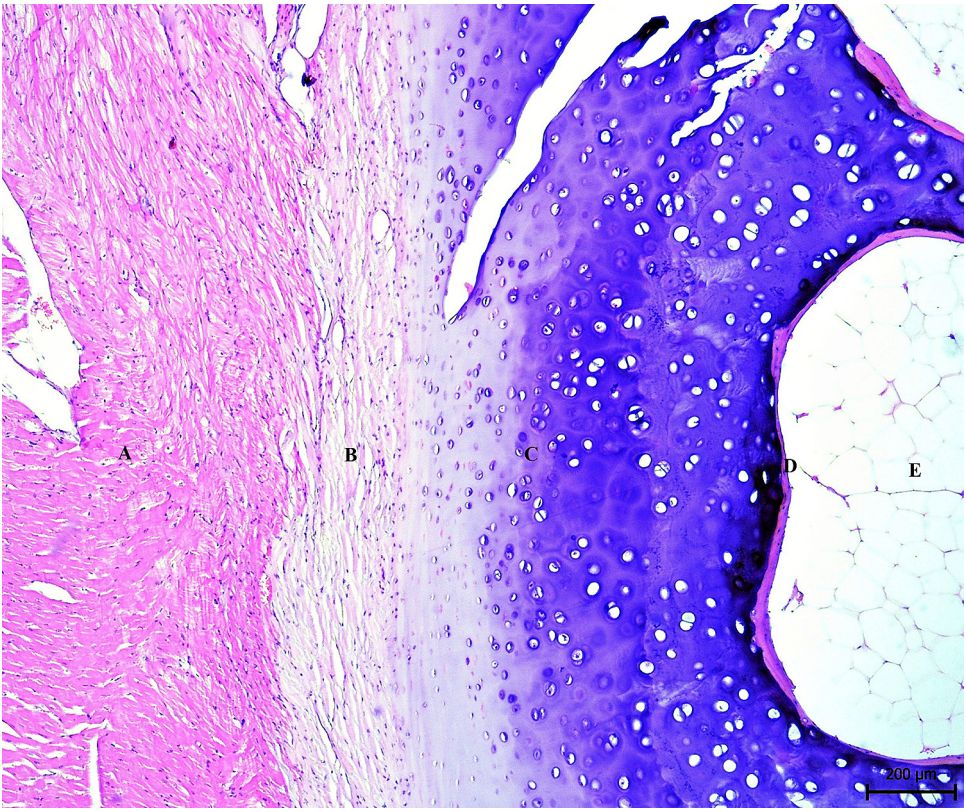

Fig. 1. Presence of the all types of tissue (myocardium - A, fibrous tissue - B, cartilage tissue - C, bone tissue - D and lipids - E) in the aortic valve area. Haematoxylin-eosin stain.

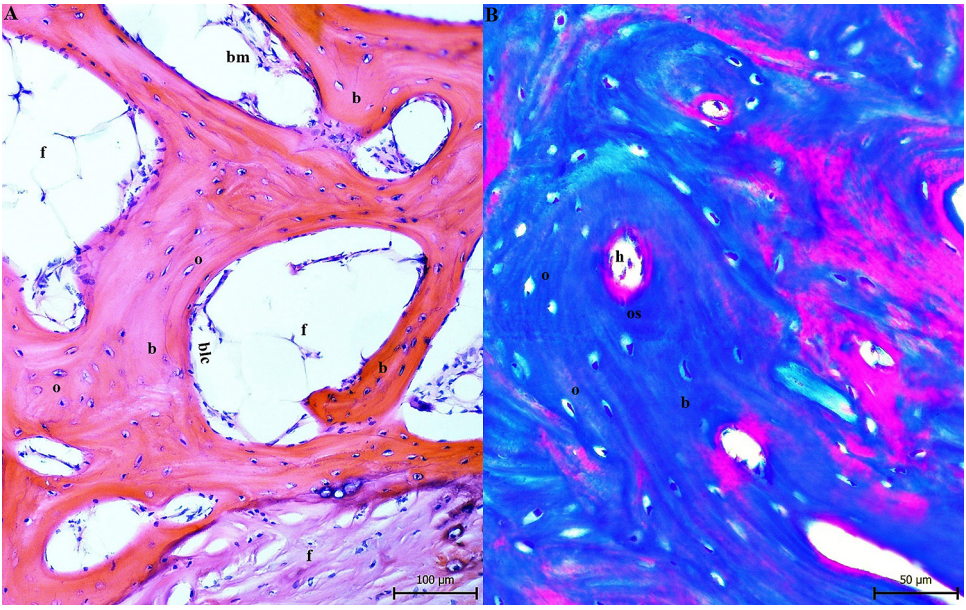

Fig. 2. Detailed view on the lamellar bone tissue in the aortic valve. Microphotography shows on the mature lamellar bone tissue (b) with osteocytes (o) and bone lining cells (blc). Bone marrow with cellular and fat components (bm, f) are organized around bone trabeculae (b) Haematoxylin-eosin stain (A). Presence of osteon (os) with Harversian canal (h). Osteocytes are well organized and lies in the lacunae. Masson trichrome stain (B).

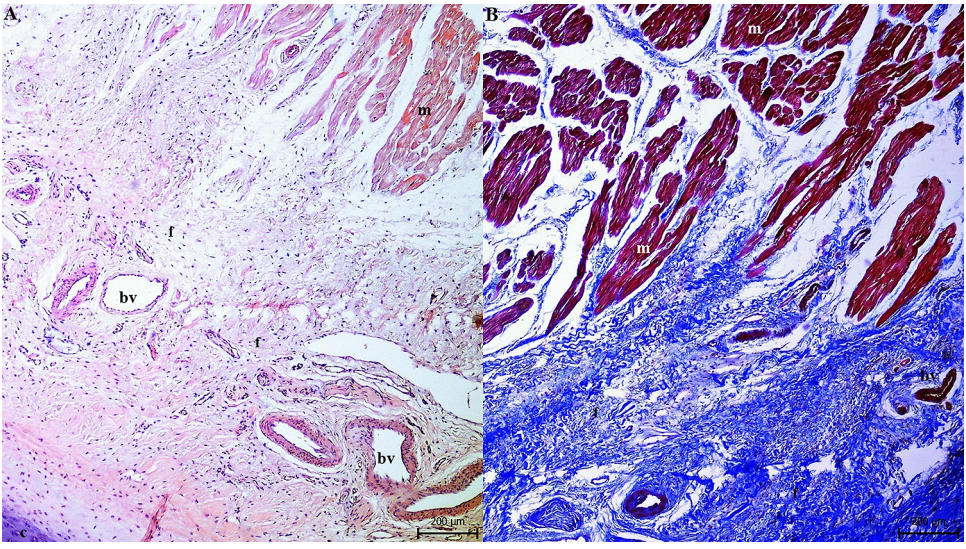

Fig. 3. Myofibrillar degenerative changes and presence subendocardial fibrosis (f) after healing of ischaemic changes. The cardiomyocytes (m) on the top of microphotography. Presence of blood vessels (bv) in the fibrous tissue. In those areas is possible to view metaplastic cartilage (c). Haematoxylin-eosin stain (A). Collagen fibres (non-specific) are accumulated and have different orientation and shape. Masson trichrome stain (B).