Journal MVZ Cordoba
2022; january-april. 27(1):e2153.
https://doi.org/10.21897/rmvz.2153

Original

Computed tomography examination of the *os cordis* in a lamb (*Ovis aries* Linnaeus, 1758)

Catia H. de Almeida Lima Massari[1]; Adriano Ferreira-Silva[1]; Henrique I. Riceti-Magalhães[1]; Dara R. Souza-Silva[1]; Maria A. Miglino[1]*.

[1]Universidad de São Paulo, Facultad de Medicina Veterinaria y Zootecnia, Departamento de Cirugía, Programa de Posgrado en Anatomia de los Animales Domésticos y Silvestres. Av. Prof. Dr. Orlando Marques de Paiva, Butantã, São Paulo/SP, Brasil.
*Correspondencia: miglino@usp.br

Received: September 2020; Accepted: June 2021; Published: November 2021.

ABSTRACT

Objective: The present paper aims to investigate the role of computed tomography as an imaging technique of diagnosis in the identification of the *os cordis* in ovine, and also how this anatomical structure is morphotopographically characterized in macro and microscopic contexts, seeking to contribute for its functional understanding. **Materials and method:** The heart of a young male ovine had been donated to the Laboratory of Animal Anatomy of the Surgery Department of the FMVZ/USP, first being subject to a *post-mortem* examination by means of the cardiac "shedding" (transverse cross sectioning of the heart). A tomographic examination of the anatomic specimen was carried out, as well as the dissection and histological analysis of the collected sample. **Results:** The results indicate the presence of an osseous structure of 6.39 mm in length, located in the aortic valve of the heart, next to a ring comprised by three valve flaps, tendinous cords, and papillary muscles. The histological findings consist of fibrous connective tissue, cancellous bone tissue, and calcified hyaline cartilage wherein the cardiomyocytes are attached. **Conclusions:** It is concluded that computed tomography, even though seldom applied to animals of zootechnical interest, poses as an effective tool for the visualization of the *os cordis* in lambs.

Keywords: Anatomy; heart; ovine; radiology; Veterinary Medicine (*Source: DeCS*).

RESUMEN

Objetivo: El presente trabajo tiene como objetivo investigar el papel de la tomografía computarizada como una técnica de diagnóstico por imagen en la identificación del hueso cardíaco en el ovino, y también cómo esta estructura anatómica se caracteriza morfotopográficamente en contextos macro y microscópicos con el objetivo de contribuir a la comprensión funcional de la misma. **Materiales y método:** El corazón de un ovino macho joven fue donado al Laboratorio de Anatomía Animal del Departamento de Cirugía de la FMVZ/USP, después de haber sido sometido a un examen *post-mortem* a través de una "defoliación" cardíaca (sección transversal del corazón). Se realizó un examen

How to cite (Vancouver).
Massari CH, Ferreira-Silva A, Riceti-Magalhães HI, Souza-Silva DR, Miglino MA. Computed tomography examination of the *os cordis* in a lamb (*Ovis aries* Linnaeus, 1758). Rev MVZ Cordoba. 2022; 27(1):e2153. https://doi.org/10.21897/rmvz.2153

ISSNL: 0122-0268

tomográfico de la muestra anatómica, así como la disección y el análisis histológico de la muestra recolectada. **Resultados:** Los resultados indican la presencia de una estructura ósea de 6,39 mm de longitud, ubicada en la válvula aórtica del corazón junto a un anillo que consta de tres válvulas, cuerdas tendinosas y músculos papilares. Los hallazgos histológicos consisten en tejido conectivo fibroso, tejido óseo esponjoso y cartílago hialino calcificado, al que están unidos los cardiomiocitos. **Conclusión:** Se concluye que la tomografía computarizada, aunque todavía se aplica poco a animales de interés zootécnico, se muestra como una herramienta eficaz para la visualización del hueso cardíaco en cordero.

Palabras clave: Anatomía; corazón; Medicina Veterinaria; ovino; radiología (*Fuente: DeCS*).

INTRODUCTION

In developing countries, ovine breeding is fundamentally oriented towards the security of livelihoods, whilst it is not related to the intensive commercial exploitation. In those countries, sheep and lamb are tied to meat and cheese production (of the Pecorino, Roquefort, and Manchego types), besides leather and wool to a certain extent - while the offer and demand of milk is virtually inexpressive (1). Sheep production is present in all continents, and in the Brazilian territory, it has also been practiced by low-income rural families that are vulnerable in terms of investment policies (2). As they are domestic ruminants and are important in cattle breeding, the form-function complex of the body structures of this species deserves to be studied. Within the context of scientific research, the ovine heart is widely used as a research model for human heart (3,4), especially in view of its great morphological and arterial distribution similarities (5). Additionally, this anatomical structure could be used for pre-clinical tests of cardio-vascular devices, developing new interventional and operational techniques, as well as for educational and training purposes (6).

According to Saunders et al. (7), Andreas Vesalius depicted in his book that the heart bone is a cartilaginous plate found to the right of the aortic ring in young ruminants and is considerably calcified in older animals. At times, there is a smaller plate found to the left of the aortic ring. In his turn, Galen, following Aristotle's teachings, described this bone and claimed to have found it in the heart of an elephant that was being prepared for the Roman Emperor's table. Vesalius would state that, in order to see it, one needed but to examine the heart of a decrepit cow. The heart bone, especially that of the stag, was much sought after by physicians to be used as "medicine", as it was assumed to have miraculous properties and was therefore sold at exorbitant prices. Vesalius was one of the first to deny that such bone possessed any therapeutic property.

Still according to Saunders et al. (7), the so-called fibrous trigone located amid the atrioventricular ostia is, in human species, the anatomical element which corresponds to the heart bone that, in the ox and in a number of ruminants, occupies the same position. Mohammadpour (8) studied 50 hearts of native caprines and 50 hearts of Lori-Bakhtiari ovines (an Iran native breed) with ages between 1.5 and 2 years and verified that the *os cordis* has been present in the right antimere of the heart in 52% of the sheep and 44% of the goats, respectively. There is no information in the literature pursuant to the presence of the percentage of heart bone in ovines from Brazil.

Thus, the objectives were to investigate the role of computed tomography as a diagnostic imaging technique in the identification of the *os cordis* in ovine, and also analyze how this anatomical structure is morphotopographically characterized in macro and microscopic contexts.

MATERIALS AND METHODS

Ethical aspects. The study was approved by the Ethics Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science of the University of São Paulo (USP, Brazil): 1632060420.

Heart examination and laboratory methods. The heart of a male ovine of approximately four months of age (*Ovis aries*) had been donated to the Laboratory of Animal Anatomy of the Department of Surgery of the School of Veterinary Medicine and Animal Science of the University of São Paulo, originated from human slaughter for feeding purposes in a rural property located in the city of Taguaí, São Paulo, Brazil. A *post-mortem* examination would then be carried out for inspection of the organ in observance of the technical standardization of meat inspection by means of the cardiac "shedding" (transverse cross sectioning of the heart) for the survey of possible cysticerca. The heart muscle was sectioned in the transverse direction, from surface to depth, in order to reduce it to a broad, fine, seamless slide that would provide the largest possible area of examination for surveying the presence of live or calcified larvae on the surface exposed, in accordance with the criteria of the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA). The heart was first frozen (-20°C), then allocated in a bowl and immersed into 10% formaldehyde, where it remained for 10 days. A tomographic examination of the heart was carried out under a Phillips® Brilliance tomograph with 64 rows of detectors belonging to the Service of Diagnostic Imaging of the University of São Paulo. The acquisition settings were 50mA and 80kV with 0.8mm thickness per slice. All image evaluations and image savings were performed via RadiAnt DICOM Viewer 2020.1 software (digital imaging and communications in medicine).

Dissection had been carried out and a single intracardiac bony structure would be carefully excised next to the aortic cardiac valve. A photographic record on the macroscopic anatomy was made. Histological analysis was performed by the decalcification process (demineralization) of calcified cartilage and bone, with subsequent Masson's Trichrome staining. In order to enable the examination of bone tissue or cartilaginous tissue with areas of calcification, before embedding and cutting the tissue, decalcification was performed to remove tissue calcium by immersing the sample fragments with a maximum thickness of 5 mm in formic acid solution.

Anatomical nomenclature. The results are descripted in accordance with the International Committee on Veterinary Gross Anatomical Nomenclature (ICVGAN) (9).

RESULTS

Under radiological anatomy, the tomographic images obtained in this study indicated the presence of a high-density structure, with a mean attenuation value of 163.7 Hounsfield units (UH). It is important to point out that, radiologically, those structures that present attenuation rates above 100 UH under X-rays are deemed to be calcified (10). This structure measured approximately 6.39 mm in length and was located at the aortic valve of the ovine heart (Figures 1-5).

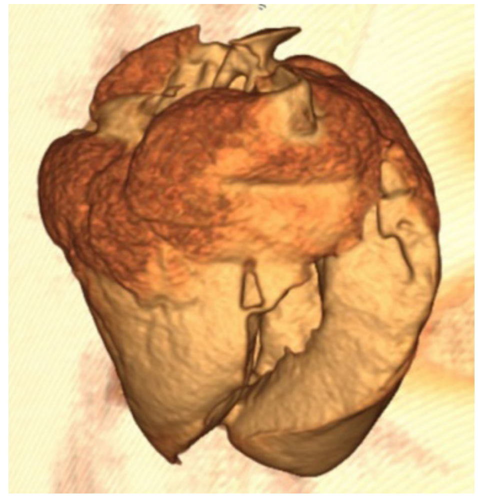

Figure 1. Three-dimensional volumetric reconstruction of an ovine heart which had been subject to the process of cardiac "shedding" (transverse cross sectioning of the heart) in inspection.

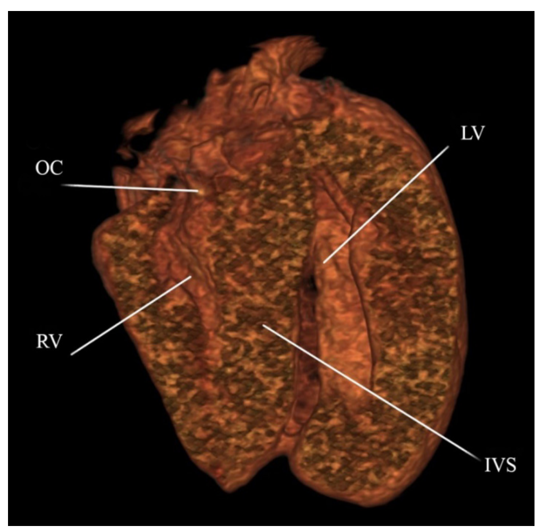

Figure 2. Localization of the *os cordis* at the base of the aortic valve of an ovine heart. RV = right ventricle; LV = left ventricle; IVS = interventricular septum; OC = *os cordis*.

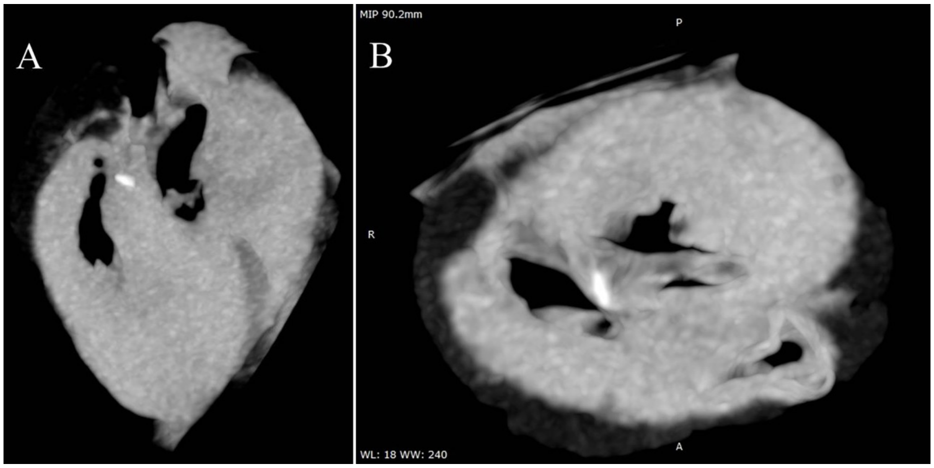

Figure 3. Localization of the *os cordis* (high-density structure) at the base of the aortic valve in longitudinal scan (A) and cross-sectional scan (B) of an ovine heart.

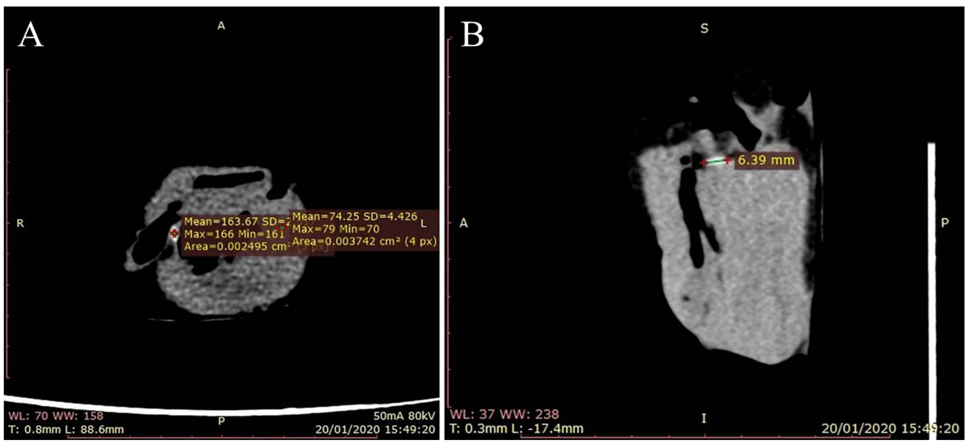

Figure 4. Measuring of the attenuation coefficient (value in Hounsfield units) of the *os cordis* of an ovine heart, according to its calcified nature (A). Measuring of the *os cordis* at the base of the aortic valve by means of the computed tomography (B).

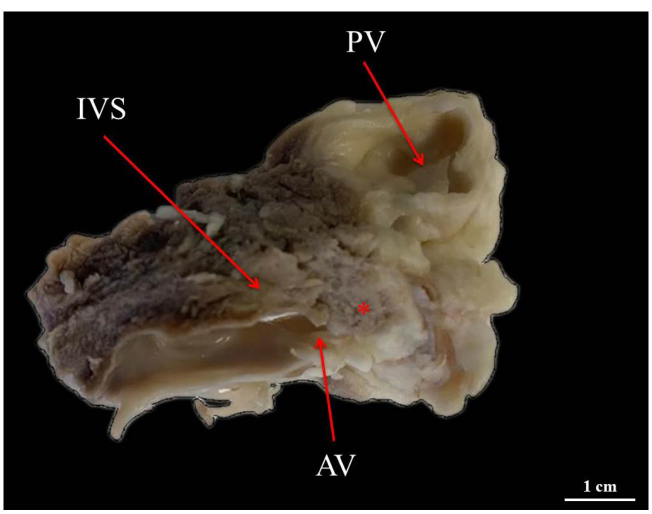

Figure 5. Cranial view of the region of the aortic valve of an ovine heart. IVS = interventricular septum with fibers that are supported in the heart bone; AV = aortic valve; PV = pulmonary valve; * = *os cordis*.

In the macroscopic anatomical study of the aortic valve complex (Figures 6 and 7), it was observed that the rigid structure of the *os cordis* belonged to a ring that was also comprised by three valve flaps, by the tendinous cords, and by the papillary muscles. That bone measured nearly 3.0 cm in length by 0.5 cm in width and was, therefore, larger than in the CT scan, because this imaging examination is only capable of discerning the calcified portion of the structure, and the total length of the *os cordis* may be overestimated by the difficult discrimination with the adjacent fibromuscular planes.

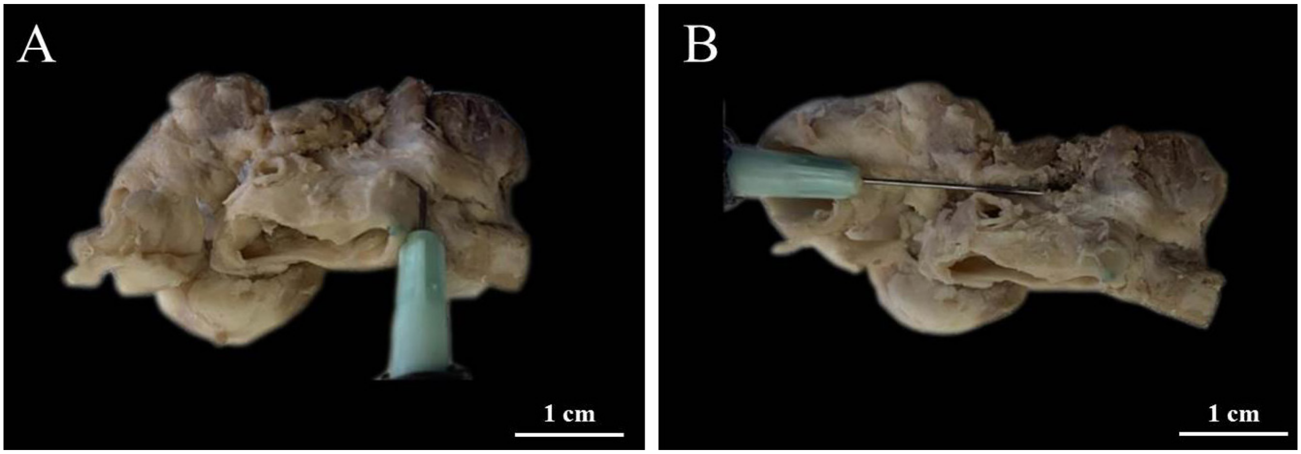

Figure 6. Cranial view showing the needle through the *os cordis* (A), and part of the musculature of the interventricular septum (at the subjacent needle) inserting in the *os cordis* (B) of an ovine heart.

Figure 7. Dorsal view of the region of the aortic valve (A) and the *os cordis* individually dissected (B) - it is seen that its limits are hard to define due to the junction of the cardiac musculature and the fibrous tissue of the valve ring.

Under optical microscopy (Figure 8), the histological findings of the *os cordis* consisted in fibrous connective tissue, cancellous bone tissue (osteocytes distributed in irregular pattern within the extracellular matrix), and calcified hyaline cartilage, wherein the cardiomyocytes are found to be attached to this rigid anatomical structure. Thus, the bony tissue that composes the cardiac bone seems to be formed by the process of endochondral ossification, which begins over a mold of hyaline cartilage, similar in shape to the bone that will be formed. In the results, it was possible to observe the calcified cartilage zone, in which the mineralization of the thin tabs of cartilaginous matrix occurs and the apoptosis of the chondrocytes, along with the ossification zone, in which bone tissue appears properly, ends. In addition, although there is still no universally accepted hypothesis for the mechanism of calcification in this particular anatomical structure, it is known that calcification begins by the deposition of calcium salts on the collagen fibrils, a process that appears to be induced by proteoglycans and matrix glycoproteins.

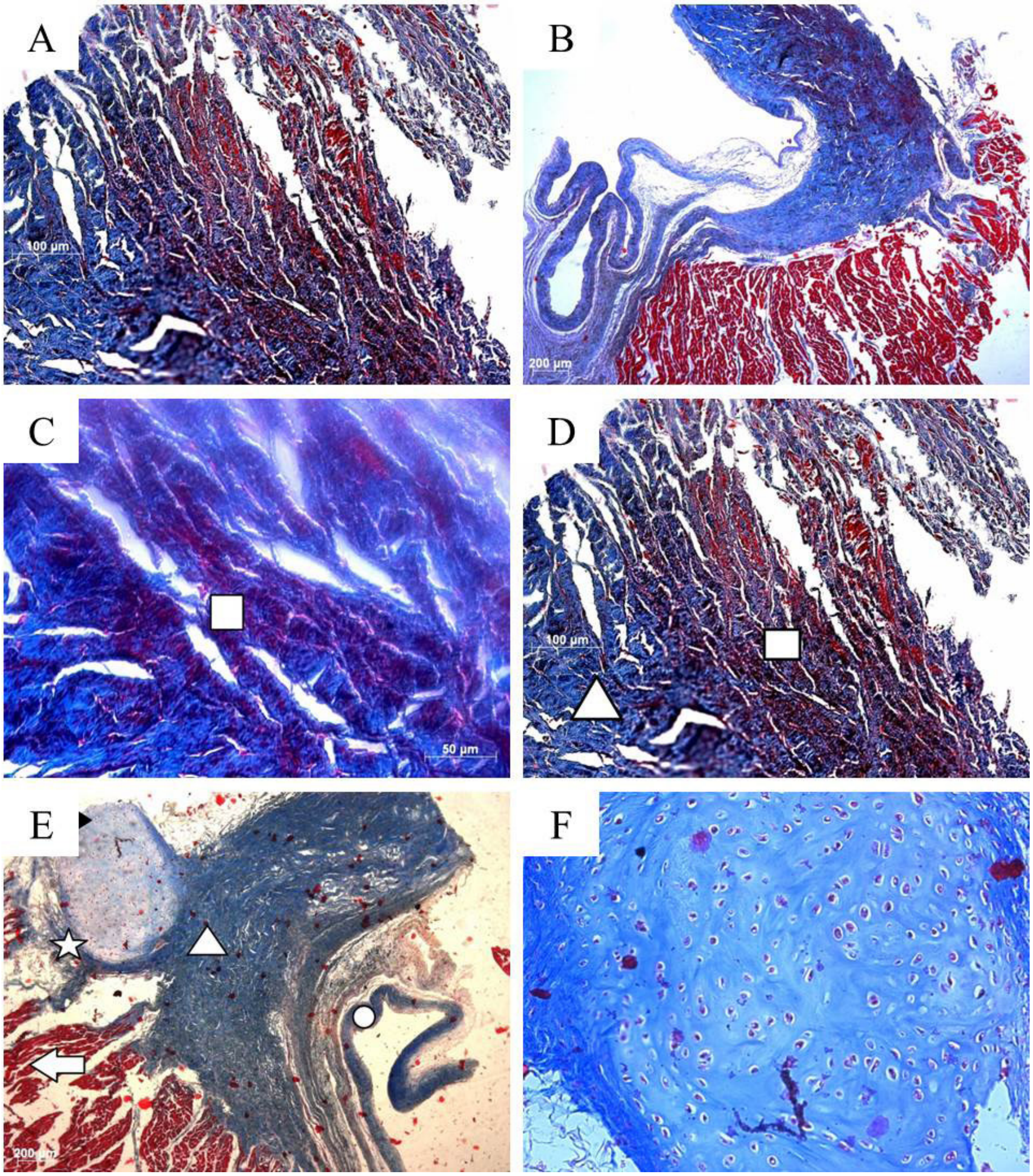

Figure 8. Photomicrographs of *os cordis* of the ovine heart: (A) fibrovascular cardiac stroma; (B) heart valve lumen; (C) collagen fibers entwined in amorphous fundamental substance (square); (D) thick bundle of collagen fibers of the valve ring (triangle) and collagen fibers entwined in amorphous fundamental substance (square); (E) presence of a hyaline cartilage nest (black arrow) and of heart muscle fibers (white arrow) that insert into the valve fibro-cartilaginous ring (triangle), collagen bundles around the ectopic hyaline cartilage (star), besides osteocytes and collagen bundle of the valve flap (white circle); and (F) in a larger degree of magnification, the presence of osteocytes and chondrocytes are highlighted.

DISCUSSION

Under radiological anatomy, the tomographic images obtained in this study are in accordance with Frink and Merrick (11) regarding the topographical anatomy of this bone for indicating its localization deep in the atrial septum and directly adjacent to the atrioventricular node.

The computed tomography (CT scan) provides advantage to the anatomical study when compared to the conventional radiography by virtue of two characteristics intrinsic to the technique, namely its tomographic nature and the high resolution of the contrast. Unlike radiographies, which are two-dimensional (flat) representations, the parts of the animal body under CT scan are examined in a series of fine sections, thus eliminating the overlap of structures and providing leeway for the three-dimensional reconstruction. The volume of data obtained under a CT scan may be reformatted in multi-plane images as three-dimensional (3D), this allowing for better representations of structural anatomical relations and, as a complement, the study of the cardiac structures can be conducted in live animals, with no need for a necropsy to be made.

The histological findings of the *os cordis* are in accordance with the literature of Abolhasanzadeh, Mastary Farahani, and Alexanian (12). These authors indicate the presence of hematopoietic bone tissue in the *os cordis*, demonstrating a functional activity of that tissue, whose physiological importance is not utterly clarified yet.

It is known that not all bones are part of the locomotor system. The bones classified as splanchnic develop normally within the viscera, away from the rest of the skeleton. In veterinary anatomy, the most significant examples of splanchnic bones are the penile bone (and its female equivalent, the clitoral bone) in dogs; the rostral bone in swine; and the heart bone in ruminants (13).

The heart bone is widely known in domestic ruminants, such as bovines, caprines and ovines, besides its also having been reported in dromedaries (14, 15). Furthermore, there are reports of that bone in chimpanzees as well, even though these animals are not ruminants (16). That mineralized anatomical structure is precociously developed in those species, located next to the semilunar cusps of the aortic valve.

In bovines, the heart bone is comprised by two areas of ossification (two ossicles) around the left and right coronary cusps (17). Nevertheless, in the lamb in this study, this bone structure is formed by a single area of ossification. The function of that small bone is to structurally support this valve that separates the left cardiac ventricle from the aortic artery. Physiologically, the aortic opens during the ventricular systole, thus allowing the passage of blood from the left ventricle to the systemic circulation, and closes during the ventricular diastole, thus preventing blood reflux. It is comprised by a support ring, where three cusps are fixated: the right coronary cusp (from which the right coronary artery originates), the left coronary cusp (from which the left coronary artery originates), and the non-coronary cusp.

As for the development of the heart bone in ovines, as well as in other ruminants, it is known to be a normal process of embryonic formation. Nevertheless, no records were found to describe the stages through which the organism evolves from conception to the specific formation of that structure. During the gastrulation phase, the mesoderm subdivides in three areas: paraxial, intermediate, and lateral. The formation of extra-embryonic coelom divides the lateral mesoderm in somatic mesoderm (parietal) and visceral mesoderm (splanchnic plate). The somatic plate gives rise to the bones, whilst the splanchnic plate gives rise to the heart, among many other structures. Therefore, it is suggested herein that the heart bone is formed from the association between these two plates of the lateral mesoderm, thus comprising a bone tissue structure entwined with the heart musculature.

Since the comparative anatomy permits comparisons to be established between anatomical aspects of different animal species, it is observed that, in dogs and human beings, the presence of cardiac mineralization denotes a pathological process.

According with Cardoso et al. (18), this pathology consists in the abnormal deposition of minerals in body tissues, whether previously injured or not.

Studies showing aortic and cardiac mineralization in dogs describe this manifestation as relatively rare in chronic degenerative alterations of the cardiovascular systems in aged canines, without clinical significance (19). Yet, in humans, the development of valve calcification resembles the atherosclerotic process, and it is started by epithelial injury (20). Moreover, the calcification of the coronary arteries is reflective of an advanced stage of this disease (21). In fact, some studies demonstrated that factors leading to valve calcification are similar to the risk factors for coronary diseases and atherosclerosis – age, male gender, body weight, diabetes, and dyslipidemias (22-27). However, the pathological mechanisms of degenerative sclerosis and precise association with the atheromatous process, as well as a complete list of causative factors, have not been clearly determined yet (20).

In conclusion, radiological anatomy allows studying the bodily structures of both cadavers and living animals by means of the imaging of the normal morphology of different species. Imaging relies on computed tomography, which, although seldom applied to animals of zootechnical interest, has been expanded in the university, in the interface of descriptive anatomy to imaging diagnostics. Thus, CT is capable of visualizing the *os cordis* in lambs, and this can improve the bulk of knowledge regarding the hemodynamic function of the heart and collaborate for the field of bio-prosthetics.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

To Prof. Dr. Lucas Martins Soldera, professor at The State University of Maringá, for helping in the acquisition of the sample on a sheep farm, at Taguaí/SP. To Prof. Dr. Claudio Campi de Castro and to Mr. Antonio Carlos Matioli for the partnership with the Diagnostic Imaging Service of the University of São Paulo; and to Mrs. Marta M. S. Righetti, histology technician at Biomedical Sciences Institute (ICB-III) of the University of São Paulo. To Mr. José Miguel Velásquez Salazar, veterinarian in Colombia, graduated from the University of Antioquia, for the translation of this manuscript into Spanish language.

REFERENCES

1. Mohapatra A, Shinde AK, Singh R. Sheep milk: A pertinent functional food. Small Ruminant Res. 2019; 181:6-11. https://doi.org/10.1016/j.smallrumres.2019.10.002

2. Hermuche PM, Maranhão RL, Guimarães RF, De Carvalho OA, Gomes RA, Paiva SR, McManus C. Dynamics of sheep production in Brazil. ISPRS Int J Geo-Inf. 2013; 2(3):665-679. https://doi.org/10.3390/ijgi2030665

3. Butters TD, Aslanidi OV, Zhao J, Smaill B, Zhang H. A novel computational sheep atria model for the study of atrial fibrillation. Interface Focus. 2013; 3(2):20120067. https://doi.org/10.1098/rsfs.2012.0067

4. Rienzo M, Imbault J, El Boustani Y, Beurton A, Sampedrano CC, Pasdois P, et al. A total closed chest sheep model of cardiogenic shock by percutaneous intracoronary ethanol injection. Sci Rep. 2020; 10(1):1-3. https://doi.org/10.1038/s41598-020-68571-5

5. Monreal G, Sherwood LC, Sobieski MA, Giridharan GA, Slaughter MS, Koenig SC. Large animal models for left ventricular assist device research and development. Asaio J. 2014; 60(1):2-8. https://doi.org/10.1097/MAT.0000000000000005

6. Katz MG, Kendle AP, Fargnoli AS, Mihalko KL, Bridges CR. Sheep (*Ovis aries*) as a model for cardiovascular surgery and management before, during, and after cardiopulmonary bypass. J Am Assoc Lab Anim Sci. 2015; 54(1):7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4311734

7. Saunders JD, O'Malley CD. Andreas Vesalius de Bruxelas. De humani Corporis Fabrica. Epitome. Tabulae Sex. Unicamp; 2002.

8. Mohammadpour A, Arabi M. Morphological study of the heart and *os cordis* in sheep and goat. Indian Vet J. 2007;84(3):284-287. https://profdoc.um.ac.ir/paper-abstract-103021.html

9. International Committee on Veterinary Gross Anatomical Nomenclature. Nomina Anatomica Veterinaria. 6th ed. Hannover: Editorial Committee; 2017. http://www.wava-amav.org/wava-documents.html

10. Nute JL, Le Roux L, Chandler AG, Baladandayuthapani V, Schellingerhout D, Cody DD. Differentiation of low-attenuation intracranial hemorrhage and calcification using dual-energy computed tomography in a phantom system. Invest Radiol. 2015; 50(1):9. https://doi.org/10.1097/RLI.0000000000000089

11. Frink RJ, Merrick B. The sheep heart: Coronary and conduction system anatomy with special reference to the presence of an *os cordis*. Anat Rec. 1974; 179(2):189-199. https://doi.org/10.1002/ar.1091790204

12. Abolhasanzadeh A, Mastary Farahani R, Alexanian S. Histological study of *os cordis* of sheep. Feyz. 2000; 3(4):25-29 http://feyz.kaums.ac.ir/article-1-476-en.html

13. König HE, Liebich HG, editors. Veterinary anatomy of domestic animals: Textbook and colour atlas. Georg Thieme Verlag; 2020.

14. Akhtar S, Hossain FM, Siddiqui MS, Alam M, Islam MN. Morphology and Morphometrical studies on Semi lunar Heart valves of Indigenous Cattle (*Bos indicus*). Int J Nat Sci. 2011; 1(1):7-11. https://doi.org/10.3329/ijns.v1i1.8608

15. Ghonimi W, Balah A, Bareedy MH, Abuel-Atta AA. *Os cordis* of the mature dromedary camel heart (*Camelus dromedaries*) with special emphasis to the cartilago cordis. J Veterinar Sci Technolo. 2014; 5(4):1. https://doi.org/10.4172/2157-7579.1000193

16. Moittié S, Baiker K, Strong V, Cousins E, White K, Liptovszky M, Redrobe S, Alibhai A, Sturrock CJ, Rutland CS. Discovery of *os cordis* in the cardiac skeleton of chimpanzees (*Pan troglodytes*). Sci Rep. 2020; 10(1):1-1. https://doi.org/10.1038/s41598-020-66345-7

17. Almeida MC, Sánchez-Quintana D, Davis N, Charles FR, Chikweto A, Sylvester W, Loukas M, Anderson RH. The ox atrioventricular conduction axis compared to human in relation to the original investigation of Sunao Tawara. Clin Anat. 2020; 33(3):383-393. https://doi.org/10.1002/ca.23524

18. Cardoso PGS, Pinto MPR, Moroz LR, Fontes TN, Santos RS, Freitas JL, Nogueira VA, Peixoto TC. Dystrophic mineralization in uremic dogs: na uptade. Pesq Vet Bras. 2019; 39(11):889-899. https://doi.org/10.1590/1678-5150-pvb-6250

19. Schwarz T, Sullivan M, Störk CK, Willis R, Harley R, Mellor DJ. Aortic and cardiac mineralization in the dog. Vet Radiol Ultrasoun. 2002; 43(5):419-27. https://doi.org/10.1111/j.1740-8261.2002.tb01028.x

20. Galas A, Hryniewiecki T, Michałowska I, Kępka C, Abramczuk E, Orłowska-Baranowska E, Rużyłło W. Aortic valve calcification in 499 consecutive patients referred for computed tomography. Arch Med Sci 2015; 11(5):952-957. https://doi.org/10.5114/aoms.2015.47874

21. Otsuka F, Sakakura K, Yahagi K, Joner M, Virmani R. Has our understanding of calcification in human coronary atherosclerosis progressed? Arterioscler Thromb Vasc Biol. 2014; 34(4):724-736. https://doi.org/10.1161/ATVBAHA.113.302642

22. Owens DS, Budoff MJ, Katz R, Takasu J, Shavelle DM, Carr JJ, Heckbert SR, Otto CM, Probstfield JL, Kronmal RA, O'Brien KDO. Aortic valve calcium independently predicts coronary and cardiovascular events in a primary prevention population. JACC: Cardiovasc Imaging. 2012; 5(6):619-625. https://doi.org/10.1016/j.jcmg.2011.12.023

23. Sato Y, Jinnouchi H, Sakamoto A, Cornelissen A, Mori M, Kawakami R, Kawai K, Virmani R, Finn AV. Calcification in human vessels and valves: from pathological point of view. AIMS Mol Sci. 2020; 7(3):183-210. https://doi.org/10.3934/molsci.2020009

24. Zheng KH, Tsimikas S, Pawade T, Kroon J, Jenkins WS, Doris MK, White AC, Timmers NK, Hjortnaes J, Rogers MA, Aikawa E. Lipoprotein (a) and oxidized phospholipids promote valve calcification in patients with aortic stenosis. J Am Coll Cardiol. 2019; 73(17):2150-2162. https://doi.org/10.1016/j.jacc.2019.01.070

25. Mantovani A, Pernigo M, Bergamini C, Bonapace S, Lipari P, Valbusa F, Bertolini L, Zenari L, Pichiri I, Dauriz M, Zoppini G. Heart valve calcification in patients with type 2 diabetes and nonalcoholic fatty liver disease. Metabolism. 2015; 64(8):879-887. https://doi.org/10.1016/j.metabol.2015.04.003

26. Sakamoto A, Guo L, Virmani R, Finn AV. Is there a role for activated platelets in progression of aortic valve calcification? Eur Heart J. 2019; 40(17):1374–1377. https://doi.org/10.1093/eurheartj/ehy696

27. Carrai P, Camarri S, Pondrelli CR, Gonnelli S, Caffarelli C. Calcification of cardiac valves in metabolic bone disease: an updated review of clinical studies. Clin Interv Aging. 2020;15:1085-1095. https://doi.org/10.2147/CIA.S244063