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Distribution of coronary arteries and histopathological analysis of three-toed sloth (*Bradypus variegatus*) heart

Distribuição das artérias coronárias e análise histopatológica do coração de *Bradypus variegatus*

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Abstract: The three-toed sloth (*Bradypus variegatus*) has been a victim of disorderly human interventions, leading to an increase in accidents and diseases for the species. This study aimed to provide a description of the coronary arteries and to highlight the histopathological findings in the sloth's heart. This information should be beneficial for medical clinics dealing with these species. Eighteen *B. variegatus* specimens were dissected after natural death. Fifteen of them, comprising five adult males, nine adult females, and one juvenile, were fixed with formaldehyde and preserved in saline solution. In two males and one female, red-coloured latex was injected through the left common carotid artery to make the coronary arteries more visible. In the female, blue-coloured latex was also injected through the right external jugular vein to aid in arterial identification. The chests were opened to evaluate and extract the heart for a detailed description of the coronary arteries. For histopathological analysis, histological slides were prepared from four hearts collected from four animals (three adults and one juvenile). Arterial analyses revealed that sloths possess two coronary arteries: one on the right and one on the left side, each of which supplies blood to their respective surfaces. The heart is composed of the endocardium, myocardium, and epicardium, similar to mammals in general. However, necrosis of endocardial tissue and cardiac muscle fibres, as well as myxomatous degeneration of valves and thrombi in vascular lumens, were observed. These necrotic events, more extensive in nature, are related to infarctions, while the more subtle ones may be associated with stressful situations experienced by the individuals.

Keywords: Xenarthra, three-toed sloth, coronary arteries, histopathology, heart.

Resumo: A preguiça-comum (*Bradypus variegatus*) tem sido vítima das investidas antrópicas desordenadas, o que tem elevado o número de acidentes e doenças para a espécie. Propôs-se com esse estudo, realizar uma descrição das artérias coronárias, assim como apontar os achados

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histopatológicos do coração do bicho-preguiça, a fim de favorecer o diagnóstico por imagem, semiologia e clínica médica das espécies. Foram dissecados 18 exemplares de *B. variegatus* obtidos após morte natural. Quinze deles, sendo 5 machos e 9 fêmeas adultas e 1 jovem, foram fixados com formaldeído e conservados em solução salina. Em 2 machos e 1 fêmea, foi injetado, a partir da artéria carótida comum esquerda, látex corado em vermelho, para tornar as coronárias mais evidentes. Na fêmea também foi injetado através da veia jugular externa direita, látex corado em azul, para evitar equívoco na identificação arterial. Os animais tiveram o tórax aberto a fim de avaliar e retirar o coração para descrição detalhada das artérias coronárias. Para análise histopatológica, foram confeccionadas lâminas histológicas oriundas de corações coletados de 4 animais (3 adultos e 1 jovem). Com base nas análises arteriais, as preguiças possuem duas coronárias: uma a direita e uma a esquerda, que irrigam suas respectivas faces. O coração é composto por endocárdio, miocárdio e epicárdio, semelhante aos mamíferos em geral. Todavia, observou-se necroses endocárdicas, de fibras musculares cardíacas, além de degenerações mixomatosas de válvulas e trombos no lúmen vascular. Esses eventos necróticos, mais extensos, estão relacionados a infartos, enquanto os mais discretos podem ser associados a situações de estresse vivenciadas pelos espécimes.

Palavras-chave: Xenarthra; preguiça-comum; vascularização; histopatologia; coração.

1. Introduction

The heart is a pulsatile muscular organ composed of three layers: the endocardium, which is more internal and formed mainly of endothelial tissue; the myocardium, an intermediate stratum that contains cardiac muscle fibres; and the epicardium, the visceral serous leaflet of the pericardium, which surrounds the organ. From ventricular systole onwards, blood leaves the heart through the vessels towards the organ itself and the entire body, and then returns to it, in atrial diastole(1). Along its path, blood transports respiratory gases, nutritional substances needed by cells, metabolic excreta, and hormones. It also participates in important activities for the body, such as thermal regulation and standardising the concentration of ions in body fluids(2).

Due to its extremely important role in the cardiovascular system and amid the increasing incidence of heart diseases worldwide, the heart has been the target of research(3, 4). In this context, the present study examined the heart of the three-toed sloth (*Bradypus variegatus*) to describe its coronary arteries as well as histopathological findings. *B. variegatus* has a strong occurrence in northeastern Brazil(5-7). Disorderly anthropogenic actions have promoted an increasing number of accidents and illnesses in the species, which highlights the importance of understanding their organ systems. This information should be beneficial for medical clinics that care for these animals(8-10).

2. Materials and methods

This study was authorised by the Ethics Committee on the Use of Animals (CEUA) of the Federal Rural University of Pernambuco (UFRPE, no. 50/2018). It is registered in the National Genetic Heritage and Associated Traditional Knowledge Management System (SinGen) under number A21069D. It also received approval from the Wild Animal Screening Center (CETAS) of the State Environment Agency (CPRH, no. 02/2017) and the Biodiversity Authorization and Information System of the Chico Mendes Institute (SisBio/ICMBio, no. 46665/10). The anatomical terms used in this paper follow the determinations of the 'International Committee on Veterinary Gross Anatomical Nomenclature', Nomina Anatômica Veterinária, 2017.

Eighteen *B. variegatus* corpses (after natural death) from CETAS of CPRH were used to describe the coronary arteries. Fifteen animals – five adult males, nine adult females, and one juvenile – were fixed with 20% formaldehyde and preserved in tanks of 30% saline solution, according to the methodology of Alcântara et al.[11]. Two adult male cadavers and one female cadaver were injected through the left common carotid artery with latex stained with red chequered paint. The solution filled the arch of the aorta and the ascending aorta, as well as its immediate branches. The female was also injected through the right external jugular vein with latex containing blue chequered paint to highlight the heart bypass veins and thus avoid any mistake in arterial identification.

The animals that were injected with the latex were fixed exactly like the other specimens that were not injected. Thus, the coronary arteries became turgid and marked in red, enabling a better description and the ability to obtain photographic records of cardiac vascularisation. The heart was accessed through a median sagittal incision of the thorax and reflection of the skin and muscles and removal of ribs. Then, the heart was removed from the cavity to carry out a detailed description of the angioarchitecture of the coronary arteries. The procedures took place in the Anatomy Area of the Department of Animal Morphology and Physiology (DMFA) of UFRPE.

The microscopic characteristics of the heart were also evaluated. For this endeavour, the heart was collected from four specimens, including one juvenile (sloth 1) and three adults (sloths 2, 3, and 4). The corpses were found at CETAS of CPRH shortly after natural death and had not been previously frozen. The heart was removed as described above in the Anatomy Area of DMFA of UFRPE.

The heart of each animal was fixed in 10% buffered formaldehyde with 0.01 M sodium phosphate (PBS, pH 7.3). Then, it was embedded in paraffin and processed in the Pathology Laboratory of the Department of Medicine of the Veterinary Department (DMV) of UFRPE. Four-micron-thick sagittal sections were stained with haematoxylin and eosin. The sections were viewed at 400× total magnification with a MOTIC® BA300 microscope coupled to a digital camera (Moticam® 2300) and connected to a microcomputer.

3. Results

Based on the analysis of the coronary arteries, in sloths, these vessels emerge from the ascending aorta, in the sinus, one right and one left. They provide a balanced circulation, without one standing out in relation to the other. Immediately after the origin of the right coronary artery, a vessel emerges, giving rise to branches that irrigate the cranial portion of the right side of the heart. From this point, a circumflex vessel originates, encircling the right auricle and giving off a branch to it. Descending vessels also emerge from the circumflex vessel, reaching the right cardiac side, including the subsinuosal interventricular branch (Figure 1). A paraconal interventricular branch arises from the left coronary artery. It irrigates the left side of the heart, reaching the apex. A circumflex vessel also originates from this coronary artery, from which a branch exits to the left atrium and branches to the cranial and middle portions of the left heart surface (Figure 1).

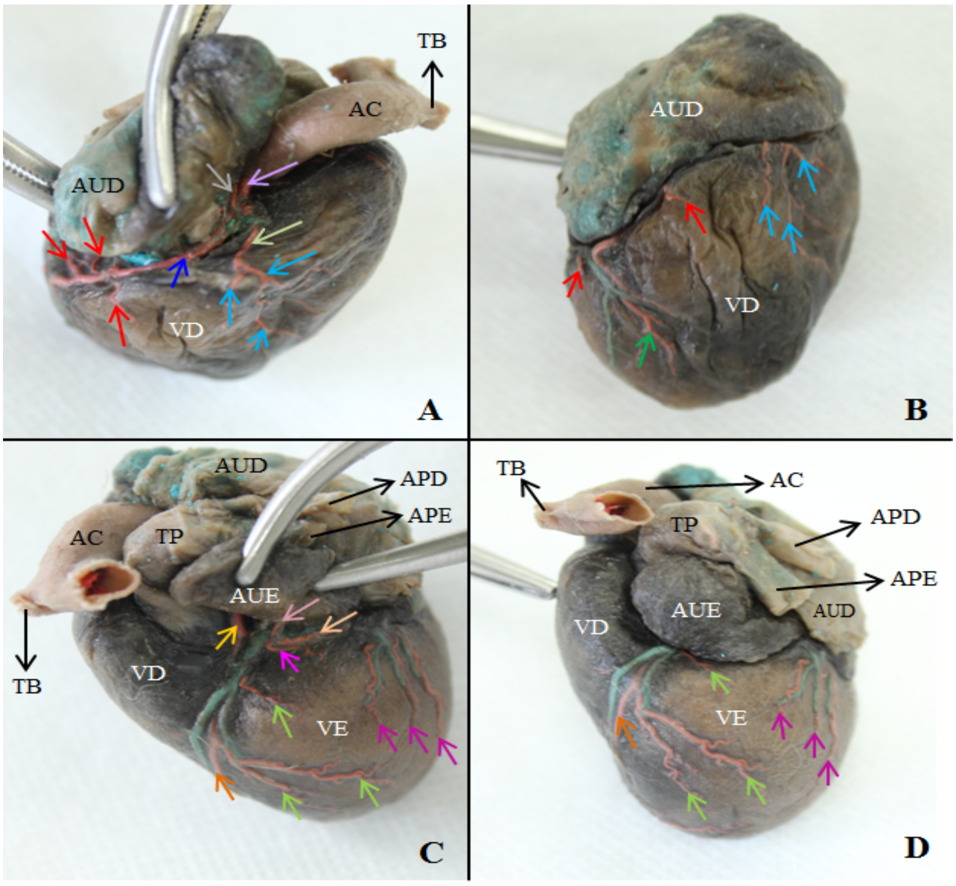

Figure 1 Photomacrograph of the heart of an adult three-toed sloth (*Bradypus variegatus*) with reduced angioarchitecture of the coronary arteries. (A and B) Origin and branching of the right coronary artery (→). (C and D) Origin and branching of the left coronary artery (→). Right atrium (AUD), left atrium (AUE), aortic arch (AC), brachiocephalic trunk (TB), pulmonary trunk (TP), right pulmonary artery (APD), left pulmonary artery (APE), right pulmonary ventricle (VD), and left ventricle (VE). A vessel emerges from the right coronary arteries directed towards the cranial portion of the right side of the heart (→), where it gives off branches (→). Circumflex branch of the right coronary arteries (→), from which an artery arises to the right atrium (→) and branches to the right cardiac surface (→). Subsinuosal branch (→). Paraconal branch (→), from which vessels depart that are distributed across the left side of the heart (→). Circumflex branch of the left coronary arteries (→), which originates an artery to the left atrium (→) and vessels to the corresponding cardiac surface (→).

According to histological data, the B. variegatus heart does not demonstrate discrepancies in relation to the general characteristics observed for other mammals. The organ has a typical epithelial lining, a typical endothelial lining (endocardium), a cardiac muscle layer (myocardium), and, externally, a layer of connective tissue with serous lining (epicardium). There is little accumulation of adipose tissue surrounding the heart.

In terms of histopathological findings, sloth 1 displayed degenerated cardiac muscle fibres that formed a thick area. The left atrioventricular valve, in turn, demonstrated thickening of its wall through a myxomatous degenerative process called endocardiosis, with collagen flexibility and deposition of fibrous material (Figure 2). In sloth 2, areas of necrosis were evident in the internal metallic layer of the thoracic aorta artery, which demonstrated the presence of a thrombus inside it. There was also necrosis of Purkinje nerve fibres and evidence of an inflammatory process, namely an increase in perivascular nuclei between cardiac muscle fibres, associated with fibroblasts/inflammatory cells. Congested cardiac vessels could also be observed (Figure 3). Sloths 3 and 4 showed extensive necrosis of many cardiac muscle fibres. In sloth 3, there was loss of the heart's architecture. Sloths 3 and 4 exhibited valvular endocardial necrosis and the presence of thrombi adhered to the endothelium and free in the vascular lumen, as well as damage to the muscle fibres surrounding the thrombotic vessels. These histological findings are indicative of infarction (Figure 4).

4. Discussion

Coronary arteries in sloths arise from the aortic sinus, one on the right and one on the left, as in the crab-eating raccoon (Procyon cancrivorus)[12], the paca (Agouti paca)[13], the South American fur seal (Arctocephalus australis)[14], the southern tamandua (Tamandua tetradactyla)[15], and birds such as ostrich (Struthio camelus)[16]. In the last two, as well as in the sloth, the coronary arteries give off circumflex branches. However, in anteaters, these vessels originate the paraconal and subsinuosal branches, from which vessels arise that vascularise the ventricles[15]. In bradypodids, the paraconal branch emerges directly from the left coronary artery and not from its circumflex branch; this anatomy also occurs in the South American fur seal[14]. Correia-Oliveira et al.[17] noted the absence of the right circumflex coronary branch in 100% of the European rabbits (Oryctolagus cuniculus) they trained; moreover, 86.66% of males and 93.33% of females lacked the left branch. In the capuchin monkey (Sapajus apella), it was possible to notice the subsinuosal branch originating from the right coronary artery and anastomosing with the paraconal branch, which also occurred for the right and left circumflex branches. However, the left circumflex and paraconal branches are not branches of the right coronary artery[18].

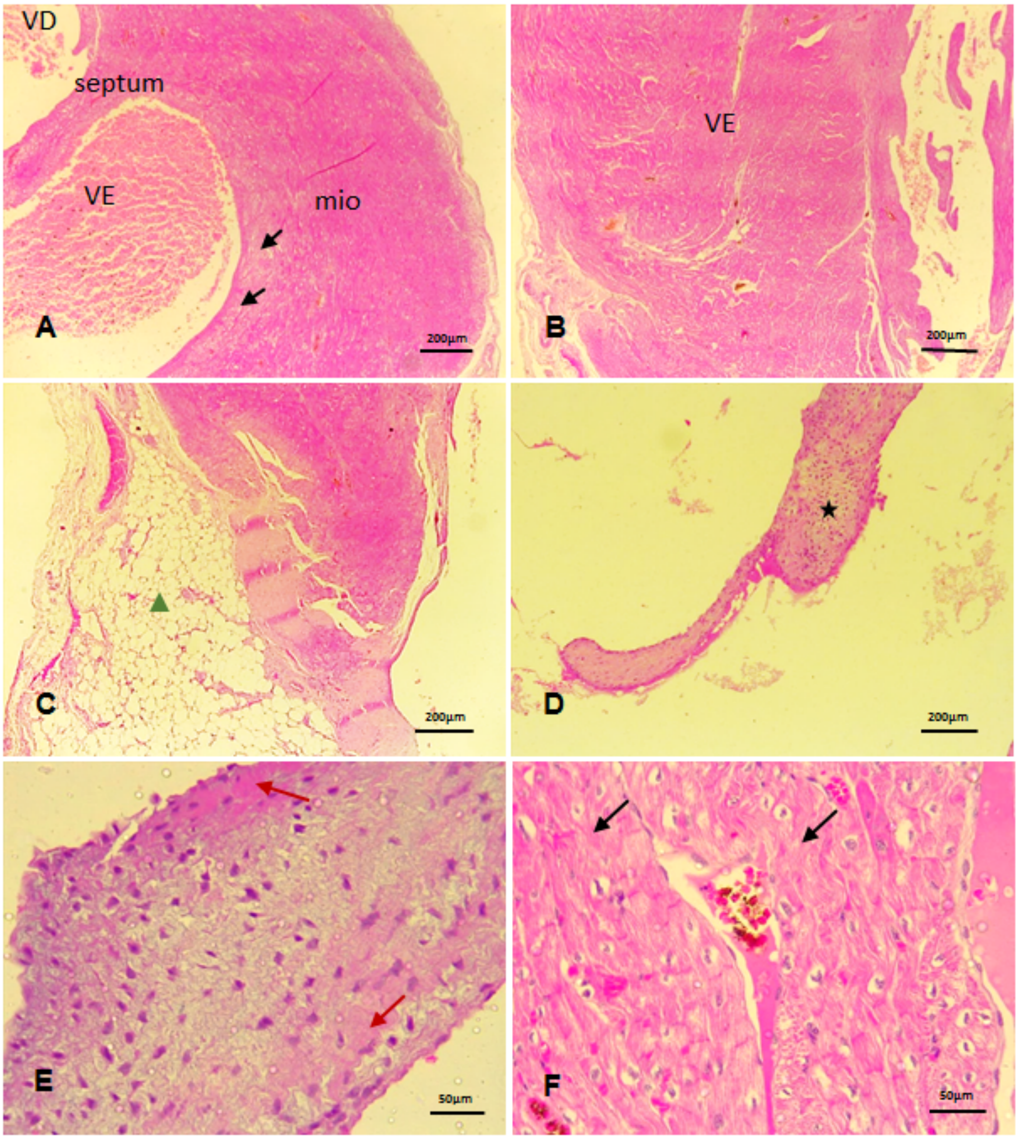

Figure 2 Photomicrographs of the heart of three-toed sloth (Bradypus variegatus) 1, which was routinely processed in paraffin and whose sagittal sections were stained with haematoxylin and eosin. (A) Interventricular region with septum separating the two cardiac ventricular chambers: right (VD) and left (VE). Thick area of myocardium (mio) with degenerated cardiac muscle fibres (→). Scale bar represents 200 µm. (B) Detail of the cardiac muscles of the left ventricle (VE); the muscles of the trabeculae carneae cannot be visualised. Scale bar represents 200 µm. (C) Atrial region showing presence of adipose tissue in the epicardium (▲). Scale bar represents 200 µm. (D) Left atrioventricular valve with thickening of its wall resulting from a myxomatous degenerative process called endocardiosis (★). Scale bar represents 200 µm. (E) Detail of the valve endothelium with thickening of the left atrioventricular valve, displaying collagen degeneration and deposition of fibrous material (→). Scale bar represents 50 µm. (F) Degeneration of cardiac muscle fibres (→). Scale bar represents 50 µm.

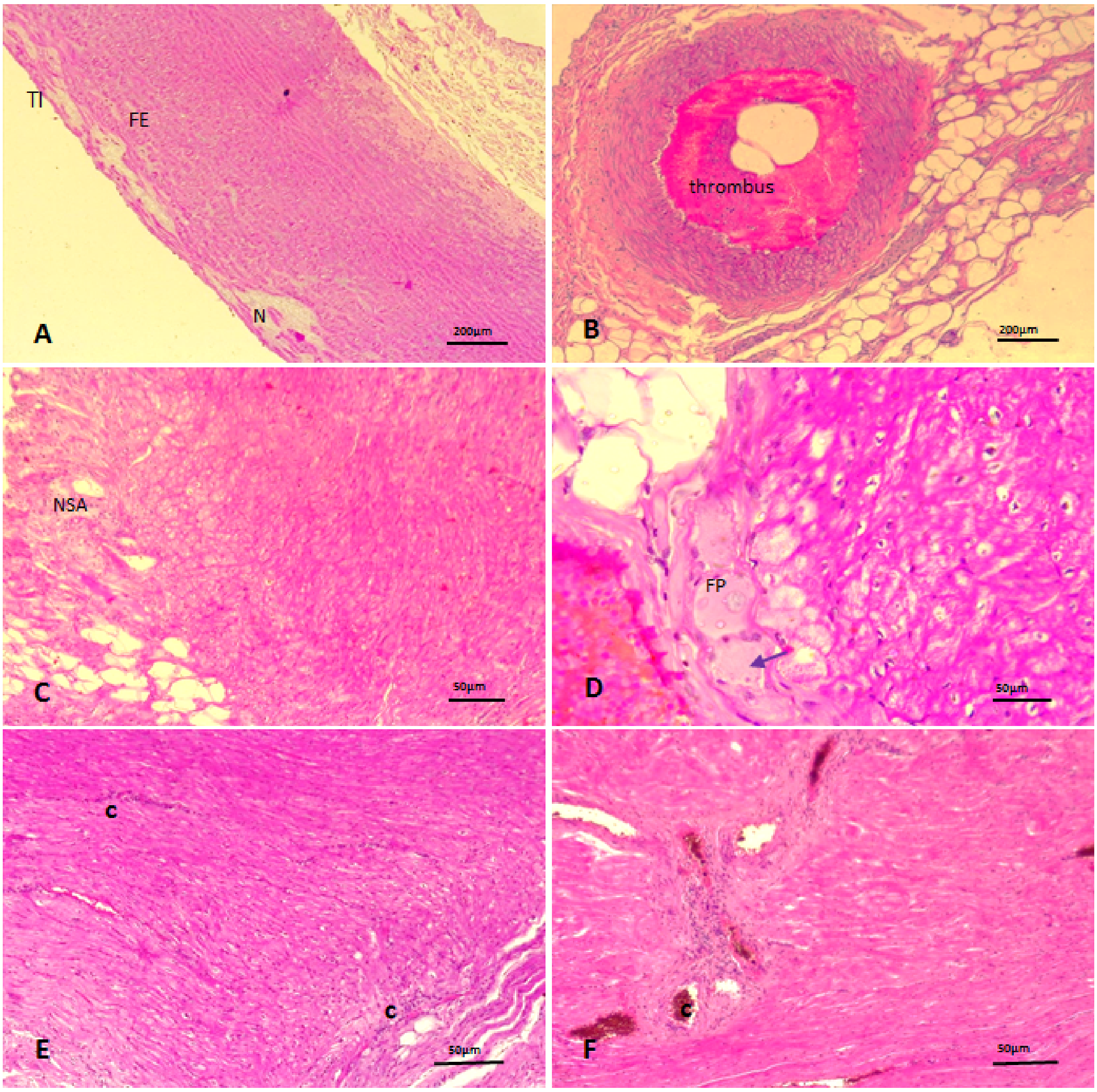

Figure 3 Photomicrographs of sagittal sections of the three-toed slot (*Bradypus variegatus*) heart, which was routinely processed in paraffin and stained with haematoxylin and eosin. (A) Thoracic aorta with its tunica intima (TI) intact and metallic fibres (FE) covering the entire media layer. However, in some areas, there was necrosis (N) in the internal metallic layer. Scale bar represents 200 µm. (B) Lumen of the thoracic aorta artery with presence of arterial thrombus. Scale bar represents 200 µm. (C and D) Sinoatrial node (NSA) and details of Purkinje cells (FP) with necrotic nerve fibres (→). Scale bar represents 50 µm. (E and F) Increase in number of perivascular nuclei between cardiac muscle fibres (fibroblasts/inflammatory cells) and congestion of cardiac vessels (c). Scale bar represents 50 µm.

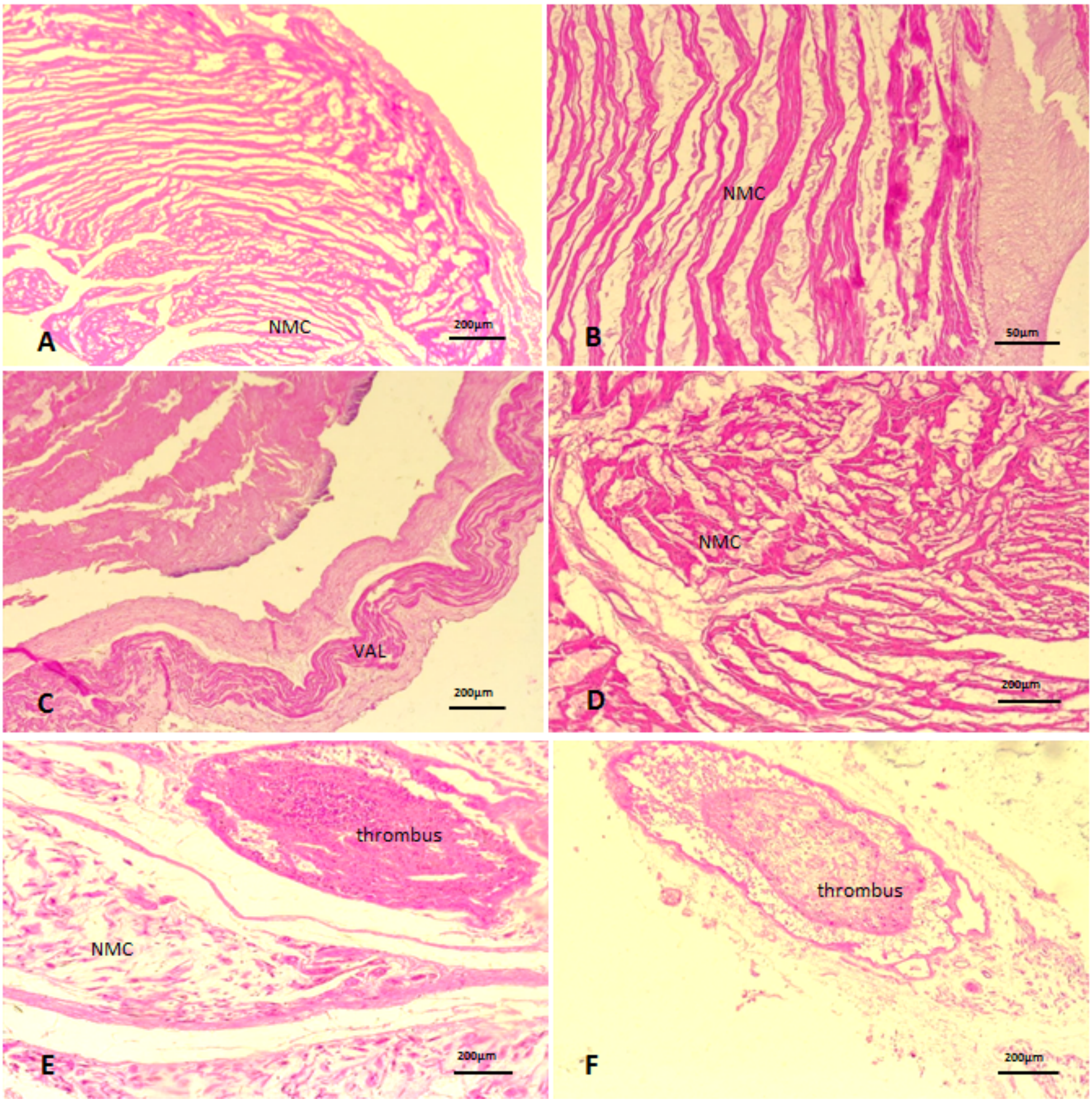

Figure 4 Photomicrographs of sagittal sections of the three-toed sloth (*Bradypus variegatus*) heart, which was routinely processed in paraffin and stained with haematoxylin and eosin. (A and B) Lower magnification view of the heart of sloth 3, showing loss of organ architecture and details of necrosis of numerous cardiac muscle fibres (NMC), associated with infarction. Scale bar represents 200 µm and 50 µm, respectively. (C) Focus of valve-related endocardial necrosis (VAL). Scale bar represents 200 µm. (D) Necrotic lesion of cardiac muscle fibres (NMC) in sloth 4. Scale bar represents 200 µm. (E and F) Presence of a thrombus attached to the vascular endothelium and free in the vascular lumen, respectively. A necrotic lesion of cardiac muscle fibres (NMC) surrounded by thrombotic vessels is also visible. Scale bar represents 200 µm.

There are variations in coronary branches between groups and even between individuals of the same species. As noted by Pinto Neto et al.[19] in goats (Capra aegagrus hircus), the left coronary artery can originate the paraconal or subsinuosal branches, which are located in the subsinuosal groove. In the xenarthrans used in this study, on the cardiac surface, the subsinuosal and paraconal branches occupy the grooves that receive these same names and irrigate, respectively, the right and left ventricle, as seen in the paca[13] and the southern tamandua[15]. In the ringed seal (Phoca hispida), the subsinuosal branch originates from a circumflex branch of the left coronary artery[20]. For crossbred cattle (Bos taurus), the paraconal and subsinuosal branches originate exclusively from the left coronary artery and send vessels to both ventricles[21]. In the marsh deer (Blastocerus dichotomus), however, the paraconal and subsinuosal grooves are occupied by the branches of the left coronary artery, the paraconal and circumflex branches, respectively. A vessel emerges from the right coronary artery and ends cranially in the heart[22].

In sloths, the paraconal branch is longer, reaching the cardiac apex. In the southern tamandua, the circumflex branch of the right coronary artery reaches the apex and, less frequently, the paraconal groove[15]. Based on the data obtained in the present study for B. variegatus as well as the study by Pinheiro et al.[15] with T. tetradactyla, coronary irrigation is balanced in Pilosa animals, with no predominance of one coronary artery over the other in terms of their irrigation areas. There was an exception in one of the tamanduas investigated: the authors found that the right coronary artery protruded from the left, which has also been recorded in human hearts[23]. This anatomy differs from domestic mammals such as the cat (Felis catus)[24, 25] and the goat[19] and wild mammals such as the capybara (Hydrochoerus hydrochaeris)[26] and the tiger (Panthera tigris)[27], where the predominance is from the left coronary artery.

In general, the aspects highlighted regarding the sloth's coronary arteries are very similar to the characteristics of these vessels in the ostrich, except for the subsinuosal branch reaching the apex and the fact that the right circumflex artery produces branches directed to the left cardiac face in the ostrich[16]. This different morphological approach between birds and mammals is related to the fact that these animals are at the same evolutionary stage[28], even though they have different reptilian ancestors.

The histopathological investigation of the three-toed sloth heart revealed that although the types of tissues observed were similar to those seen for other mammals, necrotic areas could be described in different regions and there were related thrombi in the aorta and smaller vessels associated with cardiac vascularisation. Endocardiosis of the left atrioventricular valve was also identified. Researchers indicated that this pathology involving the valves of both antimeres of the heart was the most prevalent heart disease among dogs (Canis lupus familiaris) with and without a defined breed treated at the University Veterinary Hospital of the Federal University of Santa Maria[29]. The high occurrence of myxomatous valve degeneration has also been described by Yamato et al.[30] for poodles and by Castro et al.[31] for various dog breeds. Involvement of the right atrioventricular valve is often considered a secondary condition due to left heart failure; it results in changes in the left ventricle and pulmonary hypertension[32]. This relationship explains the occurrence of bilateral valve impairment, as seen in dogs[29-31], but also points to left unilateral primary damage, which is reported more commonly, as shown in one of the sloth hearts examined in the present study.

Cases of necrosis in cardiac tissues of different species are sometimes associated with the action of pathogens or food poisoning[34-37]. However, in the necrotic events seen in the sloth hearts, there were no structures compatible with the stage of development of any infectious agent. These mammals are well adapted to consuming toxic leaves as a way to reduce competition with other folivores[38-40].

Stress has also been indicated as a triggering factor for cardiovascular problems[41]. In adverse conditions, living beings develop strategies to maintain haemostasis, which are reflected in physiological compensations related, above all, to the heart, the respiratory rate, blood pressure, and body temperature[42]. However, when stress is temporary, somatic and psychological damage can be noticed[43]. Batista et al.[43] provided a good description of this phenomenon in the collared peccary (Tayassu tajacu). They reported stress syndrome: after necropsy and guidance for microscopic analysis of the organs, there was degeneration, necrosis, and retraction of skeletal and cardiac muscle fibres.

Sloths are very specific eutherians in terms of habits and morphophysiological characteristics. In recent years, they have been losing a substantial part of their territory due to anthropic actions. Hence, they are increasingly found closer to urban centres, where they are exposed to many dangers and are at an increased risk of illnesses and accidents[8-10, 44-45]. The constant stress that these animals experience might have an intimate relationship with the various necroses, inflammatory processes, and degeneration seen in the sloths' hearts in the present study.

The existence of thrombi in the vessels responsible for cardiac circulation has a specific association with myocardial infarction[46]. This relationship could be seen in the sloth: in specimens whose hearts exhibited vascular thrombotic events, there were many necrotic muscle fibres, which denote infarction. Thrombosis is considered one of the most relevant cardiovascular complications and is related to morbidity and mortality. Atheromas are considered an important predisposing factor to the development of thrombi[47]. In sloths, however, thrombotic events seem to be conditioned by other conditions because atheromas were not observed, probably due to the slow absorption of nutrients caused by low metabolism that does not favour the accumulation of fat in these animals[48, 49]. When analysing the anatomical and physiological aspects and lifestyle habits of sloths, they spend long periods in safety, motionless, which could compromise the circulatory system if it were not for the presence of admirable networks on the thoracic and pelvic limbs and the duplicity of the caudal vena cava that fragments the passage of blood, guaranteeing excellent tissue transparency and drainage. However, these vascular projects also function as blood reservoirs[50, 51]. This anatomy may favour stasis, which is also linked to thrombus formation. Additional research is needed to identify the exact causes related to the serious cardiovascular problems of sloths identified in this study. Nevertheless, the peculiar vascular characteristics associated with the stress resulting from anthropogenic tensions are a reasonable cause.

5. Conclusion

Based on the analysis of cardiac vascularisation, there was not a predominance of one coronary artery of the ascending aorta over the other. The right and left coronary arteries supply blood to their respective sides of the heart. Necrosis of cardiac tissues is a recurring concern in B. variegatus that is associated with thrombosis, infarction, and stress. Heart diseases, such as endocardiosis, are less common. This information is crucial to understanding the heart health of sloths.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Priscilla Virgínio de Albuquerque, research and writing (review and editing). Sandra Maria de Torres, investigation. Emanuela Polimeni de Mesquita, investigation. Júlio Cézar dos Santos Nascimento, conceptualization. Apolônio Gomes Ribeiro, conceptualization. Joaquim Evêncio Neto, conceptualization. Gilcifran Prestes de Andrade, conceptualization. Adelmar Afonso de Amorim Júnior, conceptualization. Marleyne José Afonso Accioly Lins Amorim, project administration.

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