

Morphometric analysis of the His bundle (atrioventricular fascicle) in humans and other animal species, associated with electrophysiological variables. Histological and immunohistochemical study

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Abstract

His bundle is a part of the specialized electrical conduction system that, in the normal or abnormal hearts, provides connection between the atrial and ventricular myocardial compartments. The aim of this study was to perform a morphometric analysis of the characteristics of His bundle and its association with predetermined electrophysiological variables in humans, dogs, horses, and pigs. We used five hearts of the species studied. Histological sections of 5 μm thickness were obtained and stained with hematoxylin-eosin and Masson's trichrome. We also used the desmin and PAS method for precise identification of cells. His bundle was longer in horses (2.85 x 0.82 mm) and pigs (1.77 x 0.44 mm) than in dogs (1.53 x 0.26 mm) and humans, which was the shortest (1.06 x 0.23 mm). In His bundle cells, the area and diameters were significantly larger in pigs and horses than in humans ($p < 0.001$) and dogs ($p < 0.001$). We have found two patterns of organization of the components of His bundle: Group I, with large cells and a high amount of collagen fibers in ungulates (pigs and horses); group II, with smaller cells and less amount of collagen fibers in humans and dogs. Documenting differences in cell size in His bundle allows us to obtain an additional, alternative identification criterion to commonly used ones such as anatomical location. Morphological characteristics of His bundle and its cells in the different species studied coincide with rapid or slow transmission of the electrical impulse when compared with the predetermined electrophysiological variables.

Introduction

Described initially by Wilhelm His in 1893, the His bundle (HB) is the continuation of the atrioventricular (AV) node and from where the right and left branches of this bundle emerge, at level of the interventricular septum crest; from here the electrical impulse is transmitted from the atria to the ventricles (Uhley and Rivkin 1960; Bishop and Cole 1967; Bharati et al. 1991; James 2002; Vigmond and Stuyvers 2016; Duan et al. 2017). The so-called HB is a part of the specialized electrical conduction system that, in the normal heart, provides the connection between the atrial and ventricular myocardial compartments (Tawara 1906; Tawara 2000; Cabrera et al. 2020). Only until the findings of Tawara (1906; 2000), it was possible to recognize what were the limits of the HB (known at that time as AV penetrating bundle), which made the transition from the AV node when the bundle penetrates the insulating tissues of the central fibrous body and extending posteriorly to the crest of the muscular ventricular septum where it branches (Tawara 1906; Tawara 2000; James 2002; Cabrera et al. 2020). HB cells in humans are small, pale, and with few myofibrils and in bigger mammals they have the same characteristics but are more sizeable (Truex and Smythe 1965; Bishop and Cole 1967; Bharati et al. 1991; Eliška 2006). HB cells in pigs and horses closely resemble Purkinje cells (PC) but are smaller (Mettam 1928; Glomset and Glomset 1940; Bishop and Cole 1967; Bharati et al. 1991). It has been reported that the cells of HB and its branches have a high concentration of glycogen in dogs and horses (Uhley and Rivkin 1960; Bishop and Cole 1967).

Few studies have observed the morphometric parameters of HB and its cells, but it has been indicated that this structure is 1 to 1.5 mm in length in dogs and 0.25 to 0.75 mm in length in humans (Ho et al.

1995). This indicates that the HB in the dog is longer than in the man, possibly because the central fibrous body is more extensive in the dog (Tawara 1906; Tawara 2000; Ho et al. 1995).

This research underlines the importance of studying the components of the His bundle in different species and using these characteristics as a model for clinical study in human hearts. The histological characteristics of this bundle have been described, but very little has been written about its objective data. Our aim was to perform a morphometric analysis of the characteristics of the HB and its association with the predetermined electrophysiological variables in 4 species.

Materials And Methods

Sample processing and staining

We analyzed five hearts of human males obtained from autopsies of the Institute of Legal Medicine and Forensic Sciences of Bucaramanga, Colombia (adults between 20–60 years old and medium size between 60–80 Kg). Likewise, we obtained hearts of other animals for study: five male pig hearts (weighing 85–90 kg and an average age of 5 months old), five hearts of male horses (weighing 250–300 kg and 2.5–3.5 years old), and five hearts of male dogs, who underwent autopsy in small animal clinics in the city of Bucaramanga-Colombia (medium-sized adults weighing 8–19 kg and aged between 5 and 12 years old).

We presented the work to the Ethics Committee of the Cooperative University of Colombia and dated April 16, 2018 Act 003 and they gave us the resolution where it appears that it was approved by that Ethics Committee, through bioethical concept No.

014-2018. This document states that we complied with the scientific, technical and administrative standards for health research of the Colombian Ministry of Health and with the principles of the Declaration of Helsinki for humans and provisions in animals. In this document it is stated: "The project is governed under current Colombian regulations for the use of material from human and animal corpses". Regarding the authorization of the study in humans, we consider that we do comply with the requirements, since it was approved by the Ethics Committee. For its approval in that Committee, all the appropriate documentation that they requested was provided and also the samples were obtained from the Institute of Legal Medicine and Forensic Sciences of Colombia, which have the chain of custody of cadaveric material. In addition, will comply with resolution 008430 of 1993, decree 2164 of 1992, and Law 10 of 1990 of the local Ministry of Health and with the principles of the Declaration of Helsinki. Additionally, they comply with National Law 84 of 1989, which corresponds to the "National Statute for the Protection of Animals", in Chapter VI of the use of animals in experiments and research.

Five cuts of the atrioventricular area per heart were made and the samples obtained for the study included the union between the interatrial and interventricular septum; the cutting area extended two centimeters on each side of the partition. Samples were fixed in a 5% formaldehyde solution, labeled for identification, and included in paraffin. Histological sections of 5 µm thickness were obtained with a

microtome and stained with hematoxylin-eosin and Masson's trichrome. To improve the identification of HB cells, immunohistochemically staining was also performed with clone D33-IR606 of Anti-Human Desmin (DAKO Corporation) to visualize intermediate myofilaments (desmin) and compare them with surrounding cardiomyocytes. We also used a PAS (periodic acid-Schiff) method to visualize the amount of glycogen present in these cells and facilitate identification.

Image assessment and histo-morphometric analysis.

Each sample obtained was analyzed histologically and morphometrically, comparing the procedures with conventional and immunohistochemical staining. Samples were evaluated using a Leica DMD108 optical microscope (Leica Microsystems, Wetzlar, Germany). Computerized morphometric study was performed using Image-Pro Plus 7.0 software (Media Cybernetics, Silver Spring, MD, USA). 200 micrographs were studied under various morphometric parameters. In HB, we analyzed area, mean diameter, fascicle thickness, percentage of connective tissue, percentage of fundamental substance, and percentage of cells at 4X or 10X magnification. In the HB cells, in the surrounding cardiomyocytes and in PC, we also measured area, maximum diameter, minimum diameter, mean diameter, and roundness at 20X or 40X magnification.

Statistical analysis

Descriptive statistics and hypothesis testing were performed using SPSS 20 software (SPSS, Chicago, IL, USA) and Microsoft Excel 2013. Statistical significance was set at $p < 0.05$. Continuous variables were expressed as mean and 95% confidence interval. Descriptive statistics were calculated for each morphometric parameter and the Kolmogorov-Smirnov normality test was performed for each sample. In case of quantitative variables, when comparing two independent groups and with a small sample Mann-Whitney U test was chosen and Student T test was chosen when the sample was large. In case of quantitative variables, after a normal distribution between species, ANOVA test was used, and when its distribution was not normal, the nonparametric Kruskal-Wallis test was chosen. Data were expressed as mean and standard deviation (SD) for all measured lengths.

Results

HB is responsible for transmitting the electrical impulse from the atria to the ventricles where it connects with the Purkinje cells and is the natural continuation of the AV node for rapid and synchronous activation of the ventricles. Its histological structure is difficult to identify, and special techniques are needed to identify it. The different proportions of its components directly influence the speed of transmission of the nervous stimulus. In animal species, this proportion is different and that causes the nervous stimulus to be transmitted at a different speed.

We have carried out a histological and morphometric analysis to classify the different components in an objective way. We have found two patterns of organization of the components of HB: Group I, with

smaller cells and less amount of collagen fibers in humans and dogs; group II, with large cells and a high amount of collagen fibers in ungulates (pigs and horses)

Humans

HB is a thin bundle surrounded by a thin capsule of connective tissue and has a diameter between 0.41–0.77 mm and 1.06 x 0.23 mm in length revealed with Masson's Trichrome and Hematoxylin eosin technique (Table 1) (Fig. 1a, b). The percentage of collagen fibers in HB was 36.9%, with respect to the cells (Fig. 2a).

Table 1

General summary of morphometric parameters of the His Bundle in humans, dogs, horses, and pigs.

Parameters	Humans	Dogs	Horses	Pigs
Area. μm^2 (SD)	622,464.6 (490,712.1)	244,818.5 (20,715.3)	801,243.6 (336,140.3)	1,178,203.3 (582,320.1)
Mean diameter. μm (SD)	579.50 (181.18)	544.99 (152.49)	857.49 (149.58)	1,031.46 (149.88)
Fascicle thickness. μm (SD)	344.85 (92.55)	308.54 (66.12)	506.69 (91.97)	654.05 (153.88)
% collagen fibers	36.9	25.8	43.2	84.7
% cells	60.1	74.2	56.8	15.3
SD: standard deviation.				

Cells were in the central portion, following a longitudinal arrangement most of the time, forming rows 2 to 3 cells thick arranged between the collagen fibers. They occupy $185.669 \mu^2$ of area (75%) with respect to the His bundle. They were rounded or spindle-shaped cells measuring $15 \times 11 \mu\text{m}$ (Fig. 3a, b). Other parameters were also measured (Table 2). They had a pale cytoplasm because it contains few myofibrils compared to cardiomyocytes found in the myocardium. In its cytoplasm we found abundant amounts of desmin filaments that are part of its cytoskeleton, but, on the other hand, they contain a small amount of glycogen, as observed with the PAS technique. These two techniques were important to be able to identify these cells (Fig. 4). No nerve fibers were found inside and the periphery of the bundle.

Table 2

General summary of the morphometric parameters of His bundle cells in humans, dogs, horses, and pigs

Parameters	Humans	Dogs	Horses	Pigs
Area. μm^2 . (SD)	226.35 (112.17)	166.26 (63.39)	436.48 (330.48)	530.79 (272.83)
Max. Diam. μm . (SD)	17.83 (4.69)	15.87 (3.20)	23.61 (9.09)	27.29 (7.84)
Min. Diameter. μm . (SD)	13.53 (3.10)	11.94 (2.49)	18.73 (7.29)	20.83 (6.40)
Mean diameter. μm . (SD)	15.56 (3.64)	13.82 (2.61)	21.23 (8.16)	23.99 (6.92)
Roundness. μm . (SD)	1.04 (0.04)	1.07 (0.03)	1.06 (0.03)	1.06 (0.04)
SD: standard deviation.				

Dogs

HB is a thin bundle which has a fine capsule of connective tissue that surrounds it and has a diameter between 0.46–0.72 mm and 1.53 x 0.26 mm in length with Hematoxylin eosin and Masson's Trichrome (Table 1) (Fig. 5a, b). The percentage of collagen fibers in HB was 25.8%, with respect to the cells (Fig. 2c).

Cells were in the central part, following a longitudinal and transverse arrangement, forming rows 3 to 4 cells thick arranged between the collagen fibers. They occupy 220.764 μm^2 of area (44%) with respect to the His bundle. They were 16 x 13 μm round or spindle cells (Fig. 3c, d). Other parameters were also measured (Table 2). They had few myofibrils compared to the surrounding cardiomyocytes, so their cytoplasm was pale. We found large amounts of desmin filaments in its cytoplasm that are part of its cytoskeleton, but on the other hand, they contain a small amount of glycogen, as observed with the PAS technique. These two techniques were important to be able to identify these cells (Fig. 4). No nerve fibers were observed in the heart of this species.

Horses

HB is a thin bundle surrounded by a thin capsule of connective tissue and has a diameter between 0.73-1 mm and 2.85 x 0.82 mm in length revealed with Masson's Trichrome and Hematoxylin eosin technique (Table 1) (Fig. 5c, d). The percentage of collagen fibers in HB was 43.2%, with respect to the cells (Fig. 6a).

Cells were in the central portion, following a longitudinal arrangement most of the time, forming rows 3 to 5 cells thick arranged between the collagen fibers. They occupy $206.027 \mu^2$ of area (55%) with respect to the His bundle. They were spindle-shaped cells measuring $17 \times 12 \mu\text{m}$ (Fig. 3e, f). Other parameters were also measured (Table 2). They had a pale cytoplasm because it contains few myofibrils compared to cardiomyocytes found in the myocardium. In its cytoplasm we found abundant amounts of desmin filaments that are part of its cytoskeleton, but, on the other hand, they contain a small amount of glycogen, as observed with the PAS technique. These two techniques were important to be able to identify these cells (Fig. 4). We found many nerve fibers inside and the periphery of HB (Fig. 6b).

Pigs

HB is a thin bundle which has a fine capsule of connective tissue that surrounds it and has a diameter between 0.92–1.25 mm and $1.77 \times 0.44 \text{ mm}$ in length with Hematoxylin eosin and Masson's Trichrome (Table 1) (Fig. 1c, d). The percentage of collagen fibers in HB was 84.7%, with respect to the cells (Fig. 6c).

Cells were in the central part, following a longitudinal and transverse arrangement, forming rows 4 to 5 cells thick arranged between the collagen fibers. They occupy $131.180 \mu^2$ of area (22%) with respect to the His bundle. They were $35.8 \times 20.9 \mu\text{m}$ round or spindle cells (Fig. 3g, h). Other parameters were also measured (Table 2). They had few myofibrils compared to the surrounding cardiomyocytes, so their cytoplasm was pale. We found large amounts of desmin filaments in its cytoplasm that are part of its cytoskeleton, but on the other hand, they contain a small amount of glycogen, as observed with the PAS technique. These two techniques were important to be able to identify these cells (Fig. 4). No nerve fibers were observed in the heart of this species. HB presented nerve fibers in its inside and periphery (Fig. 1c, d).

We compared each component of the HB, finding that the thickness of His fascicle is larger in pigs than in humans ($p = 0.035$) and in dogs ($p = 0.018$). The percentage of collagen fibers inside the HB was higher in pigs than in humans ($p = 0.001$) or dogs ($p = 0.007$), while the percentage of cells in HB was higher in humans than in pigs ($p = 0.013$) (Fig. 2, 6).

We next studied HB cells to uncover any differences between the species studied. Regarding HB cells, the area and diameters were significantly larger in pigs and horses than in humans ($p < 0.001$) and dogs ($p < 0.001$), and the maximum diameter of these cells was also larger in pigs than in horses ($p = 0.029$). Cells were rounder in humans than in pigs ($p = 0.049$), in horses ($p = 0.036$), and in dogs ($p < 0.001$).

Description of the data of HB cells in the patterns suggested by us in different groups described by the species also correlated with the size of the P cells in the AV node, which were large in pigs and horses and small in humans and dogs (Table 3).

Table 3
Mean values of AV node P cells parameters in humans, dogs, horses, and pigs

Species	Area ($\mu\text{m}^2/\text{SD}$)	Maximum diameter ($\mu\text{m}/\text{SD}$)	Minimum diameter ($\mu\text{m}/\text{SD}$)	Mean diameter ($\mu\text{m}/\text{SD}$)
Humans	18.65 (5.29)	6.74 (0.86)	4.08 (0.83)	5.23 (0.84)
Dogs	204.22 (63.80)	18.38 (2.8)	13.37 (2.43)	15.6 (2.44)
Horses	322.31 (71.93)	23.15 (2.48)	18.24 (2.32)	19.06 (2.39)
Pigs	843.17 (306.59)	37.46 (7.38)	28.65 (5.51)	32.19 (6.18)
SD: standard deviation.				

Differentiation between HB cells in the different species showed large cells in pigs (Fig. 7a) and horses (Fig. 7b) and small cells in humans (Fig. 7c) and dogs (Fig. 7d). This provides us with alternative criteria for identification and differentiation between these cells.

Discussion

Study of His bundle is gaining importance because it is the natural continuation of the conduction system from the supraventricular portion of the heart and allows depolarization of the large ventricular mass to the most distal portions.

Few previous reports have described the morphometry of HB, and in most of them it has been indicated that this bundle is longer in dogs (1-1.5 mm) than in humans (0.25–0.75 mm) because the central fibrous body is more extensive in canines (Tawara 1906; Tawara 2000; Ho et al. 1995; De Almeida et al. 2020]. However, in other studies, in humans, morphometric values of HB have been reported higher than described by most of the authors and what was found in our study (1.06 x 0.23 mm), where measurements of 2.68 x 3.7 mm (Cabrera et al. 2020), 11.3 x 1.8 mm (Kistin 1949) and HB width of 1-1.5 mm (Shimada and Arita 1996) are indicated, despite which based on the initial descriptions of Tawara (1906) (Kawashima and Sasaki 2011). In Tawara's work (1906; 2000), it was indicated that HB in humans is smaller than in dogs, cats, and ungulates; but the morphometric parameters of this bundle were not reported, only the measurements of the right and left branches in humans, cats and calves were described. It was also indicated that in a heart of an adult large breed dog, the HB measured 3 x 0.7 mm, which contrasts with our results (1.53 x 0.26 mm) which were described in medium breed dogs.

Several studies have indicated that HB and its branches are mainly composed of PC, but smaller than those found in the final portion of the conduction system (Glomset and Glomset 1940; Bishop and Cole 1967; James 1970; James and Sherf 1971; Bharati et al. 1991; Nabipour 2004). In most studies this has been revealed via electron microscopy analysis, but differences have been found in certain characteristics, so we prefer to simply call them HB cells to distinguish them from the cells of the final portion of the conduction system. Previous reports describe HB cells as quite sizable in large mammals and small in humans and dogs (Glomset and Glomset 1940; Bishop and Cole 1967; Bharati et al. 1991; Eliška 2006), and these cells have also been described as elongated and oblong in humans (James and Sherf 1971). These findings coincide with our study in that we found HB cells to have at an equally large size as described, being larger in ungulates and small in humans and dogs, which allows them to be classified into two groups as we have suggested. In humans, HB has been described as a cord-shaped structure with a diameter of 4 mm and a fascicle thickness of 0.7 mm (Waller et al. 1993; Randhawa et al. 2017). In our research, we found the same HB structure, but with a significantly smaller diameter and thickness of the fascicle than had been indicated (0.4 and 0.2 mm respectively).

In pigs and horses, a large number of nerve fibers have been found inside HB, as well as increased connective tissue compared to the surrounding cardiac fibers (Mettam 1928; Glomset and Glomset 1940; Bishop and Cole 1967; Bharati et al. 1991]. In humans, nerve fibers inside the HB and the periphery are also documented (Montoya and Ynaraja 1992; Waller et al. 1993) and little connective tissue inside HB has been described in dogs (Montoya and Ynaraja 1992). In our study, we detected a large amount of nerve fibers present inside the HB in horses and especially in pigs, but we found no nerve tissue in humans or dogs. These nerve fibers contribute to the stimulation or decrease of pacemaker activity generated by the sinus node. We also observed a large amount of connective tissue within HB in pigs and horses, which is largely generated by the central fibrous body where the bundle is located.

In dogs and horses, different authors have reported that HB cells are rich in glycogen (Uhley and Rivkin 1960; Bishop and Cole 1967; Gianni et al. 2018). We sought to identify cells using PAS method in the four species studied, which showed negative in all samples analyzed, suggesting that the glycogen levels in these cells are very low to zero since the test is specific for this substance. Using desmin as an alternative method of immunohistochemical identification, we were able to observe positive for HB cells compared with cardiomyocytes, allowing them to be fully recognized.

AV node is responsible for receiving electrical impulses from the sinus node and one of its main functions is to produce a delay in the transmission of that electrical impulse to the ventricles for its protection. In horse literature at rest, we find a long PR segment (0.14 seconds) on the electrocardiogram (Corredor-Matus et al. 2005; Mira et al. 2016; Mitchell 2019; Van Steenkiste et al. 2020) that coincides with a large number of collagen fibers and large cells in HB reported in our study, which would explain a slower transmission of the electrical impulse when atrial to ventricular activation occurs. Horses belong to group I suggested by us in HB and although pigs also belong to this group due to the similar histological characteristics found, we observed a short PR segment at rest (0.08 seconds) on the electrocardiogram (Fernandez et al. 2003; Wang et al. 2015; Zhang et al. 2016), which would indicate a faster transmission

of the electrical impulse in the atrioventricular zone similar to that described in the species belonging to group II as we will indicate later. What does coincide with our histological findings in HB and AV nodes in horses and pigs at rest is that in these species the heart rate (38–56 beats in horses and 55–86 beats in pigs) (Fernandez et al. 2003; Guerrero et al. 2009; Mira et al. 2016; Zhang et al. 2016) is slower, possibly due to the amount of collagen fibers present in these structures of the conduction system. This indicates that the transmission of the electrical impulse through HB in these species could be slower, which would justify the large number of nerve fibers that help make the impulse's passage more effective. This has also been indicated by other authors who report that the high presence of collagen within the HB can minimize or even prevent the propagation of electrical impulses (James and Sherf 1971; Vijayaraman et al. 2018). In species belonging to group II, a similar PR segment has been reported at rest (0.10 seconds in humans and 0.09 seconds in dogs) (Tilley et al. 2008; Carrillo et al. 2011; Pérez-Riera et al. 2011), coinciding with the little amount of collagen fibers and small cells inside the HB in our study, which would allow a faster transmission of the electrical impulse in the atrioventricular area. The heart rate in humans (60–100 beats) and in dogs (80–120 beats) at rest (Mishra and Rath 2011; Vargas-Pinto et al. 2017) is faster than in group I species, which also coincides with the small cells and the few collagen fibers described by us in HB, allowing a rapid transmission of the electrical impulse. This presence of compacted cellular tissue with many specialized cellular connections can likewise facilitate the propagation of this impulse (James and Sherf 1971; Vijayaraman et al. 2018).

Regarding ventricular activation in group I species, we found in previous reports a wide QRS complex in horses (0.19 seconds) and narrow in pigs (0.05 seconds) at rest (Fernandez et al. 2003; Corredor-Matus et al. 2005; Wang et al. 2015; Mira et al. 2016; Zhang et al. 2016; Mitchell 2019; Van Steenkiste et al. 2020), which could be correlated with the characteristics of Purkinje fibers (PF) in both species, being PC very large and surrounded by thin sheaths of collagen fibers (Canale et al. 1986; Ono et al. 2009), despite the difference in duration of the QRS complex in horses and pigs (Fernandez et al. 2003; Corredor-Matus et al. 2005; Wang et al. 2015; Mira et al. 2016; Zhang et al. 2016; Mitchell 2019; Van Steenkiste et al. 2020). Thanks to these characteristics, these fibers have conduction properties and facilitate rapid propagation of the electrical impulse, like in humans (Oosthoek et al. 1993; Ono et al. 2009; De Almeida et al. 2015). In group II species, the electrocardiographic and histological characteristics of HB are very similar and allow rapid ventricular activation. Although a medium-sized QRS has been reported in humans (0.12 seconds) and narrow in dogs (0.04–0.06 seconds) (Tilley et al. 2008; Carrillo et al. 2011; Pérez-Riera et al. 2011), the low amount of collagen fibers and cellular compaction with a large number of specialized connections allow rapid depolarization of the ventricular myocardium and its consequent rapid transmission of the electrical impulse.

Electrical impulses progress from the branches of HB towards PF to finally allow ventricular contraction, but these fibers also have a determining role in generating ventricular arrhythmias, which can be observed at the electrocardiographic level (Li et al. 2015; Aouadi et al. 2019). For this reason, we reiterate the importance of the histological study of HB, as with a working knowledge of the cellular and tissue structure associated with cardiac physiology, it is possible to delineate the sites of arrhythmia production in different species.

Conclusions

HB cells represent cells with their own entity, different from PC, based on their morphometric and histological characteristics. HB in humans and dogs refers smaller cells, the less the amount of collagen fibers, and absence of nerve fibers. That coincides with the faster transmission of the electrical impulse in the atrioventricular area. HB in horses and pigs refers large cells, high amount of collagen fibers, and large amount of nerve fibers that coincide with the slow transmission of the electrical impulse in the atrioventricular area.

Declarations

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Author contributions

FGT and ARS conceived and designed the study. FGT performed the experiments. FGT wrote the first draft of the manuscript. FGT and ARS revised the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets in this study are available from the corresponding author on reasonable request.

Compliance with ethical standards

The authors declare that all experiment protocols were approved by the Ethics Committee of Universidad Cooperativa de Colombia (No. 014-2018) and will comply with resolution 008430 of 1993, decree 2164 of 1992 and Law 10 of 1990 of the local Ministry of Health and with the principles of the Declaration of Helsinki. Additionally, they comply with National Law 84 of 1989, which corresponds to the "National Statute for the Protection of Animals", in Chapter VI of the use of animals in experiments and research.

Conflict of Interest

The authors declare that they have no conflict of interests.

Consent to participate Not applicable.

Consent for publication All authors give consent for publication.

Code availability Not applicable.

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Figures

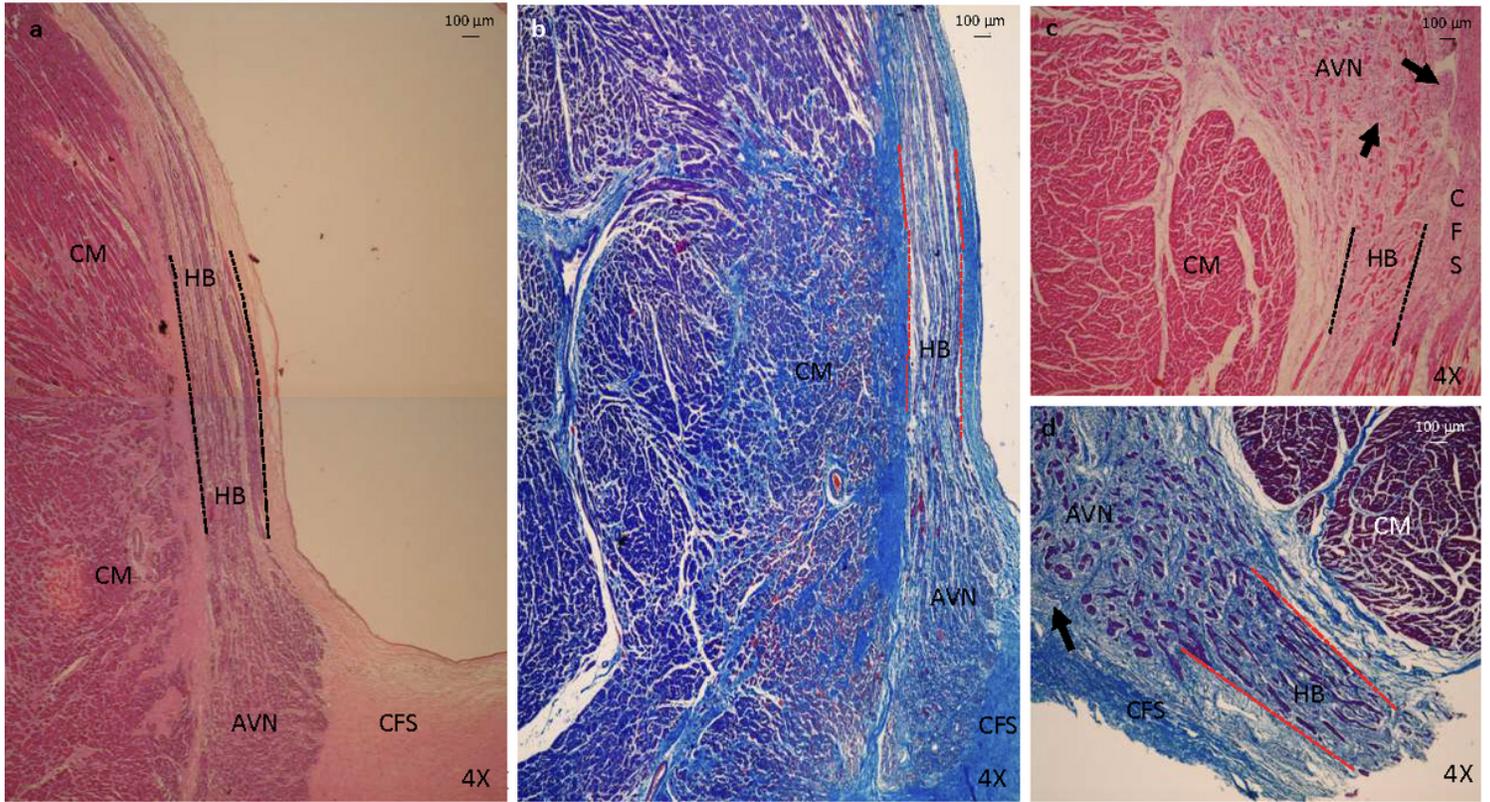


Figure 1

His bundle in humans (a, b) and pigs (c, d) stained with hematoxylin-eosin (a, c) and Masson's trichrome (b, d). The arrows indicate nerve fibers inside and on the periphery of His bundle in pigs. HB: His bundle; CFS: cardiac fibrous skeleton; CM: cardiomyocytes; AVN: atrioventricular node.

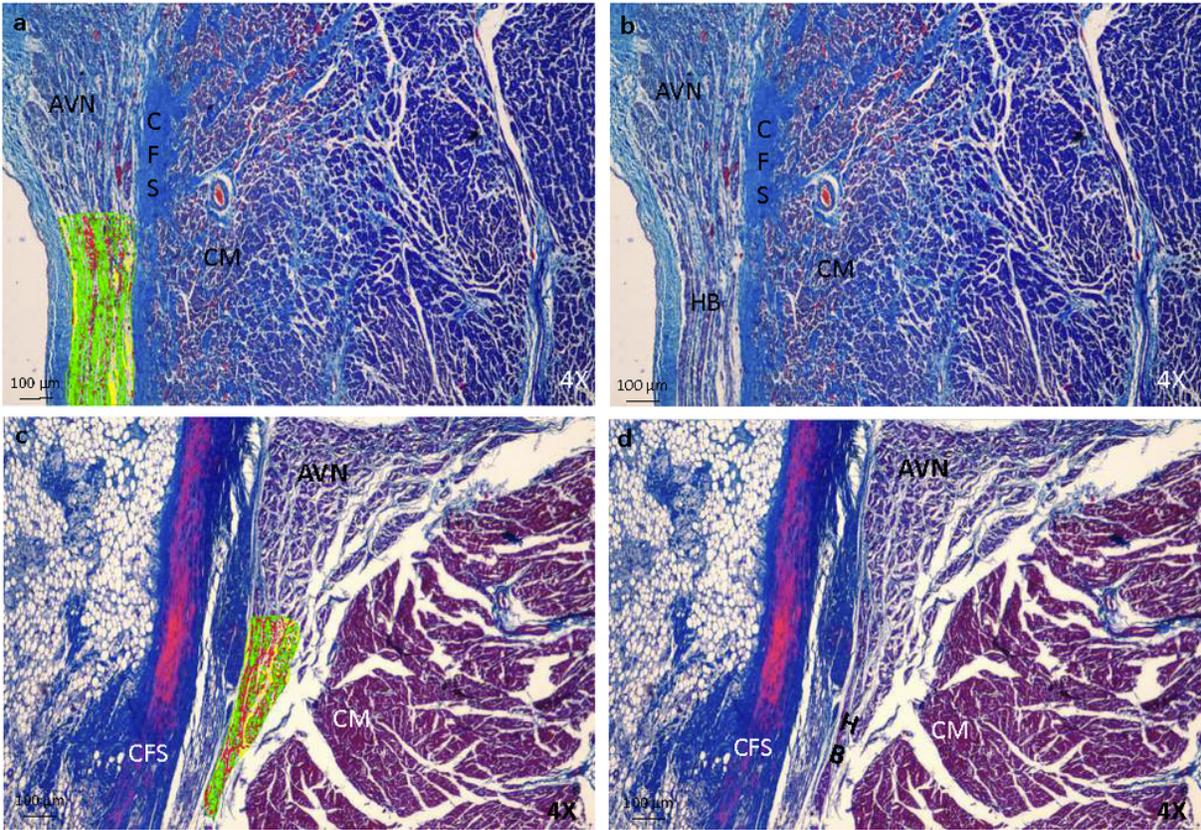


Figure 2

His bundle (HB) stained with Masson's trichrome in humans (a, b) and dogs (c, d). Morphometric analysis (a, c) compared to the original micrograph (b, d) is observed. Note that the red color indicates the percentage of collagen fibers, and green the percentage of cells inside the His bundle. CFS: cardiac fibrous skeleton; CM: cardiomyocytes; AVN: atrioventricular node.

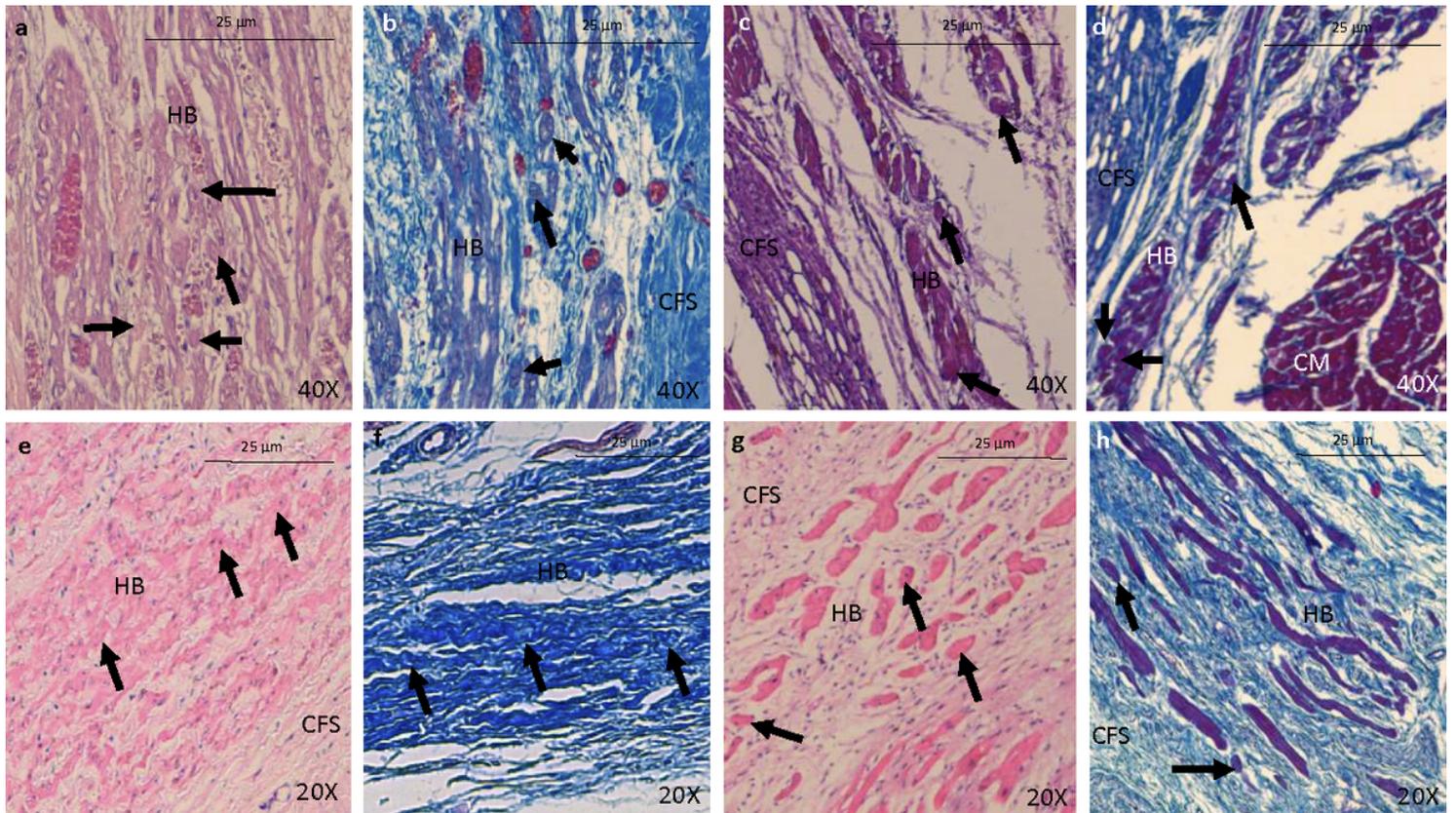


Figure 3

His bundle in humans (a, b), dogs (c, d), horses (e, f) and pigs (g, h) stained with hematoxylin-eosin (a, c, e, g) and Masson's trichrome (b, d, f, h) at high magnification. Arrows indicate His bundle (HB) cells. CFS: cardiac fibrous skeleton; CM: cardiomyocytes.

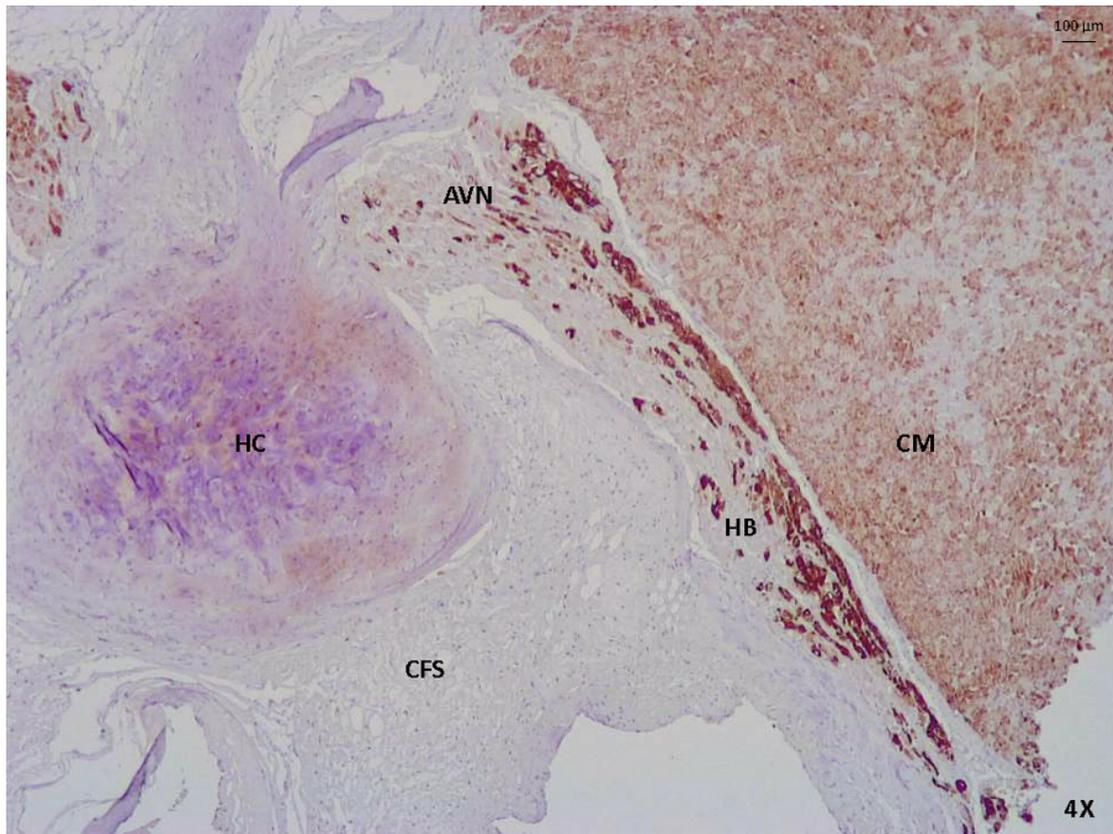


Figure 4

Identification of AV node cells and His bundle cells in dogs by desmin immunohistochemical staining. HB: His bundle; AVN: atrioventricular node; CFS: cardiac fibrous skeleton; HC: hyaline cartilage; CM: cardiomyocytes.

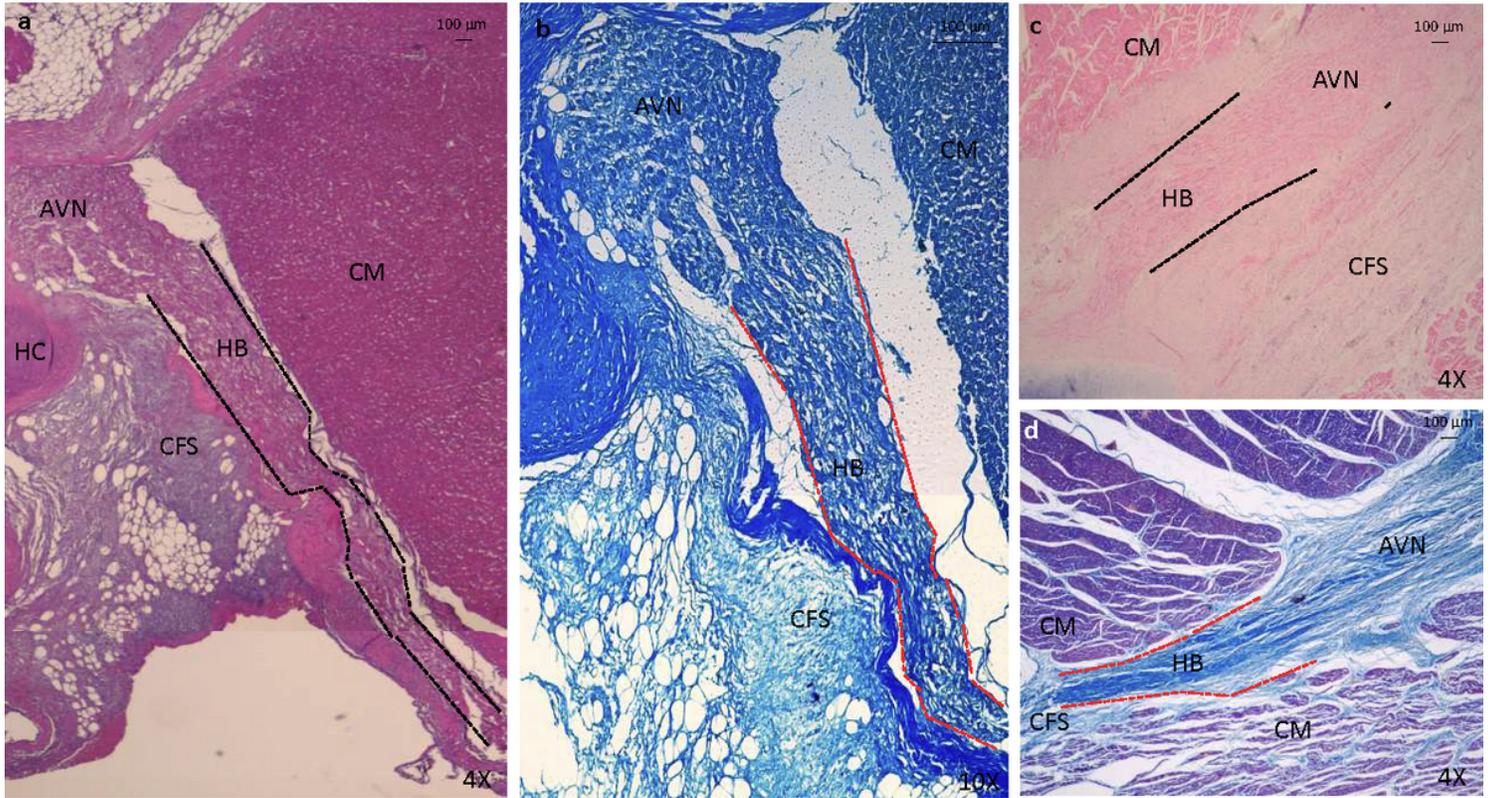


Figure 5

His bundle in dogs (a, b) and horses (c, d) stained with hematoxylin-eosin (a, c) and Masson's trichrome (b, d). HB: His bundle; CFS: cardiac fibrous skeleton; CM: cardiomyocytes; AVN: atrioventricular node; HC: hyaline cartilage.

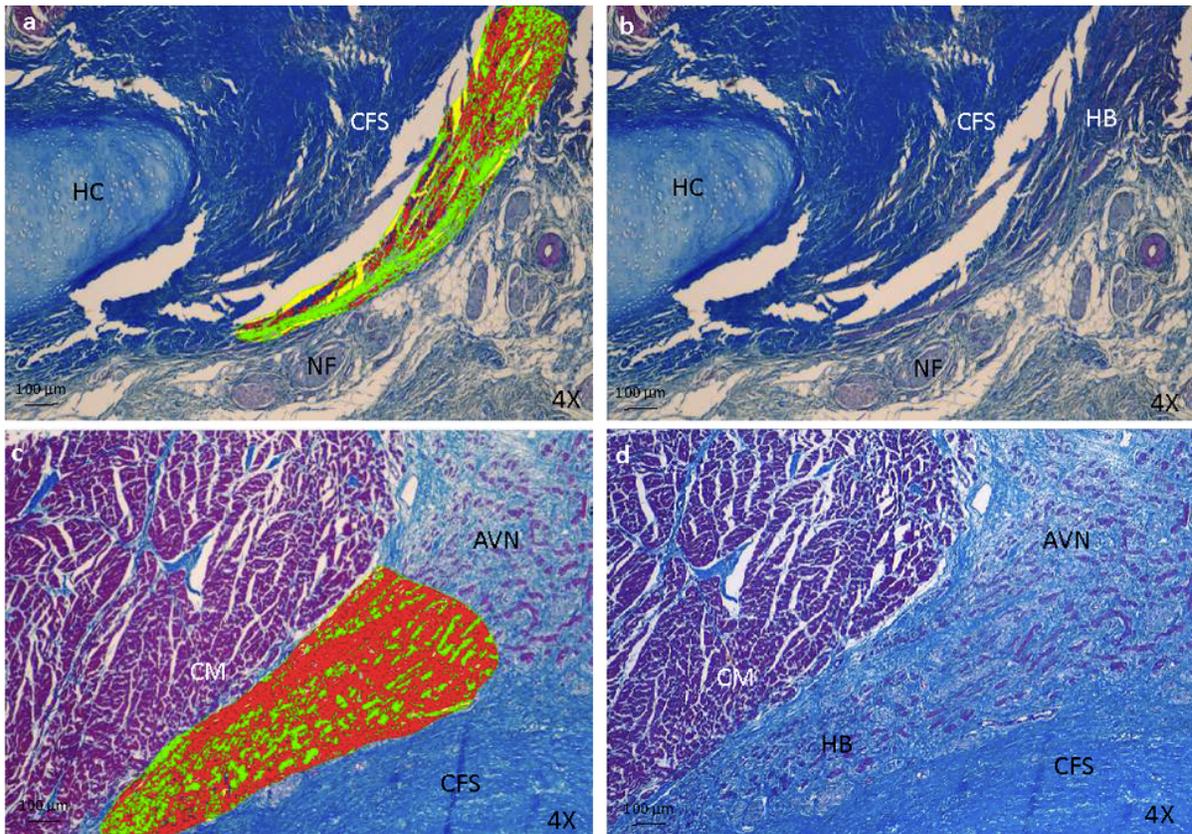


Figure 6

His bundle (HB) stained with Masson's trichrome in horses (a, b) and pigs (c, d). Morphometric analysis (a, c) compared to the original micrograph (b, d) is observed. Note that the red color indicates the percentage of collagen fibers, and green the percentage of cells inside the His bundle. CFS: cardiac fibrous skeleton; CM: cardiomyocytes; AVN: atrioventricular node; HC: hyaline cartilage; NF: nerve fibers

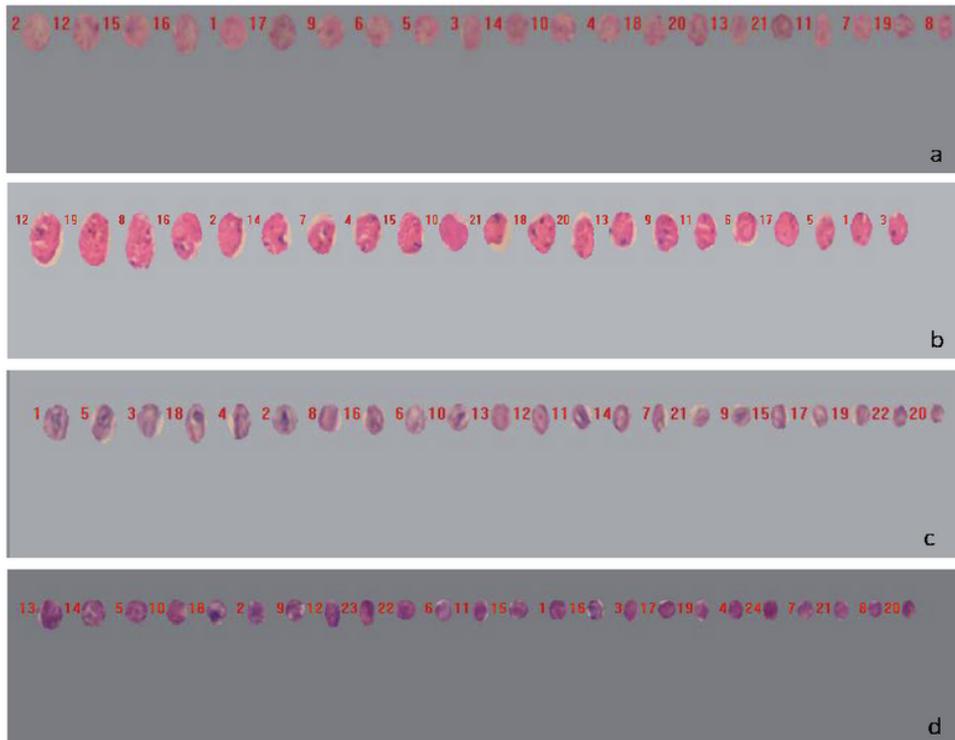


Figure 7

Comparison of the size of His bundle cells in different species. Morphometry of His bundle cells in horses (a), pigs (b), humans (c) and dogs (d).