Histomorpho Quantitative Characterization of Spleen in Horses


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ABSTRACT

Being the largest organ of the lymphatic tissue the spleen works through the white immune defense and through the red pulp in the storage of blood, especially in horses great physical ability that allows greater blood redistribution in order to meet the high oxygen of the tissues. In this way, this study aimed to morphoquantitatively analyze structures of horse spleen through histological and stereological techniques. The fres spleens were subjected to dehydration in increasing ethanol, clearing in xylol, and impregnated embedded in paraffin. Next, using the manual microtome, cut into 5mm thick and stained with Hematoxylin & Eosin and Trichrome Azan. Photomicrographs of five random fields were with Olympus® BX51 microscope and analyzed with the aid of the image analyzer STEPanizer®. The parameters found for the constituents of spleen tissue were descriptive analysis of the data, for obtaining the mean and standard error of Microscopically, spleens consisted of red pulp, trabeculae, connective tissue and white average proportion of red pulp was 66.16%±1.12, trabeculae 11.20%±1.00, connex 8.73%±0.53 and of white pulp 4.39% ± 0.28.

INTRODUCTION

The spleen is an organ formed by a fibroelastic capsule, which emits trabeculae that assist in conducting vessels and nerves, and by a stroma, consisting of a network of fibers and reticular cells providing support to the splenic parenchyma that is formed by white and red pulp. Besides the hematopoietic function in newborns, which can also be performed in adults, the spleen is also involved in defending the body through the production of lymphocytes and antibodies (BACHA & BACHA, 2003). In mammals, the spleen has a similar architecture but the arrangement and proportion of cells vary according to the species (HARTWIG & HARTWIG, 1985).

Based on diverse morphologies evident among different organic systems of horses, the present study aimed to provide histomorphometric data to subsidize future researches, since there is no data in the literature concerning the volume density of structures compounding the spleen of these animals. To this end, a morphological and stereometric study was conducted with horse spleen in order to determine its tissue arrangement and composition.

MATERIAL AND METHODS

Five spleens were collected from horses used to pull wagons, during necropsies of educational interest in the Laboratory of Veterinary Pathology (Ethics Committee of the Institute of Biological Sciences, University of Brasilia - Brazil, Protocol. 51203/2010). The animals were adults of both sexes, had no history of heart or lymphatic conditions. During necropsy, with the animal in the right lateral decubitus an incision was made in the ventral midline, and disarticulating forelimbs and hindlimbs. The access to the abdominal cavity was made by incising the abdominal muscles, and spleen was taken out together with the omentum. Macroscopically we evaluated the shape, the edge of the organ, color, consistency, cutting surface and absence of injuries.

For quantitative analysis of composition of spleens, these were cross-sectioned, fixed in 10% formaldehyde solution for 48 hours and random fragments were subjected to conventional histology: dehydration in increasing ethanol, clearing in xylol, and impregnation and embedding in paraffin. Next, using the manual microtome (Leica RM 2125RT), cut into 4mm thick and stained with Hematoxylin & Eosin and Trichrome Azan for disclosure and differentiation of structural elements and connective tissue in these organs.

To evidence the cell types compounding the spleen, the immunohistochemical technique was accomplished. To this end, antigen retrieval was performed, using citrate buffer (pH = 6) in a water bath for three minutes, blocking endogenous peroxidase with 4% hydrogen peroxide volume 30 for 20 minutes, washing of slides in distilled water and reduction of nonspecific markings from the application of 1% fraction V Bovine
Serum Albumin (BSA). This same solution was also used to dilute the CD20 monoclonal primary antibody (M774, DAKO) at a concentration of 1:200, followed by incubation in a moist chamber overnight at 4°C. On the next day, slides were washed in distilled water and post-primary blocking was done, washed with distilled water and incubated with secondary antibody, both at room temperature for 30 minutes. The developer solution was prepared with diaminobenzidine (DAB) and applied on the slides for about 30 seconds. Counterstaining was performed with hematoxylin for 50 seconds, followed by washing with water and drying in air stream for subsequent passage in alcohol, xylol and mounting with Entellan.

Photomicrographs of five random fields were captured with Olympus® BX51 microscope and analyzed with the aid of the image analysis software STEPanizer®. The parameters found for the constituents of spleen tissue were evaluated by descriptive analysis using the GraphPad Prism® 6.01.

**Results:**

Microscopically, we observed a thin capsule lining the entire organ, which was externally formed by smooth muscle fibers and connective tissue comprised of elastic and collagen fibers, with predominance of the latter (Figure 1B). The capsule emitted into the parenchyma prominent trabecular septa with irregular and sizes and shapes incomplete distributed throughout the organ, without a definite pattern, subdividing it into smaller and irregular compartments (Figure 1A).

In the parenchyma, the white pulp was characterized by dense lymphoid tissue (Figure 1C). Splenic corpuscles were composed of cells arranged in different layers. The centrally arranged cells were clear with vesicular nuclei and those more peripheral were dark, with nuclei well-stained with hematoxylin. The composition, nature and distribution of cellular components in the splenic corpuscle were formed by the germinal center and the outer ring.

The red pulp occupied the regions between the splenic corpuscles and trabeculae, consisting of loose lymphoid tissue throughout the parenchyma forming the splenic sinuses which in turn involved the spleen cords. These were continuous with variable thickness and made up of a loose network of cells and reticular fibers interspersed with macrophages, lymphocytes, plasmocytes and erythrocytes. The splenic sinusoidal capillaries were lined by elongated endothelial cells, with major axis parallel to the sinusoid. This thin and incomplete wall was surrounded by discontinuous basal lamina and by reticular fibers arranged in the transverse direction and in different directions. Through the quantification of lymphocytes stained by immunohistochemistry, it was possible to count $17.0\pm1.1$ of these cells/100µm² (Figure 2).

![Fig. 1: Photomicrographs of horse spleen stained with Trichrome Azan (A) and H&E (B and C).](image-url)
A- Photomicrograph showing the emission of trabecular septa (+) by the capsule. Trichrome Azan. B- Photomicrograph showing the composition of the splenic capsule. Fibrous connective tissue (star) and smooth muscle (asterisk). Hematoxylin & eosin. C-Photomicrograph showing the white pulp (arrow), red pulp (x) and trabecular septa (+). Hematoxylin & eosin.

Fig. 2: Photomicrograph of the white pulp stained with immunohistochemistry for CD-20 showing lymphocytes.

Microscopically, spleens consisted of red pulp, trabeculae, connective tissue and white pulp. The average proportion of red pulp was 66.16%±1.12, trabeculae 11.20%±1.00, connective tissue 8.73%±0.53 and of white pulp 4.39% ± 0.28 (Graph 1).

Graph 1. Volume density of red pulp, trabeculae, connective tissue and white pulp of horse spleen.

Discussion:
The quantification of lymphoid tissues may be accomplished by manual morphometry or semi-quantitative systems (HALASZ et al., 1993). These methods allow the correlation and knowledge of several parameters as well as provide three-dimensional estimates, and are important for a better classification of lymphoid compartments (MELO-JUNIOR et al., 2001; LIMA et al., 2002). The morphometric study of the splenic immunoarchitecture proved important for understanding the mechanisms of defense when the organism is exposed to pathogen agents (BRITO et al., 2005; ROTH et al., 2006).

In horses, two or three muscle layers are oriented perpendicular to each other along the connective tissue of the capsule and its trabeculae, forming a thicker capsule, thus allowing a more effective contraction and expulsion of blood accumulated inside the organ (BACHA & BACHA, 2003). This is the mechanism that occurs during hemorrhage, during the exercise, during the administration of certain anesthetics and under stress. The amount of blood expelled from the spleen by a contraction depends on the blood storage capacity of the species. In the case of horses, the volume of blood stored is about 1/3 of the total circulating (SWENSON & REECE, 2004). The presence of hematopoietic cells in the spleen reveals its ability to generate red blood cells, which tends to decline and become inactive over the years (PACHECO et al., 2003).
A common characteristic of animals that require large blood supply is the presence of large amounts of trabeculae in the splenic parenchyma; these consist mainly of smooth muscle and connective tissue. Although there are no comparative data for this species in the literature we found that as in dogs (Valli et al., 2002), due to the large amount of trabeculae in the parenchyma (tab. 1), the spleen of horses also has a high expandability with the purpose of storing large amount of erythrocytes, as well as a very high contraction capacity to meet the demand for blood. Otherwise it was observed in rats and mice (Cesta, 2006; Valli et al., 2002) a little amount of trabeculae, since rodents do not have such contraction capacity.

Conclusion:
A quantitative and descriptive evaluation characterized and defined that the spleen of horses has a very peculiar arrangement. Structurally, this organ showed red pulp, trabeculae, connective tissue and white pulp. The volume density of its main components was responsible for hematopoiesis, defense system and splenic contraction. Our results may be used as a reference for future work.

REFERENCES