

Histomorphological Studies on the Spleen of Pig (*Sus scrofa*)

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How to cite this paper: Kaur, H., Singh, O., & Pathak, D. (2020). **Histomorphological Studies on the Spleen of Pig (*Sus scrofa*)**. *International Journal of Livestock Research*, 10(12), 68-72. doi: <http://dx.doi.org/10.5455/ijlr.20201008035904>

Received : Sep 29, 2020

Accepted : Nov 24, 2020

Published : Dec 31, 2020

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Abstract

The present study was conducted to study the histomorphology of the spleen of pig (n=12). The pig spleen was enclosed by connective tissue capsule that was composed of collagenous, elastic and reticular fibers along with smooth muscle fibers. The capsule was made up of two layers, outer fibrous connective tissue and inner muscular layer of smooth muscle fiber. Adipose tissue was also observed in capsule. The branching connective tissue trabeculae extended from the inner muscular layer of capsule into splenic parenchyma and divided it into smaller compartments by forming a net like framework which was composed of reticular fibers, smooth muscle fibers and collagen fibers. The splenic parenchyma consisted of red pulp and white pulp. The white pulp was made up of two components, which were splenic nodules or Malphigian corpuscles or lymphatic nodules and peri-arterial lymphatic sheath. Peri-arterial lymphatic sheaths were abundant in spleen. Central artery was placed eccentrically and two or three central arteries were observed in the splenic nodule in present study. Splenic nodules were composed of aggregation of the lymphatic tissue which occurred as aggregation of two or three nodules. Large and abundant ellipsoids were characteristic of pig spleen and composed of macrophages, reticular cells and lymphocytes. The shape of ellipsoids varied from oval to elongated. Red pulp consisted of splenic sinuses, splenic cords and blood vessels. Splenic sinuses were poorly developed in the pig.

Keywords: Histomorphology, Spleen, Pig

Introduction

The spleen is the largest lymphoid organ and is the primary site for immune cell proliferation and differentiation and is specialized for filtration of blood. It is involved in haemopoiesis and phagocytosis of aged erythrocytes (Das *et al.*, 2005). It is comprised of two functionally and morphologically distinct compartments, the red pulp and the white pulp. Dellman and Brown (2006) classified mammalian spleens depending on the type of post capillary vessels into sinusal and non-sinusal type. In majority of domestic animals except dog non sinusal type of spleen is present. The architectural design of spleen varies due to functional reasons (Alim *et al.*, 2012). A thorough knowledge of histomorphology will be helpful in explaining the immune response of pigs to varying clinical conditions and also in understanding peculiarities of porcine immune system for applied experimental studies.

Materials and Methods

The present study was conducted on spleen of pig (n=12). The tissue samples from pig spleen were fixed in 10% neutral buffered formalin immediately after collection. After achieving the complete fixation, the tissue samples were processed for paraffin block preparation by acetone-benzene schedule (Luna, 1968). Sections of 4-5 μm were obtained on clean glass slides with the help of rotary microtome. These paraffin sections were stained with various stains to study the histomorphology of spleen.

Table 1: Histomorphological stains used on paraffin sections of spleen of Pig

Stain	Reference	Purpose
1. Hematoxylin and Eosin	Luna (1968)	Routine morphology
2. Masson's trichrome	Luna (1968)	Collagen fibers
3. Gridley's	Sheehan and Hrapchak (1973)	Reticular fibers
4. Verhoeff's	Sheehan and Hrapchak (1973)	Elastic fibers

Results and Discussion

The structure of spleen of pig consisted of capsule, trabeculae, white pulp and red pulp.

Capsule and Trabeculae

The spleen was enclosed by double layered connective tissue capsule (Fig. 1) made up of outer fibrous connective tissue and inner muscular layer of smooth muscle fibers (Fig. 2). The capsule was composed of collagenous, elastic and reticular fibers along with smooth muscle fibers. Adipose tissue was also observed in capsule.

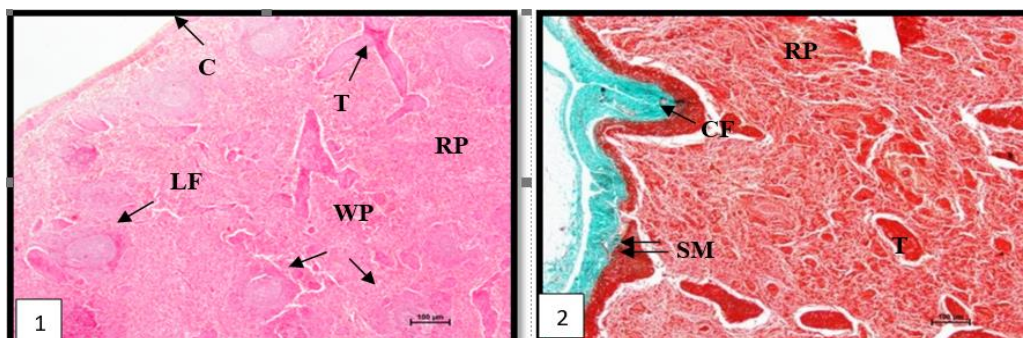


Figure 1: Section of the spleen of pig showing the connective tissue capsule (C), trabeculae (T), white pulp (WP), red pulp (RP) and lymphoid follicle (LF) (Arrow). Haematoxylin and Eosin, X 100.

Figure 2: Spleen of pig showing two layered capsule (C), trabeculae (T). The outer layer of capsule made up of collagen fibers (CF) (single arrow) and inner layer was smooth muscle layer (SM) (double arrows)(Arrow).Masson's trichrome, X 100.

Shringi *et al.* (2017) also reported that splenic capsule was composed of collagen, elastic and reticular fibers along with smooth muscle fibers in Large white Yorkshire pig. The collagen fibers were found abundantly in fibrous layer and elastic fibers were observed more in inner muscular layer of smooth muscle in the present study. Similar observations were made by Waghaye *et al.* (2017) in goat. The elastic fibers in capsule were arranged parallel to the direction of muscle fibers which might help in distention and contraction of smooth muscle. The collagen fibers in the outer layer provides firmness. The branching connective tissue trabeculae extended from the inner muscular layer of capsule into splenic parenchyma and divide it into smaller compartments by forming a net like framework (Fig 4). The trabeculae were scattered throughout splenic tissue and were composed predominantly of reticular fibers and smooth muscle fibers (Fig. 3). Reticular fibers were arranged parallel to direction of smooth muscle fibers of trabeculae. The collagen fibers were also present in the capsule and trabeculae of the pig spleen in the present study.

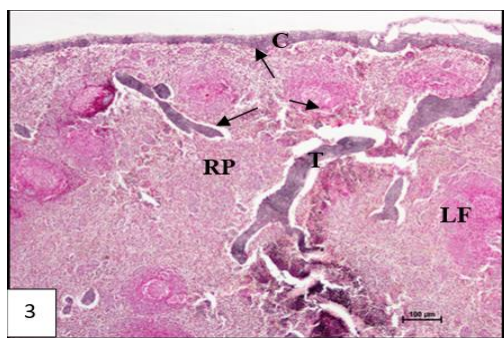


Figure 3: Section of pig spleen showing reticular fibers (RF) in the capsule (C), trabeculae (T) and white pulp (WP). (Arrow). Gridley's stain, X 40.

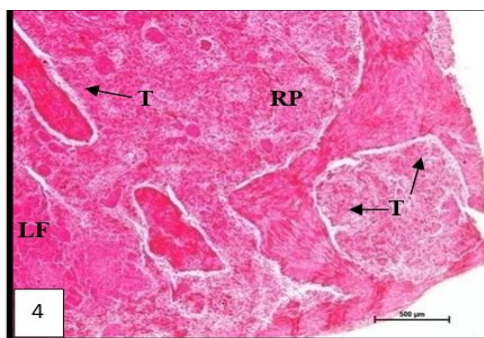


Figure 4: Section of pig spleen showing branching trabeculae (T) extending into parenchyma, lymphoid follicle (LF) and red pulp (RP) (arrow). Hematoxylin and Eosin, X 100

Parenchyma

The splenic parenchyma was composed of red pulp and white pulp.

White Pulp

White pulp consisted of lymphocytes, plasma cells, reticular cells and macrophages which were distributed throughout splenic parenchyma. The white pulp was composed of two components- splenic nodules or Malpighian corpuscles or lymphatic nodules and peri-arterial lymphatic sheath. The observations of present study were corroborated with reports made by Devi *et al.* (2016) in Marwari goat. Splenic nodules were composed of aggregation of the lymphatic tissue which occurs as aggregation of two or three nodules (Fig. 5). The splenic nodules showed germinal center which appeared as lightly stained area with loosely aggregated large lymphocytes and few plasma cells and eccentrically placed central arteries (Fig. 6).

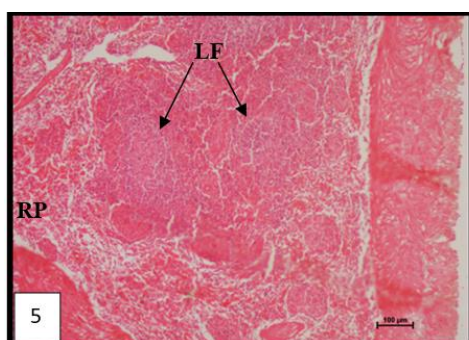


Figure 5: Section of spleen of pig showing aggregation of lymphoid follicle (LF) and red pulp (RP)(arrow). Hematoxylin & Eosin, X 100

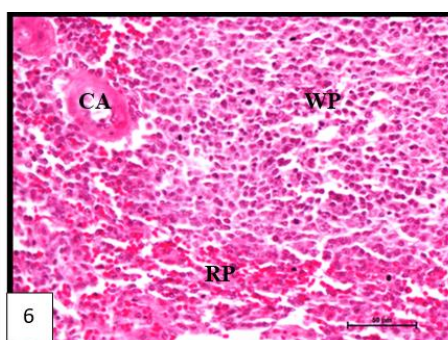


Figure 6: Section of pig spleen showing differential area of white pulp (WP) with nucleated cells and red pulp (RP) with splenic sinuses and erythrocytes. Hematoxylin and Eosin, X 1000

The peri-arteriolar lymphatic sheath was mainly made up of lymphocytes with reticular cells, macrophages and plasma cells with central artery placed eccentrically (Fig 8). The peri-arteriolar sheath was abundant in pig spleen (Fig. 7). The present study revealed that ellipsoids (pericapillary macrophage sheath) were large and abundant in the marginal zone (Fig. 10). The shape of ellipsoids varied from oval to elongated and was composed of macrophages, reticular cells and lymphocytes around the arteries. The marginal zone was made up of two or more layers of reticular and collagen fibers in spleen of pig in the study. Two or three central arteries were observed in the splenic nodule in present study which is in agreement with the observations of Das *et al.* (2005) in dog.

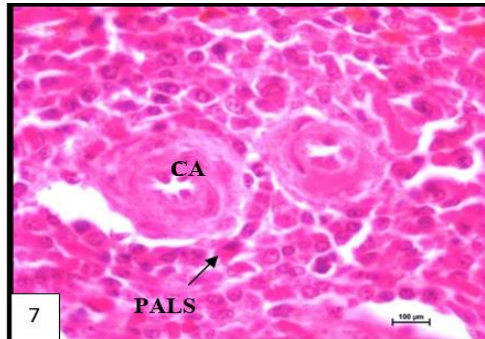


Figure 7: Section of spleen of pig showing central artery (CA) surrounded by PALS (Peri-arteriolar lymphatic sheath) (arrow). Hematoxylin& Eosin X 1000

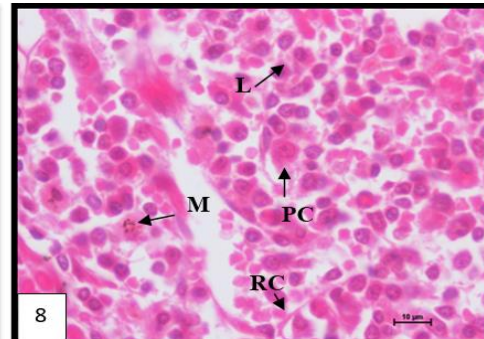


Figure 8: Section of pig spleen showing macrophages (M), plasma cells (PC), reticular cells (RC) and lymphocytes (L)(arrow). Hematoxylin and Eosin X 1000

Red Pulp

The red pulp occupied space between white pulp and trabeculae (Fig. 9). Red pulp consisted of splenic sinuses, splenic cords and blood vessels. The splenic cords were composed of reticular cells, lymphocytes, macrophages, granulocytes, plasma cells and mast cells. Reticular fibers formed the meshwork in the spleen of pig which was in accordance with Usende *et al.* (2014) as they reported that red pulp cords were consisted of reticular network with megakaryocytes in Nigerian indigenous pig. In the present study, the red pulp splenic sinuses were observed as irregular anastomosing channels between splenic cords. The splenic sinuses were poorly developed in pig and contained erythrocytes, granulocytes and circulating mononuclear cells.

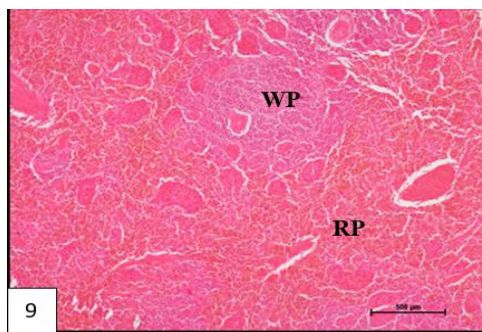


Figure 9: Section of spleen of pig showing area of red pulp (RP) and white pulp (WP). Hematoxylin and Eosin X100

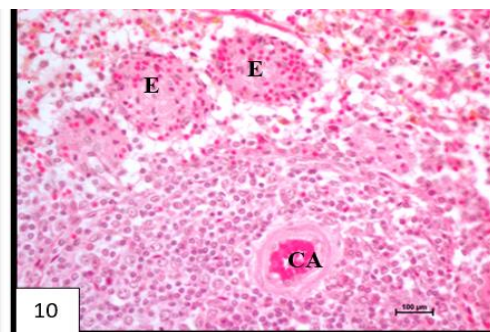


Figure 10: Section of pig spleen showing central artery (CA) with erythrocytes and ellipsoids (E). Hematoxylin and Eosin X1000

Conclusion

Spleen was enclosed by connective tissue capsule, made up of outer fibrous layer of collagen, elastic and reticular fibers and inner of smooth muscle fibers. Branching trabeculae arose from inner muscular layer of capsule and scattered throughout splenic tissue. White pulp consisted of aggregation of lymphoid follicles and large number of well-developed PALS around central arteries. Ellipsoids were large and found abundantly in marginal zone. Splenic sinuses were poorly developed in pig.

Conflict of Interests

There is no conflict of interest.

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