RESEARCH ARTICLE

Histochemical and Immunohistochemical Studies on Pig Spleen (*Sus scrofa*)

Harmanpreet Kaur[1], Opinder Singh[2]*, Devendra Pathak[3]

Abstract

Histochemical and immunohistochemical studies were conducted on spleen of 12 healthy and normal pigs. The sections were stained with various stains to study the histochemical details. The capsule and trabeculae showed moderate activity for neutral and acid mucopolysaccharides and proteins whereas white pulp showed weak activity for acidic mucopolysaccharides and moderate to strong for neutral mucopolysaccharides. The lymphocytes present in lymphoid follicle of white pulp and erythrocytes in sinuses were devoid of acid mucopolysaccharides activity but periphery showed positive reaction. The ellipsoids and lymphocytes in germinal centre showed weakly positive activity. The capsule and trabeculae showed positive reaction for phospholipids and bromophenol blue positive activity was observed throughout the spleen. Polymer based horseradish peroxidase method was used to demonstrate the presence of B-lymphocyte and T-lymphocyte in white pulp. Localization of more T-lymphocytes and macrophages in white pulp and ellipsoids implied that T-lymphocytes possibly act as major phagocytic cell in spleen.

Keywords: Histochemistry, Immunohistochemistry, Pig, Spleen.

Ind J Vet Sci and Biotech (2022): 10.21887/ijvsbt.18.1.5

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 haemopoisis 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. The red pulp is mostly involved in blood storage and phagocytosis, the white pulp is the primary region for immune response and B-lymphocyte maturation (Ikpegbu *et al.,* 2014). 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.,* 2014). The objective of the study was to ascertain the histophysiology of pig spleen, erythrophagocytic activity and localization of major phagocytic cells as apart from source of livelihood for farmers there is increasing interest and demand of pigs for biomedical research.

Materials and Methods

The present study was conducted on the spleen of pig (n = 12). The tissue samples from pig spleen were fixed in 10% neutral buffered formalin immediately after collection. The fixed tissue samples were processed for paraffin block preparation by acetone-benzene schedule (Luna, 1968). The paraffin blocks were prepared and sections of 4 to 5 µm were obtained on clean glass slides with the help of rotary microtome. These paraffin sections were stained with various stains *viz.* Periodic Acid Schiff for Neutral mucopolysaccharides (Sheehan and Hrapchak, 1973), Alcian Blue at pH 1.0 and 2.5 for Acid mucopolysaccharides, Bromophenol Blue for basic protein, Sudan Black for Lipids, Acid Haematin for phospholipids (Chayen *et al.*, 1969) and Perl's Prussian Blue for hemosiderin (Sheehan and Hrapchak, 1973) to study the histochemistry of spleen. The polymer-based Horseradish peroxidase method was used for immunostaining (Pathak *et al*., 2019). The sections in duplicate were mounted on super frost positively charged slides (Fisher Scientific). After dewaxing and rehydration, heat-induced antigen retrieval was done in citrate buffer. The sections were incubated with primary antibodies and

[1]Department of Veterinary Anatomy, Khalsa College of Veterinary and Animal Sciences, Amritsar-143001, Punjab.

[2]Department of Veterinary Anatomy, GADVASU, Ludhiana, Punjab, 141004 orcid id: 0000-0003-1050-925X.

[3]Department of Veterinary Anatomy, GADVASU, Ludhiana, Punjab, 141004 orcid id: 0000-0002-3999-3906.

Corresponding Author: Opinder Singh, Department of Veterinary Anatomy, GADVASU, Ludhiana, Punjab, 141004, e-mail: singhopinder68@gmail.com

How to cite this article: Kaur, H., Singh, O., Pathak, D. (2022). Histochemical and Immunohistochemical Studies on Pig Spleen (*Sus scrofa*). Ind J Vet Sci and Biotech. 18(1), 23-27.

Source of support: Nil

Conflict of interest: None.

Submitted: 23/07/2021 **Accepted:** 29/11/2021 **Published:** 10/01/2022

universal secondary antibodies. The cryostat sections were utilized for the demonstration of lipids and phospholipids.

Results and Discussion

The distribution of neutral and acidic mucopolysaccharides, lipids, phospholipids, and proteins was studied in pig's spleen.

Acid Mucopolysaccharides

The splenic capsule was positive for acidic mucopolysaccharides. The outer fibrous layer was strongly positive for acidic mucopolysaccharides, and the inner muscular layer was weakly positive for acidic mucopolysaccharides (Figure 1). The trabeculae extending from the capsule also showed weak activity for the presence of acidic mucopolysaccharides. The lymphocytes present in the lymphoid follicle of white pulp were devoid of acidic mucopolysaccharides activity. However, some activity was observed around the central artery and in the connective tissue framework. Ellipsoids were weakly positive for acidic mucopolysaccharides. The lymphocytes present in germinal centers were weakly positive for acid mucopolysaccharides indicating the presence of immature lymphocytes. The connective tissue fibers showed weak to moderate activity for the acid mucopolysaccharides. The erythrocytes present in the sinuses were devoid of acid mucopolysaccharides. The peripheral part of splenic sinuses was positive for acid mucopolysaccharides which may be due to a connective tissue framework.

Neutral Mucopolysachaarides

The inner muscular layer of the capsule covering the spleen was moderately positive for neutral mucopolysaccharides; however outer fibrous layer was weakly positive (Figure 2). The trabeculae extending from the capsule towards the parenchyma of the spleen also showed moderate reaction for neutral mucopolysaccharides. The lymphoid follicles present in the white pulp of the spleen were comprised of lymphocytes and strongly positive for neutral mucopolysaccharides (Figure 2). The connective tissue framework was strongly positive for neutral mucopolysaccharides in the parenchyma of the spleen. The area around blood vessels was weakly positive for neutral mucopolysaccharides. Gautam and Mishra (2015) observed strong periodic acid schiff (PAS) reaction in capsule, trabeculae, trabecular arteriole and nodular arteriole, and erythrocytes of the red pulp in prenatal goat spleen.

Lipids and Phospholipids

Fine sudanophilic lipid droplets were observed inside the capsule and trabeculae of the spleen (Figure 3). Fat lobules were present surrounding the capsule of the spleen. These lobules suggested that the spleen was embedded

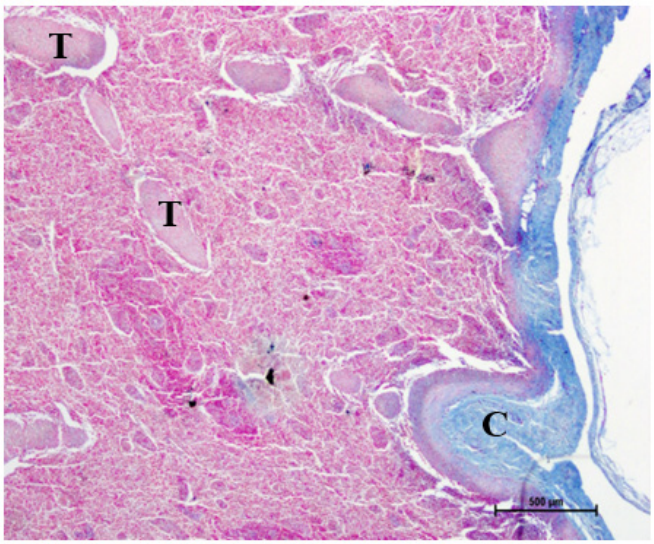

Figure 1: Photomicrograph of section of spleen of pig showing acidic mucopolysaccharide activity in capsule(c) and trabeculae (T) of pig spleen. AB/PAS X 100.

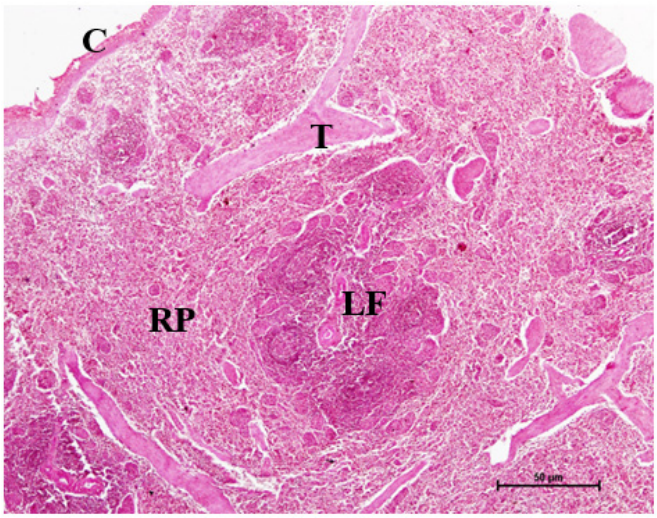

Figure 2: Photomicrograph of section of spleen showing moderate to strong activity for neutral mucopolysaccharides in capsule (C), trabeculae (T), and lymphoid follicles (LF). Neutral mucopolysaccharide activity is also seen in red pulp (RP). PAS X 100.

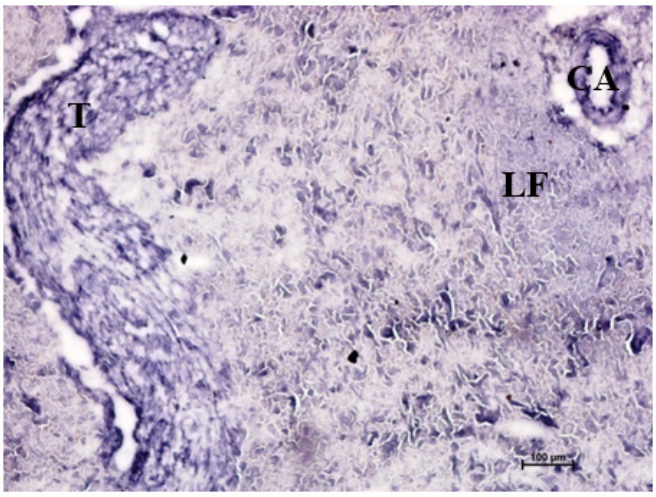

Figure 3: Section showing reaction of lipids in trabeculae (T), lymphoid follicle (LF) and central artery (CA). Sudan black X 100

in the white adipose tissue. The capsule was positive for phospholipids (Figure 4). The trabeculae extending from parenchyma showed a positive reaction for phospholipids. The presence of phospholipids was also observed around blood vessels.

Proteins

In the spleen of the pig, the bromophenol blue positive activity was observed throughout the spleen. However, the intensity of the activity varied in different structures present in the spleen. The capsule showed moderate to strong activity for proteins (Figure 5). This might be due to the presence of collagen and reticular fibers, which contain glycoproteins. The trabeculae extending from the capsule showed moderate to strong activity for proteins. The lymphoid follicles present in the pulp region showed moderate activity for proteins (Figure 6). Erythrocyte present in the sinuses showed intense strong activity for proteins. The connective tissue present in the parenchyma also showed moderate to strong positive action for proteins. In the parenchyma region, the diffused lymphocytes showed moderate protein content.

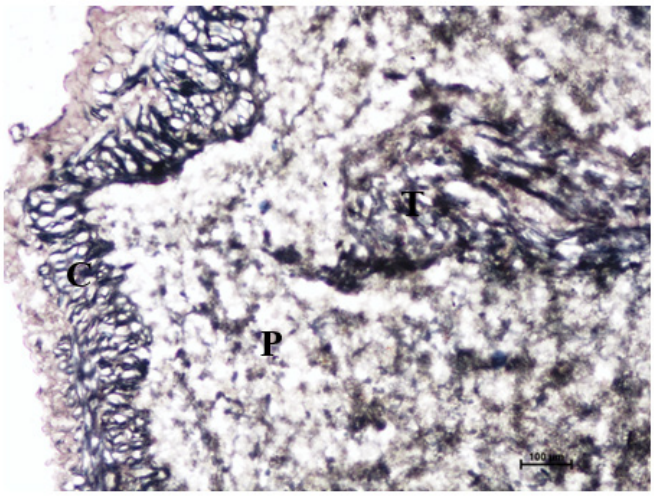

Figure 4: Photomicrograph of cryosection of pig spleen showing strong reaction of phospholipids in capsule (C), trabeculae (T) and parenchyma (P). Acid hematin X 100

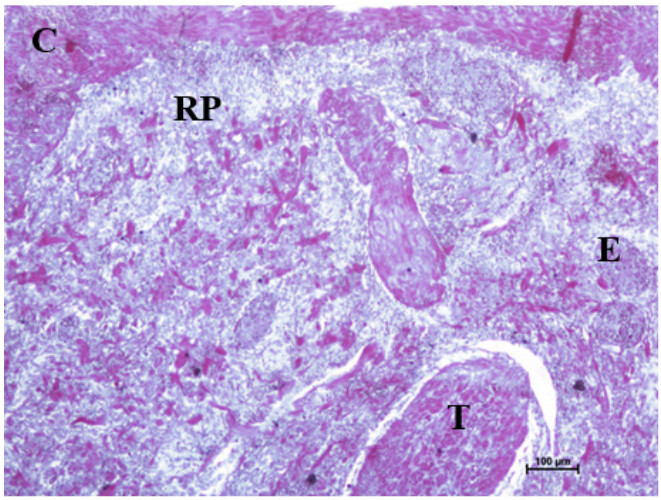

Figure 5: Photomicrograph of section showing weak reaction in red pulp (RP) and weak to moderate in trabeculae (T) and weak in ellipsoids (E). Bromophenol Blue X 100

Erythrophagocytosis

In the present study the hemosiderin pigment formed by the degradation process was found in the parenchyma of the spleen especially in red pulp (Figure 7). Macrophages engulfing the erythrocytes were observed in the sinuses. These ingested erythrocytes undergo lysosomal degradation and lead to the formation of hemosiderin pigment. Few bluish granules were observed in the splenic sinus as well as in cytoplasm of the macrophage and white pulp of the spleen (Figure 8). This indicated the active process of degeneration of erythrocytes by phagocytic cells, resulting into the formation of hemosiderin pigment. The present study observed large

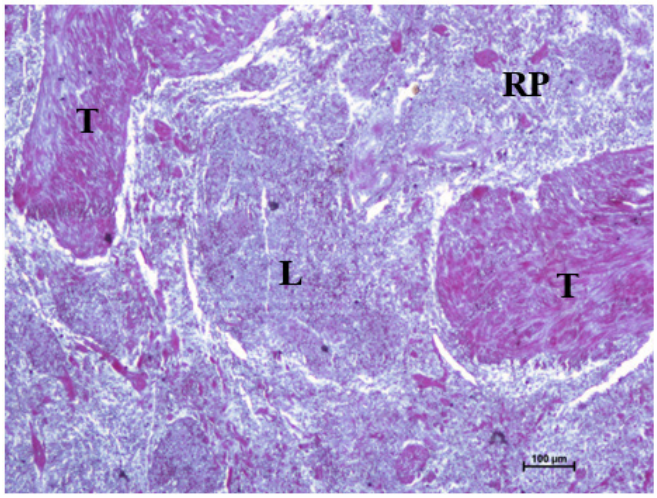

Figure 6: Photomicrograph of section of spleen showing weak to moderate protein activity in the trabeculae (T), lymphoid follicle (LF) and red pulp (RP). Bromophenol Blue X 100

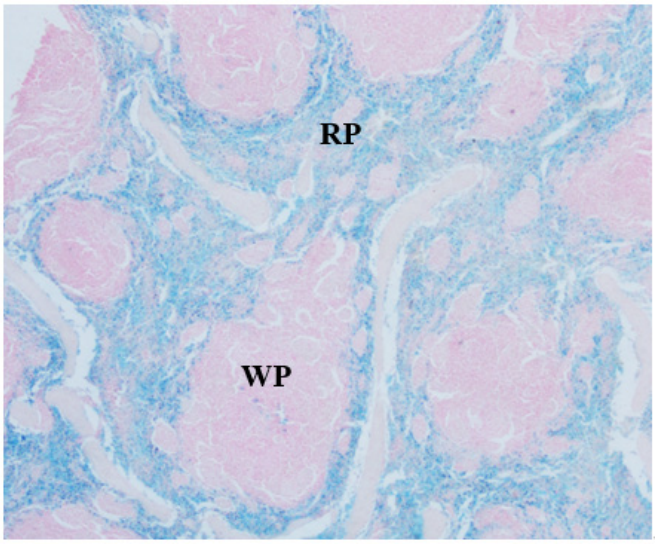

Figure 7: Photomicrograph of section of white pulp (WP) and erythrophagocytosis in the red pulp (RP) with deposition of haemosiderin pigment in pig spleen. Perl's Prussian blue X 40.

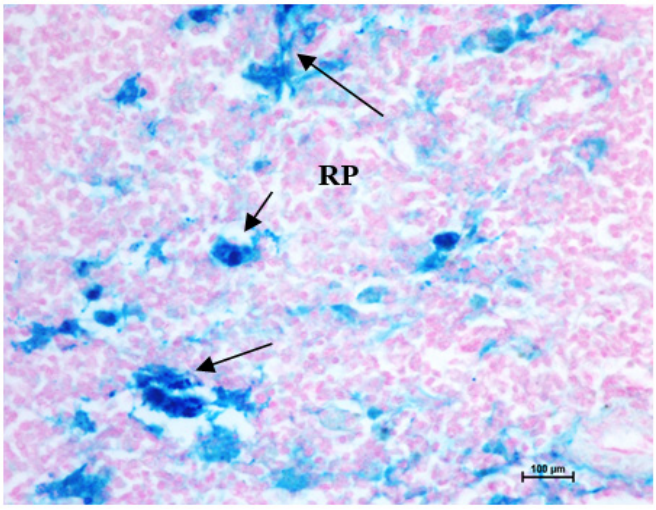

Figure 8: Photomicrograph of section of spleen showing accumulation of bluish granules of haemosiderin pigment in splenic sinuses (arrow) . Perl's Prussian blue X 400.

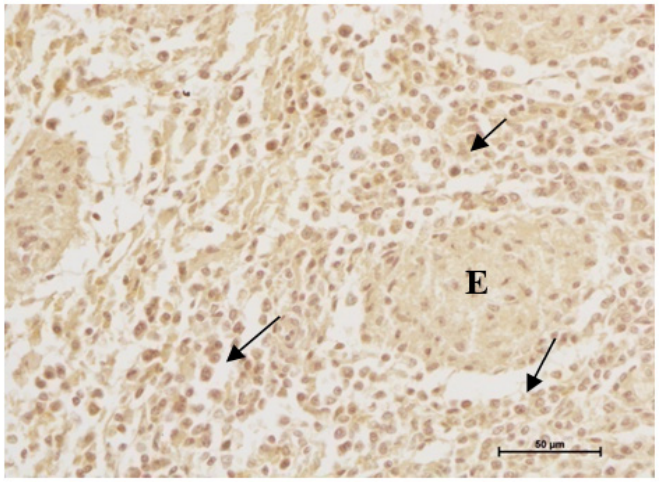

Figure 9: Photomicrograph of section immunopositive activity showing T-lymphocytes with cytoplasmic reaction in spleen present around ellipsoid (E) (arrow). IHC X 400

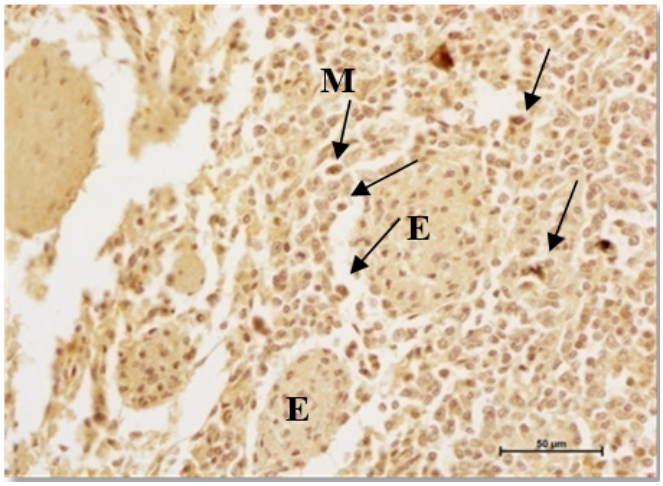

Figure 10: Photomicrograpgh of immunopositive activity showing macrophages (M) with cytoplasmic reaction around ellipsoid (E) in spleen (arrow). IHC X 1000

quantities of haemosiderin pigment in both sinuses and cytoplasm of macrophages of pig. The ellipsoids in the present study were surrounded by cells having haemosiderin pigment in the cytoplasm. The presence of a large number of macrophages around ellipsoids was confirmed by immunohistochemistry

Distribution of Lymphocytes and Macrophages

Immunohistochemical studies on the pig spleen revealed that numerous B-lymphocytes and T-lymphocytes were present in the area of the white pulp of the spleen (Figure 9). More T-lymphocytes were present around the ellipsoids (Figure 9). The maximum localization of macrophages was seen in white pulp and ellipsoids (Figure 10). So, it was concluded that T-lymphocytes possibly act as phagocytic cells in pig spleen. Similar observations were made by Cesta (2006) in the lab animals. Sheathed capillaries were found to be surrounded by reticular cells and macrophages and formed the ellipsoids. These observations were in line with the observations of Gnanadevi *et al.* (2019) in sheep and goats, which reported that macrophages were found mostly in the ellipsoid area.

Conclusion

It was concluded from the present study that the distribution of histochemical moieties in the different parts of the spleen was directly correlated with the metabolic status. Localization of more T-lymphocytes and macrophages in white pulp and ellipsoids implied that T-lymphocytes possibly acted as major phagocytic cells in the spleen, whereas phagocytes were the predominant cells involved in the process of erythrophagocytosis.

Acknowledgement

Authors are thankful to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, for providing facilities and financial assistance to carry out this work.

References

Alim, A., Nurunnabi, A.S.M., Ara, S., Mahbub, S., & Mohanta, L.C. (2012). Comparative histological study on the spleen of human (*Homo sapiens*), cow (*Bos indicus*), goat (*Capra hircus*). *Nepal Journal of Medical Sciences,* 1(2), 64-67.

Cesta, M.F. (2006). Normal structure, function and histology of the spleen. *Toxicologic Pathology,* 34, 455-465.

Chayen, J., Butcher, R.G., Bitensky, L. & Poulter, L.W. (1969). A Guide of Practical Histochemistry. Oliver and Boyd, Edinburgh, England. pp: 83-174.

Das, S.K., Alam, M.S., Islam, M.N. & Awal, M. A. (2005). Histology of the spleen of indigenous dog (*Canis familiaris*) of Bangladesh. *Journal of Veterinary Medicine,* 3(1), 59-61.

Dellman, H.D. & Brown, E.M. (2006). Textbook of Veterinary Histology. Iowa Blackwell Publishing. Edn. 6th, pp: 147-152

Gautam, A.K. & Mishra, U.K. (2015). Histochemical and transmission electron microscopic studies of spleen in prenatal stages of goat. *Indian Journal of Animal Research,* 49(1), 59-62.

Gnanadevi, R., Senthilkumar, S., Kannan, T.A. & Ramesh, G. (2019). Comparative Histoarchitectural Study of Splenic Components in Sheep and Goat. *International Journal of Current Microbiology and Applied Sciences,* 8(5), 1387-1394.

Ikpegbu, E., Nlebedum, U.C., Nnadozie, O. & Agbakwuru, I.O. (2014). The spleen of the African palm squirrel (*Epixerusebii*): A Micromorpholgical Observation. *Journal of Veterinary Advances,* 4(6), 564-569.

Luna, L.G. (1968). Manual of histological staining methods of armed forces Institute of Pathology. McGraw Hill Book Company, New York, USA, Edn. 3rd , pp: 38-196

Pathak, D., Bansal, N., Singh, O., Gupta, K. & Ghuman, S.P.S. (2019). Immunohistochemical localization of estrogen receptor alpha (ERα) in the oviduct of Indian buffalo during follicular and luteal phases of estrous cycle. *Tropical Animal Health and Production,* 51(6), 1601-1609.

Sheehan, D.C. & Hrapchak, B.B. (1973). Theory and Practice of Histotechnology. The C V Mosby Company, Saint Louis. Edn. 1st, pp: 80-172.