

Micro and Macro Anatomical Studies of Spleen at Different Stages of Development in Pati Duck (*Anas platyrhynchos domesticus*) of Assam.

J. Ahmed, Munmun Sarma, K. B DevChoudhury and Anil Deka*

Department of Anatomy & Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam, India.

ABSTRACT

The study on the micro and macro anatomy of the spleen of Pati duck of Assam is of great value regarding the diseases control regime. The study aimed to evaluate the gross and histomorphology examination of the spleen of the immune system. For this study, thirty-six numbers of Pati ducks were utilized. The duck was divided into six groups depending on its age viz., day old, 15 days, 4 weeks, 8 weeks, 16 weeks, and 24 weeks. For a collection of the spleen, a ventro median incision was given in the abdominal region, the spleen was exposed, its topography was studied *in situ* and the organ was separated from the body. The research was carried out for two years in the Department of Anatomy & Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam. The pieces of spleen were collected immediately after slaughter and fixed in a 10% neutral buffered formalin solution and were processed as per the standard technique of procedure (Luna, 1968). The paraffin blocks were sectioned in Shandon Finesse microtome at 5µm thickness and the sections were stained with Mayer's Haematoxylin and Eosin staining technique for Cellular details Van Gieson's method for collagen fiber, Gomori's method for reticular fibre, Hart's method for elastic fibre and Bielchowsky's method for axis cylinder and dendrites as per the method of Luna (1968). The spleen of Pati duck of Assam was located close to the right side of the junction between the proventriculus and gizzard adjacent to the liver in close association with the gall bladder. It was reddish brown. The spleen of Pati duck of Assam is encapsulated by a connective tissue capsule from which connective tissue fibres penetrated the parenchyma as trabeculae. Spleen parenchyma is comprised of red pulp and white pulp. It was observed that fine reticular fibres formed the meshwork which supported the splenic parenchyma.

Keywords- Pati duck, Micro, Macro, Anatomical, Studies, Spleen.

INTRODUCTION

In Assam, the Pati duck has recently been accepted as a breed that is reared by people in rural and semi-urban areas. Pati ducks produce 70-95 eggs in a year. Not much research work has been done on the lymphoid organs of Pati ducks. Lymphoid organs play an important role in the immune mechanisms of birds. The study of the lymphoid organs will enable the farmers to maintain their health following vaccine protocol, and disease control in a more scientific way which will increase productivity with less input. Also, cross-breeding of Pati ducks can be done with other high-yielding breeds of duck. The spleen is the largest organ in the lymphatic system. It is an important organ for keeping body fluids balanced. It acts as a blood filter, it controls the number of red blood cells and blood storage in the body and helps to fight infection. Further, this understanding will be helpful to investigate ways to manipulate the local immune system to have the desired effect on the systemic immune system. This may potentiate the strategies to minimize the immunization cost and disease control protocol in the poultry industry. Due to the paucity of literature on the postnatal development of the spleen of Pati duck a local variety of Assam, the present study was designed to establish some anatomical norms on spleen at different stages of development. This would help physiologists, pathologists, and poultry scientists with effective disease control regimes.

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CORRESPONDING AUTHOR: Anil Deka

E-MAIL ID: dranilvet01@gmail.com

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MATERIALS & METHOD

The present study was conducted on thirty-six numbers of Pati ducks. The duck was divided into six groups depending on its age viz., day old, 15 days, 4 weeks, 8 weeks, 16 weeks, and 24 weeks. The post-hatched experimental birds were reared in a backyard system in Hajo and Kamrup Rural, Assam. The birds were sacrificed according to the technique advocated by [3]. After exsanguinations the Pati ducks of appropriate age were sacrificed, their skin and fascia were reflected carefully without disturbing the normal arrangement of the organs, and their topography was recorded. For a collection of the spleen, a ventro median incision was given in the abdominal region, the spleen was exposed, its topography was studied *in situ* and the organ was separated from the body. For histomorphological studies tissue samples (4-5 mm) were collected from spleen from each bird of each age group and were fixed in 10 % NBF solution for 3- 4 days. The fixed tissue sample was then processed as per the standard technique and procedure [7]. The paraffin blocks were sectioned in Shandon Finesse microtome at 5 micrometers (µm) thickness and the tissue sections were stained with different stained with Mayer's Haematoxylin and Eosin staining technique, Van Gieson's method for collagen fibre, Gomori's method for reticular fibres, Hart's methods for elastic fibre and Bielchowsky's method for axis cylinder and dendrites as per the standard methods of [7].

RESULTS

The spleen of Pati duck of Assam was located close to the right side of the junction between the proventriculus and gizzard adjacent to the liver in close association with the gall bladder (Fig.1). It was reddish brown. The spleen of Pati duck of Assam has two surfaces viz. dorsal and ventral. The dorsal surface was smooth while the ventral surface was rough. There were two borders (cranial and caudal) and two extremities viz. dorsal and ventral. In the present investigation, it was found that the spleen of the Pati duck was triangular with a bulging dorsal surface giving it a curved appearance (Fig.2), and the ventral surface was cavate giving it a segmented appearance. The spleen of Pati duck of Assam is encapsulated by a connective tissue capsule from which connective tissue fibers penetrated the parenchyma as trabeculae (Fig.3). Spleen parenchyma is comprised of red pulp and white pulp. It was observed that fine reticular fibres formed the meshwork which supported the splenic parenchyma. The capsule and trabeculae comprised collagen fibres (Fig.6) and few reticular fibres (Fig. 8) and smooth muscle cells. Trabeculae were present in small patches in the day-old and 15-day-old ducklings. At 4 weeks of age, the capsule was found to have few amounts of reticular fibres. Trabeculae were observed to be penetrating the parenchyma and divided the parenchyma into incomplete lobules. However, the trabecular artery was appreciated in the trabeculae and central arteriole in the splenic nodule of 15 days old Pati duck. The connective tissue capsule was well developed at the age of 6 months and trabeculae were well developed within the organ at 16 weeks of age. Connective tissue fibres encircled splenic nodules and there was the presence of a central arteriole in a reticular capsule. The splenic parenchyma consisted of red and white pulp (Fig.3). On the day of hatching, it was observed that there was no clear distinction between the red and the white pulp. In 15 days post-hatching ducklings it was observed that splenic corpuscles were distributed independently in the parenchyma, and few central arterioles were present. A central artery was found along with several nodules in the spleen of Pati duck at the age of 4 weeks. In the age group of 2 months, it was found that there were individual nodules that were close to each other. Splenic corpuscles were distributed in the red pulp. There was no clear demarcation between white pulp and red pulp as red pulp was intermingled with white pulp in day-old ducklings. Randomly distributed white pulp contained few lymphatic nodules. There were germinal centers in the white pulp (Fig.4). In 24 weeks, old Pati ducks it was found that splenic corpuscles were not present independently rather two or three corpuscles clubbed together forming Peri arterial Lymphatic Sheaths (PALS) (Fig.5). Elastic fibres were noticed in capsule and trabeculae at 24 weeks of age (Fig.7). Nerve fibre innervations were appreciated in capsule and trabeculae (Fig.9) and were appreciated around splenic corpuscles and its quantity increased with the increase of age.

DISCUSSIONS

The spleen of Pati duck of Assam was located close to the right side of the junction between the proventriculus and gizzard adjacent to the liver in close association with the gall bladder. It was triangular and reddish brown. [1] reported that the shape of the spleen of the broiler was round, its color was reddish-brown and it lies close to the junction between the proventriculus and gizzard on the right side. However, [5] reported that in boiler chicken the shape of the spleen was spherical in different groups of broiler chickens. This difference might be due to the species variation. The spleen of Pati duck of Assam has two surfaces

namely dorsal and ventral which were following [2] who reported that in duck and goose the shape of the spleen is more triangular, with a flat dorsal surface with a curved ventral surface. The dorsal surface was smooth while the ventral surface was rough.[8] reported that the shape of the spleen in ducklings was triangular with a curved dorsal and ventral surface. It was a reddish-brown organ that lies close to the right side of the junction between the proventriculus and gizzard. This is following our findings. The spleen of Pati duck of Assam is encapsulated by a connective tissue capsule from which arises the trabeculae. These trabeculae enter the splenic parenchyma, which is divided into red and white pulp. A network of fine reticular fibers is noticed which supports the splenic parenchyma. A thin connective tissue capsule covers the splenic parenchyma. The capsule is comprised of collagen and few reticular fibers and smooth muscle cells. Trabeculae were present in small patches in the day-old and 15-day-old ducklings. At 4 weeks of age the capsule was found to have few numbers of reticular fibers were observed. The trabeculae penetrated the parenchyma and divided the parenchyma into incomplete lobules. At the age of 16 weeks, the trabecular artery was observed in the trabeculae. The connective tissue capsule was well developed at the age of 6 months in Pati duck of Assam and trabeculae were well developed within the organ at 16 weeks of age. Connective tissue fibers encircled splenic nodules and there was the presence of a central arteriole in a reticular capsule.[4] described that the avian spleen was covered by a thin fibrous capsule lined externally by a flattened layer of peritoneal mesothelium. It bears two main layers under the peritoneum, an external layer made of predominantly collagen fibers with few elastic fibers comprising about one-third of the total thickness and an internal layer composed mainly of an elastic fiber network in combination with fine collagen fibers, a few muscles fibers, and fibroblast. The inner layer comprised about two-thirds of the capsule thickness. Unlike mammalian spleen typical trabeculae were not present in avian spleen. The underlying framework of the splenic tissue consists of a network of reticular cells and reticulin fibers. This reticular area was particularly dense around the arteries of the white pulp and the capsule. Superimposed upon this framework two types of splenic tissue were found, the white pulp and red pulp. This difference in the structure of the spleen in Pati duck could be attributed to species differences. Elastic fibres were noticed in the capsule and trabeculae at 24 weeks of age. Our findings are following the findings of [6] but are different from the findings of [1] who reported that the spleen of the broiler was surrounded by a thick splenic capsule and there was a small number of trabeculae. [1] reported that the spleen of the broiler had a network of splenic tissue consisting of a network of reticular cells and fibers arranged into red pulps, which were distributed within the white pulps. The white pulp comprised a network of reticular cells and reticular fibers within which the immunocompetent cells were diffusely distributed. It contained sheathed arteries and lymphatic nodules. The red pulp of the spleen was formed from venous sinuses and an anastomosing cord of reticular cells, macrophages, lymphocytes, and blood cells. There is dissimilarity with our findings which might be attributed to the difference in species. Our research findings are following the findings of [5] who observed that histologically, the spleen of the broiler chicken was surrounded by a thick splenic capsule and a small number of trabeculae. The red pulp was less distinct and these were distributed sparsely within the white pulp, composed mostly of red blood cells. The white pulp

was composed of a network of reticular cells and reticular fibers within which small, medium, and large-sized lymphocytes and plasma cells were spread. It contained sheathed arteries and lymphatic nodules. The splenic red pulp is composed of venous sinuses and anatomizing cord of reticular cells, macrophages, lymphocytes, and blood cells.

CONCLUSION

The knowledge of gross anatomy and histomorphology of the spleen is very essential in elucidating its role in disease pathogenesis and immunology. The spleen is the largest organ in the lymphatic system. It is an important organ for keeping body fluids balanced and it acts as a blood filter as well as it controls the amount of red blood cells, and blood storage in the body, and helps to fight infection. Further, this understanding will be helpful to investigate ways to manipulate the local immune system to have the desired effect on the systemic immune system. This may potentiate the strategies to minimize the immunization cost and disease control protocol in the poultry industry. This study will be helpful to utilize the Pati duck for a breeding program being a local variety of Assam. It will help physiologists, pathologists, and poultry scientists with effective disease control regimes.

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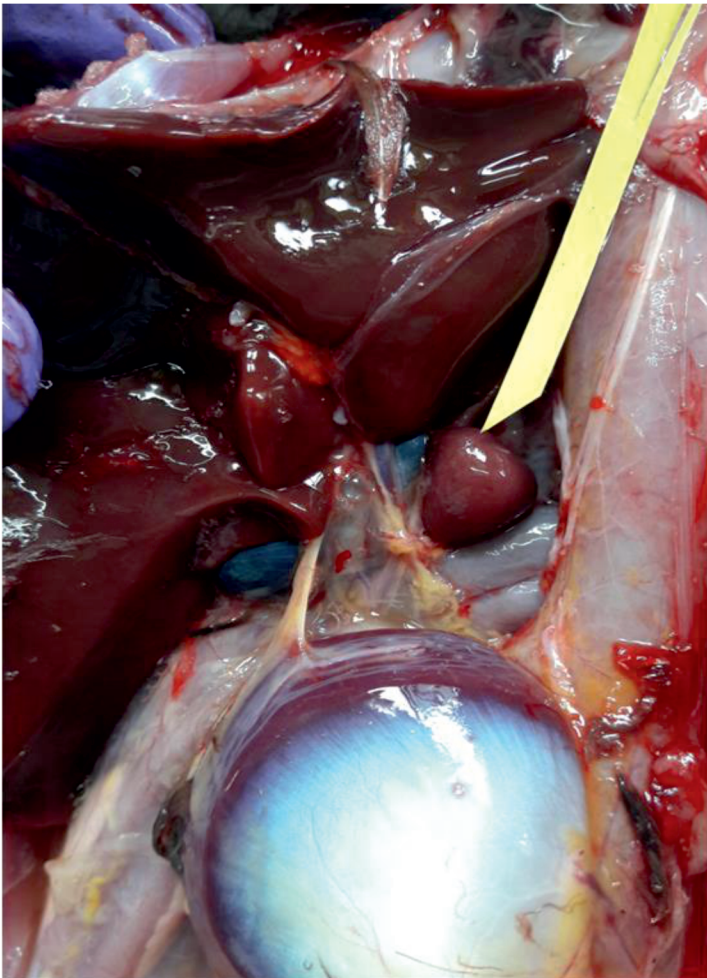


Fig.1. Photograph showing the in-situ position of spleen of Pati duck of Assam.



Fig.2. Photograph showing the ex-situ position of spleen of Pati duck of Assam.

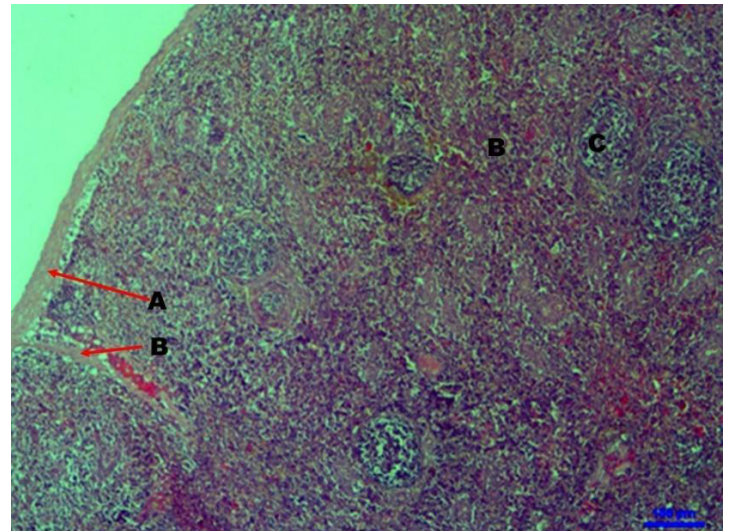


Fig.3: Photomicrograph showing the capsule (A), trabeculae (B) red pulp (B) and white pulp (C) of spleen at 16 weeks of age of Pati Duck. H&E 10X

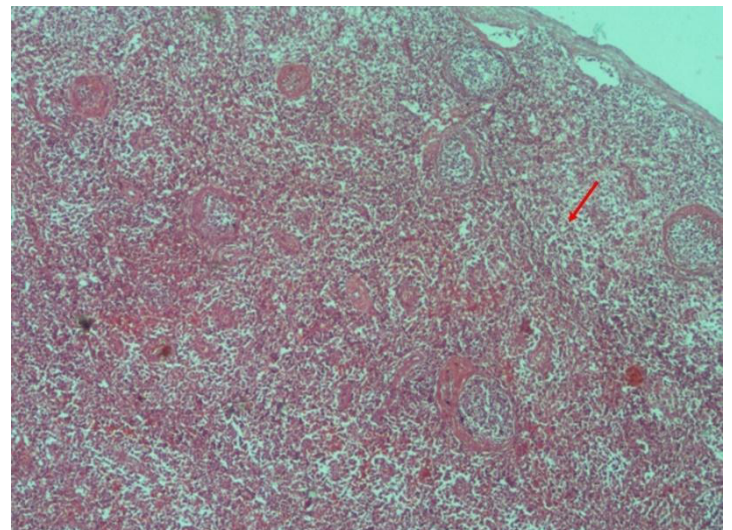


Fig.4: Photomicrograph showing the presence of germinal centres (arrow) in 8 weeks of age. H&E 10X.

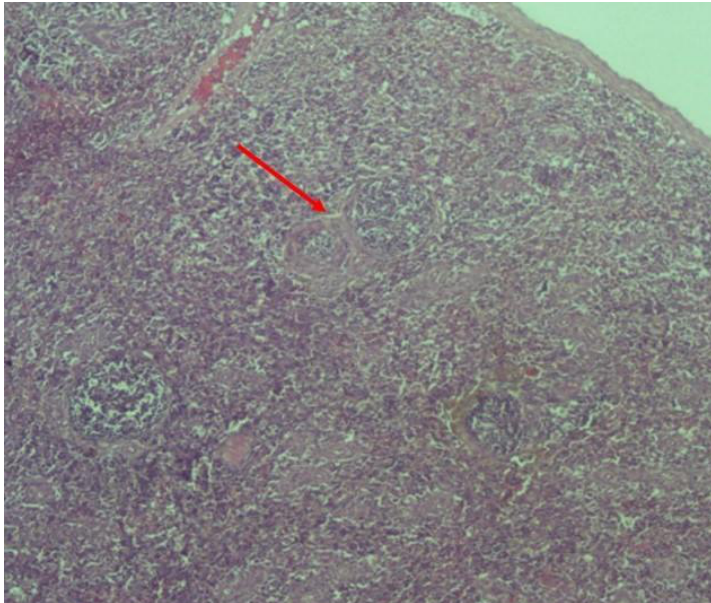


Fig.5: Photomicrograph showing the presence of splenic corpuscles clubbed together (arrow) forming the periarterial sheaths. H&E, 10X

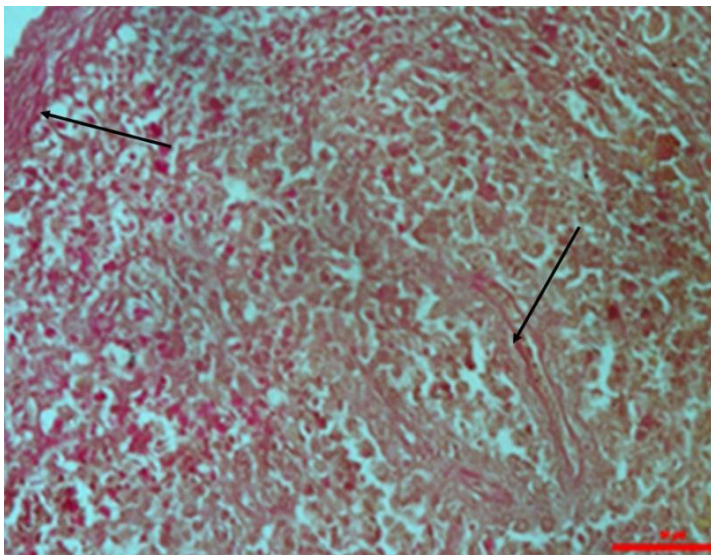


Fig.6: Photomicrograph showing the presence of collagen fibres in capsule (arrow) and trabeculae (arrow) in spleen of 24 weeks old Pati duck. Van Geison's Method 40X

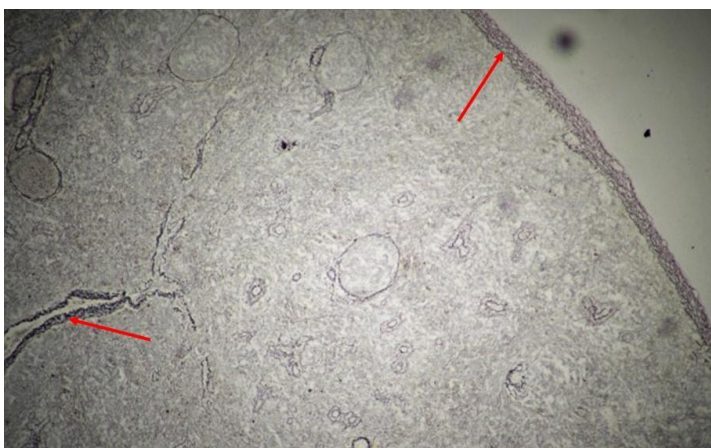


Fig.7: Photomicrograph showing the presence of elastic fibre in capsule (arrow) and trabeculae (arrow) at 24 weeks of age. Harts's Method 10X

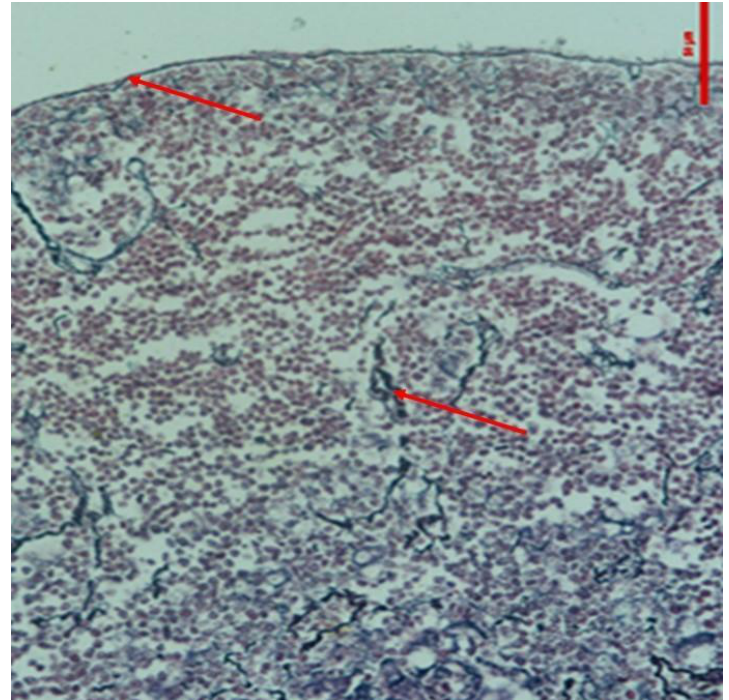


Fig.8: Photomicrograph showing the presence of reticular fibres (arrow) in capsule and trabeculae in spleen of 8 weeks of Pati duck. Gomori's method 10X.

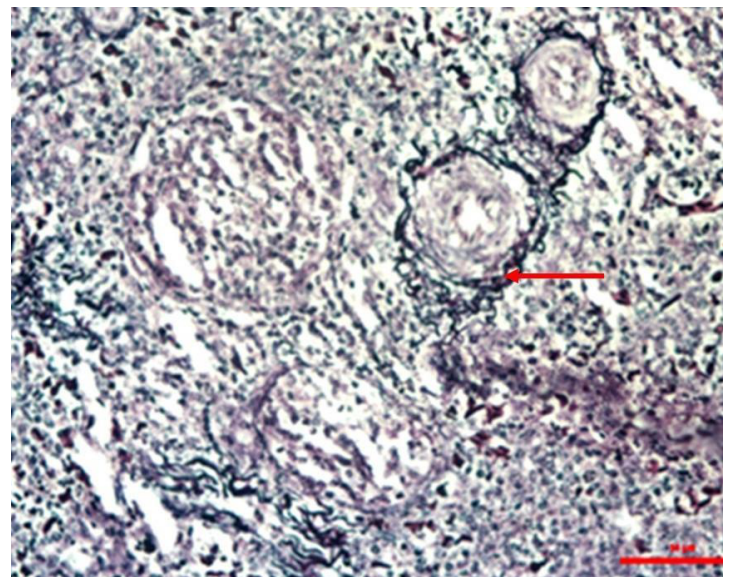


Fig.9: Photomicrograph showing the innervations of nerve fibre in around the splenic corpuscles. Bielchowsky's Method 40X.

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