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Anatomical Study of Thymus at Different Stages of Development in Pati Duck of Assam

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ABSTRACT

Background: The study on Thymus of Pati duck of Assam is of great value in regard to normal academic and vaccine production. The aim of the study was to evaluate the gross and histo-morphological examination of thymus of Pati duck at different age group.

Methods: The present studies were conducted on 36 numbers of Pati duck of Assam of irrespective of sex at different stages of development. The ducks were divided into six groups *viz.,* day old, 15th day, 4th week, 8th week, 16th week and 24th week of age. For histomorphological studies, tissue samples were fixed in 10% NBF and then processed as per the standard technique andprocedure of Luna (1968). The paraffin blocks were sectioned in Shandon Finesse microtome at 5 micrometer (µm) thickness and the tissue sections were stained with Mayer's Haematoxylin and Eosin staining technique for Cellular details, Van Gieson's method for collagen fibres, Gomori's method for reticular fibres, Hart's and Verhoeff's method for elastic fibers and Bielchowsky's method for axis cylinder and dendrites.

Result: The thymus, a primary lymphoid organ of Pati duck of Assam was arranged in two long chains on either side of the neck embedded in the sub dermal connective tissue as chains of lobes connected to each other by connective tissue. The thymus of Pati duck consisted of a capsule made up of connective tissue fibres and cells from which trabeculae entered the parenchyma dividing that organ into a number of incomplete lobules. The unique structure of the thymus, the Hassall's corpuscles, was formed by the hypertrophied reticulo-epithelial cells and was very common in the medulla.

Key words: Anatomy, Development, Different stages, Pati duck, Thymus.

INTRODUCTION

Pati duck is an indigenous breed of duck found predominantly in north eastern region of India. Pati ducks produce 70-95 eggs in a year (Kalita *et al.,* 2009) and get matured by the age of 6-8 months and the incubation period is 28 days approximately with a good health regime The main function of the thymus is chiefly to develop T lymphocytes. Once matured, these cells leave the thymus and are transported via blood vessels to the lymph nodes and spleen. T lymphocytes are responsible for cell mediated immunity, which is an immune response that involves the activation of certain immune cells to fight infection. Since there is very scanty literature on the anatomical study of the thymus of Pati duck at different stages of development being a local variety of Assam, hence the present study was designed to establish a anatomical norms on the thymus of Pati duck of Assam at different stages of development.

MATERIALS AND METHODS

The present studies were conducted on 36 numbers of Pati duck of Assam of irrespective of sex at different stages of development. The ducks were divided into six groups *viz.,* day old, 15th day, 4th week, 8th week, 16th week and 24th week of age. The present study was carried out in the Department of Anatomy and histology, College Veterinary Science, Assam Agricultural University, Guwahati, Assam in the year 2017-18.

For gross anatomical studies, after exsanguinations of the Pati ducks, their skin and fascia were reflected carefully without disturbing the normal arrangement of the organs and their topography were recorded. The skin was reflected on either side in the cervical and thoracic region and the thymus was exposed and topography was studied *in situ.*

For histomorphological studies, tissue samples (4-5 mm) were collected from the thymus of 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 of Luna (1968). The paraffin blocks were sectioned in Shandon Finesse microtome at 5 micrometer (µm) thickness and the tissue sections were stained with Mayer's Haematoxylin and Eosin staining technique for Cellular details,Van Gieson's method for collagen fibres, Gomori's method for reticular fibres, Hart's methods for elastic fibers and Bielchowsky's method for axis cylinder and dendrites.

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RESULTS AND DISCUSSION

Gross studies on thymus of pati duck

The thymus, a primary lymphoid organ of Pati duck of Assam was arranged in two long chains on either side of the neck embedded in the sub dermal connective tissue as chains of lobes connected to each other by connective tissue (Fig 1). The lobes of the thymus were located parallel to the internal jugular vein and vagus nerve which extended from the mid cervical region to the thoracic inlet (Fig 2). The total number of lobes ranged from 3 to 6 on both sides of the neck in all the age groups. The caudal most lobes were found to be largest in all the age groups and it was closely associated with the thyroid gland on either side of the neck.

The thymus, a primary lymphoid organ of Pati duck of Assam was arranged in two long chains on either side of the neck embedded in the sub dermal connective tissue as chains of lobes connected to each other by connective tissue. The lobes of the thymus were located parallel to the internal jugular vein and vagus nerve which extended from the mid cervical region to the thoracic inlet. This was not in accordance with Koch (1973) who reported that thymic lobes extended from the anterior cervical region to the anterior thoracic region. Kendall and Ward (1974) reported that the thymus gland of Q. *quelea* consisted of many lobes, well differentiated from each other in two long chains on each side of the neck. The total number of lobes ranged from 3 to 6 on both sides of the neck in all the age groups. In adults, there were 3-8 lobes of varying size and shape extending along each jugular vein as far as the thyroid gland. White (1951) reported that there were five pairs of thymic bodies in young chicken, on either side of the neck superficial to the jugular vein and vagus nerve.The caudal most lobes were found to be largest in all the age groups and it was closely associated with the thyroid gland on either side of the neck. King and McLelland (1975) observed that the thymus of birds consists of 3-8 pale, pink, flattened, irregularly shaped lobes each about 1 cm long in domestic fowl) strung along each side of the neck close to the jugular vein.

Histo-morphological studies on thymus of Pati duck

The thymus of Pati duck consisted of a capsule made up of connective tissue fibres and cells from which trabeculae entered the parenchyma dividing that organ into a number of incomplete lobules. Numerous fine connective tissue fibres penetrated the organ from the capsule forming trabeculae (Fig 3) and divided the thymus into incomplete lobules (Fig 4). The structural changes appeared around the 3 months of life and consisted of the formation of reticular fibers, the proliferation of non-epithelial cells, presence of mucous cells and small mucous cysts andan increase in the number of plasma and myoid cells. A moderate amount of collagen fibres were observed in the capsule in day old birds. Even though with the advancement of age the amount of collagen fibre in the capsule increased, it was observed that an increased amount of collagen fibres (Fig 7) were present in 15 day old ducklings. Reticular fibres were present



Fig 1: Photograph of thymus showing 4 lobes (arrow) were on either side of the neck underneath the skin in 4 weeks of age in Pati duck.



Fig 2: Photograph showing four lobes of the thymus on the left side located adjacent to the internal jugular vein. It extended from the mid cervical region to the thoracic inlet. The caudal most lobes was found to be the largest.

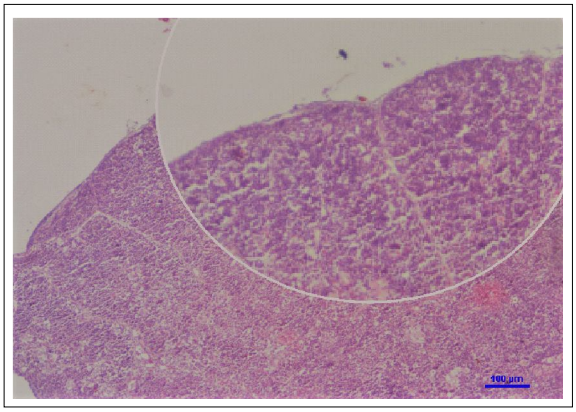


Fig 3: Photomicrograph showing the presence of numerous fine connective tissue fibres penetrating the organ from the capsule (arrow) forming trabeculae (arrow). H&E 10X.

on the inner lining of the fibrous capsule in all age groups (Fig 8). The presence of fine nerve fibre was observed in the capsule penetrating into the parenchyma through trabeculae from day old birds to 6 months of age. The lesser amount of elastic fibres was present in the inner lining of the capsule in 4 weeks of age andit increased with the advancement of age. The unique structure of the thymus, the Hassall's corpuscles, was formed by the hypertrophied reticulo-epithelial cells and was very common in the medulla. Parenchyma was not properly differentiated into cortex and medulla in 0 day old ducks. Myoid cells were also found in the medullary region at 15 days of age and 4 weeks post hatching. Plasma cells were found in the parenchyma at the age of 28 days post hatching. Lymphocytes were mainly present in different stages of maturation and the cells present were epithelial cells, dendritic cells, macrophages, fibroblasts, forming a meshwork. At the age of 4 weeks, it was observed that the thymic medulla started developing in the central portion of each thymic lobule. At the age of 4 months, it was observed that the medullary region is not only confined to the central portion of the lobule but was also observed in the peripheral region. Myoid cells and plasma cells were present in the thymic medulla along with thymocytes (Fig 5). The presence of hassal's corpuscles with a diffused group of reticular cells has been observed. In birds of 6 months of age, it was observed that several diffused forms of Hassall's corpuscles were present in the thymic cortex (Fig 6). The medullary area is not clearly

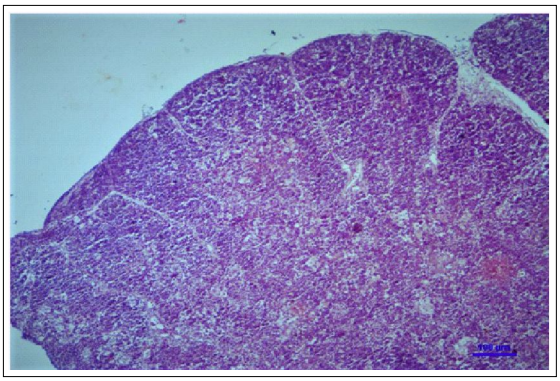


Fig 4: Photomicrograph showing the thymus encapsulated by a thin connective tissue capsule numerous fine connective tissue fibres penetrated the organ from the capsule forming trabeculae and divides the thymus into incomplete lobules (arrow). Cortex and medulla were not well differentiated at 24 weeks of age. H&E 10X.

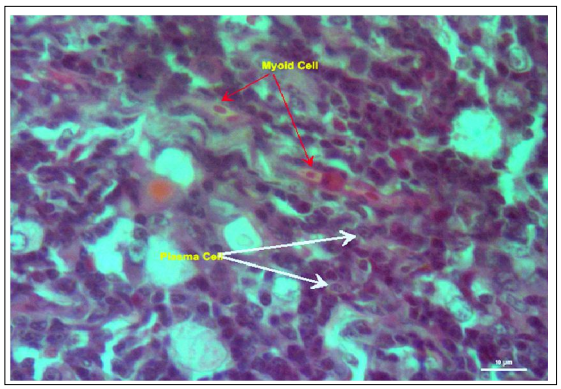


Fig 5: Photomicrograph showing the presence of myoid cell and plasma cells in the thymic medulla. H&E 40X.

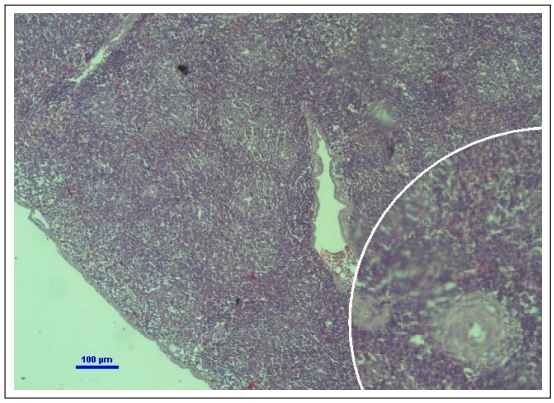


Fig 6: Photomicrograph showing the presence of Hassall's corpuscles(arrow, 40X) H&E 10X.

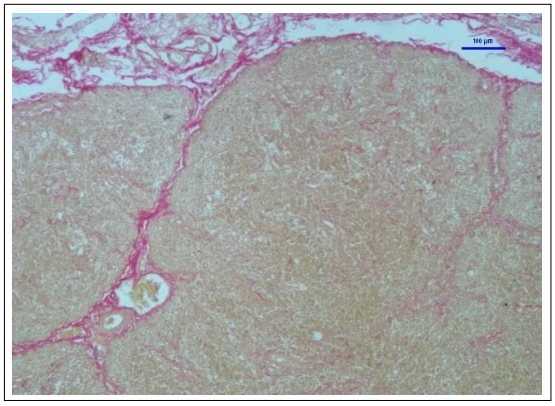


Fig 7: Photomicrograph showing abundance of collagen fibres (arrow) in the capsule and trabeculae in 16 weeks of age. Van-Geison's Method, 10X.

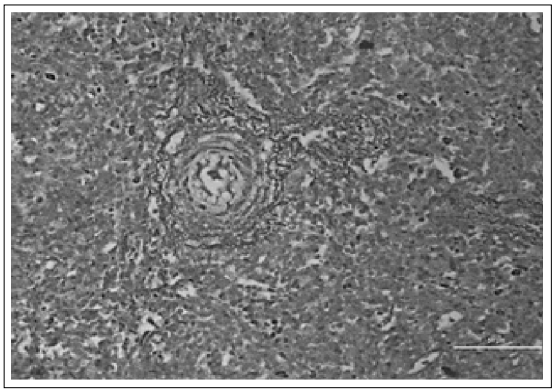


Fig 8: Photomicrograph showing the presence of reticular fibres (arrow) in the wall of Hassall's corpuscles at 24 weeks. Gomori's Method 10X.

differentiated into the central zone but it has been observed as small regions within the cortex as well towards the periphery of the cortex. The cortex appears basophilic due to the presence of densely packed small and medium sized lymphocytes. During the first week, the cortex contained huge pale eosinophilic structures with more frequently undergoing mitotic divisions. By the 5th week, basophilic cortex and an eosinophilic medulla were clear. This difference in the demarcation of cortex and medulla can be attributed to species variation. Patches of collagen fibres in a scanty amount in the parenchyma. In day old birds it was observed that there was a reticular mesh work in the trabeculae dividing it into various compartments. Reticular fibres were also seen in the wall of Hassall's corpuscles in 15 day old ducklings and ducks of 16, 8, 4 weeks. In ducks of 24 weeks of age, it was observed that reticular fibres formed a typical mesh work in the entire gland. In the present study, it was observed that at the age of 15 days elastic fibres (Fig 10) were observed in the periphery of the Hassall's corpuscles and at the age of 8 and 16 weeks, elastic fibres were seen to be sparse in cross section of artery. Myoid cells were frequently found in the medulla and in the corticomedullary region, occasionally found in the cortex. Hassall's corpuscles were observed commonly in the medulla and less frequently in the cortical and corticomedullary junction. The reticuloepithelial cells were large cells had long cytoplasmic process both in cortex and medulla. Elastic fibres were noticed in the structures dividing the thymic parenchyma into compartments and at the periphery of Hassall's corpuscles at 24 weeks of age. In day old birds nerve fibres were seen penetrating the trabeculae from the capsule into the parenchyma. The innervations of nerve fibres increased by the age of 8 weeks onwards (Fig 9). At 24 weeks of age distinct penetration of nerve fibres was noticed in the inter lobular spaces.

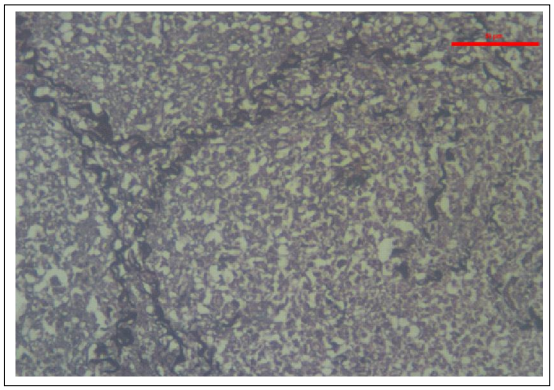


Fig 9: Photomicrograph showing the innervations of a nerve fibre (arrow) intrabeculae at 24 weeks of age. Bielchowsky's method. 10X.

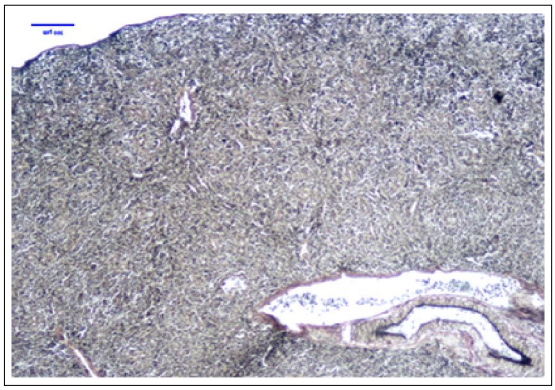


Fig 10: Photomicrograph showing the presence of elastic fibres (arrow) in capsule and wall of blood vessels at 24 weeks of age. Hart's method 10X.

The thymus of Pati duck composed of a capsule made up of connective tissue fibres as well as cells from which trabeculae entered the parenchyma dividing that organ into a number of incomplete lobules. Abundant fine connective tissue fibres penetrated the organ from the capsule forming trabeculae and divided the thymus into incomplete lobules in consonance with Sultana *et al.* (2012) observed that the thymus was encapsulated by a thin connective tissue capsule from where abundant fine septa of connective tissue invaginate to divide the organ into lobules which were not separated completely. This is in accordance with our findings. The structural changes appeared around the 3 months of life and consisted of the formation of reticular fibers, the proliferation of non-epithelial cells, presence of mucous cells and small mucous cysts andan increase in the number of plasma and myoid cells. A moderate amount of collagen fibres were observed in the capsule in day old birds. Even though with the advancement of age the amount of collagen fibre in the capsule increased, it was observed that an increased amount of collagen fibres was present in 15 day old ducklings. Reticular fibres were present on the inner lining of the fibrous capsule in all age groups. The presence of fine nerve fibre was observed in the capsule penetrating into the parenchyma through trabeculae from day old birds to 6 months of age. The lesser number of elastic fibres were present in the inner lining of the capsule in 4 weeks of age andthe presence of it increased with the advancement of age. The trabeculae were appreciated, extending from the capsule deep into the parenchyma dividing the gland into lobules in 0-day old birds up to 24 weeks of age. Akter *et al.* (2006) opined that a thin connective tissue capsule encircles the thymus. The thymus was divided into incompletely separated lobules by a numerous fine septum which originate from the thymic capsule. Each lobule is organized into a peripheral cortex and a central medulla. In our finding it was observed that the lobes were not distinctly differentiated. It is in accordance with the findings of our investigation. The parenchyma of the thymus consisted of the cortex and medulla and their proportion varied at different ages. The unique structure of the thymus, the Hassall's corpuscles, was formed by the hypertrophied reticulo-epithelial cells and was very common in the medulla. Franchini and Ottaviani (1999) found that in *Gallus domesticus* the distinct demarcation between the

cortex and medulla is lost with the advancement of age. At the age of 4 weeks, it was observed that the thymic medulla started developing in the central portion of each thymic lobule. Hassal's corpuscles were well appreciated in the thymic lobules. Myoid cells were also present in the lobules. At the age of 4 months, it was observed that the medullary region is not only confined to the central portion of the lobule but was also observed in the peripheral region. Myoid cells and plasma cells were present in the thymic medulla along with thymocytes. The presence of hassal's corpuscles with a diffused group of reticular cells has been observed. In birds of 6 months of age, it was observed that several diffused forms of Hassall's corpuscles were present in the thymic cortex. The medullary area is not clearly differentiated into the central zone but it has been observed as small regions within the cortex as well towards the periphery of the cortex. At 6 months of age, it was observed that there was distinct penetration of nerve fibre in the interlobular space. Leena *et al.* (2008) found that in newly hatched birds the thymus is comprised of pyramidal or polygonal lobules. The cortex appears basophilic due to the presence of densely packed small and medium sized lymphocytes. By the first week of age after hatching the differentiation of the cortex and medulla started and was distinct by the third week of age. During the first week, the cortex contained huge pale eosinophilic structures with more frequently undergoing mitotic divisions. By the 5th week, basophilic cortex and an eosinophilic medulla were clear. This difference in the demarcation of cortex and medulla can be attributed to species variation. Patches of collagen fibres in scanty amounts in the parenchyma. In day old birds it was observed that there was a reticular mesh work in the trabeculae dividing it into various compartments. Reticular fibres were also seen in the wall of Hassall's corpuscles in 15-day old ducklings and ducks of 16, 8,4 weeks. In ducks of 24 weeks of age, it was observed that reticular fibres formed a typical mesh work in the entire gland. Treesh *et al.* (2014) found that Hassall's corpuscles had structure less hyalinised centre and peripheral concentrically arranged reticulo epithelial cells, in addition to inter septal position of aggregation of fat cells. In the present study, it was observed that at the age of 15 days elastic fibres were observed in the periphery of the Hassall's corpuscles and at the age of 8 and 16 weeks, elastic fibres were seen to be sparse in cross section of artery. Hassall's corpuscles were observed commonly in the medulla and less frequently in the cortical and corticomedullary junction. The reticuloepithelial cells were large cells had long cytoplasmic process both in cortex and medulla. Elastic fibres were noticed in the structures dividing the thymic parenchyma into compartments and at the periphery of Hassall's corpuscles at 24 weeks of age. In day old birds nerve fibres were seen penetrating the trabeculae from the capsule into the parenchyma. The innervations of nerve fibres increased by the age of 8 weeks onwards. At 24 weeks of age distinct penetration of nerve fibres was noticed in the inter lobular spaces.

CONCLUSION

Thorough knowledge of the morphology and morphometry of Thymus is very essential in elucidating its role in disease pathogenesis and cell mediated immunity. Poultry thymus are maturation site of T lymphocytes, So, with the help of light microscope, it is easy to understand the anatomical structure of thymus of Pati duck. This study will help to utilize the Pati duck for a breeding programme as well as help physiologist, pathologist and poultry scientists for effective disease control regime.

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Conflict of interest

All authors decalre that they have no conflict of interest.

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