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Histomorphological study of spleen in post – hatched Japanese quail (*Coturnix coturnix Japonica*)

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Abstract

The present research was conducted to study age wise histomorphological changes in spleen of post-hatched Japanese quail of one to four week of age. The spleen was ensheathed by connective tissue capsule of uneven thickness and was composed of unequally distributed collagen, reticular and elastic fibres with few muscle cells. The thickness of capsule, distribution and arrangement of splenic capsular components varied with the advancement of age. Connective tissue trabeculae were poorly developed in all age group of birds. Splenic parenchyma was composed of indistinctly demarcated white pulp and red pulp in the network of reticular cells and reticular fibres. In all age group of birds, the tissues of white pulp was diffusely scattered and was associated with parenchymal vasculature. The white pulp was subdivided in to periarterial lymphatic tissue, perivenous lymphatic tissue, periellipsoidal lymphatic tissue and ellipsoids. The sizes of periarterial lymphatic tissue and perivenous lymphatic tissue increase with the age. Ellipsoids were round to oval structures composed of one to five concentric layers of reticular cells with few lymphocytes in network of reticular and collagen fibres around the penicillar capillaries. Age wise progressive increase in the number of macrophages was recorded in the periellipsoidal lymphatic tissue. The red pulp was less capacious at all stages of age and composed of cords and sinuses in the network of reticular cells and fibres.

Keywords: japanese quail, spleen, histomorphology, periellipsoidal lymphatic tissue, ellipsoids

1. Introduction

Spleen is the specialized form of connective tissue, which contain large number of lymphocytes with reticular cells and reticular fibres. The critical function of the spleen is the protection of body against destructive effects of invading foreign substances, cells or micro-organisms.

Spleen is secondary lymphoid organ, where lymphocytes respond to antigen. Spleen also acts as an erythropoetic organ besides lymphopoietic organ during embryonic stage. The quail spleen, throughout life undergoes structural changes with the advancement of age [9]. It serves as complex filter interposed in the circulation to clear the blood of particulate matter and senescent blood cells.

Most of the histomorphological studies on spleen had been carried out in chicken. Hence, the present study has been undertaken to observe the histomorphological changes with the advancement of age in post-hatched Japanese quail (*Coturnix coturnix Japonica*).

2. Materials and Methods

The present study was conducted on spleen of 48 Japanese quail of either sex. The spleen were collected by abdominal laparotomy and cranial displacement of sternum at the end of first, second, third and fourth week of age. The collected spleen samples were washed immediately with normal saline and fixed in 10% neutral buffered formalin, 10% formal saline and Bouin's fluid. The tissues pieces were processed for paraffin embedding technique [5]. The sections of 5 µm thickness were obtained and were stained using following staining procedures.

1. Haematoxylin and Eosin for normal histoarchitectural study [10]
2. Masson's trichrome for collagen and muscle fibres [5]
3. Verhoeff's elastic stain for elastic fibres [10]
4. Gomori's reticulin for reticular fibres [10].

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3. Results and Discussion

Histomorphologically, the Japanese quail spleen was composed of capsule, trabeculae, white pulp and red pulp (Fig. 1 & 2). The spleen was covered by thin fibromuscular capsule of uneven thickness which was found thicker at hilus in all age group of birds similar to the earlier reports of Venkatesan and Vijayaragavan^[15] in quail. The capsule was composed of unequal distribution of collagen, reticular, elastic fibres with few smooth muscle cells. A progressive increase in the thickness of capsule with the advancement of age was recorded, which is in accordance with the Venkatesan *et al.*^[14] in Japanese quail. The capsular components found to be varied with the advancement of age. A progressive increase in the amount of collagen fibres whereas progressive decrease in the reticular and elastic fibres was recorded (Fig. 3, 4, 5, 6, 7 & 8). However, from third week of age, the inner thin muscular layer was noticed in the splenic capsule with intermingled elastic fibres (Fig. 4 & 8). In agreement with this Venkatesan and Vijayaragavan^[15] in Japanese quail reported elastic fibres in the deeper part of splenic capsule. The results of the present study pertaining to increase in thickness of splenic capsule may be because of increase in amount of collagen fibres corroborates with the earlier findings of Venkatesan *et al.*^[14] in Japanese quail. The reticular fibres are immature stage of collagen fibres and get converted into matured collagen fibres. Possibly this might be the reason for progressive decrease in amount of reticular fibres and increase in the amount of collagen fibres in splenic capsule with the advancement of age observed during the present study. The increased amount of collagen fibres in the capsule with the advancement of age recorded during present study might be necessary to provide firmness to the organ. The distinct connective tissue trabeculae radiating from inner side of capsule were not observed whereas; poorly developed trabeculae were recorded within the splenic parenchyma in all age of birds during the present work. These observations are in line with the reports made by Baishya and Bhattacharyya^[2] in Assam fowl, Davidson *et al.*^[4] in avian species, Song *et al.*^[12] in Ostrich chicks, Khan *et al.*^[8] and Akter *et al.*^[11] in broiler. The trabecular arteries and veins were quite marked and were located beside each other is in line with the reports made by Liman and Bayram^[9] in quail and Bingol^[3] in Geese (Fig. 9, 10 & 11). The trabecular component was chiefly composed of collagen and reticular fibres (Fig. 9 & 10). However, the lattice of circularly arranged elastic fibres was found surrounding to trabecular vessels in all age group of birds (Fig. 11).

Although, Sturkie^[13] reported that, the spleen is not considered in birds to be a reservoir of blood that can quickly expel additional erythrocytes in circulation, the results of present study regarding the appearance of capsular thin inner smooth muscular layer with intermingled elastic fibres from third week of age onwards and lattice of circularly arranged elastic fibres around the trabecular vessels might be necessary to change in volume of spleen.

In all age group of birds, splenic parenchyma was composed of indistinctly demarcated white pulp and red pulp, is in line with reports made by Baishya and Bhattacharyya^[2] in Assam fowl, Liman and Bayram^[9] and Venkatesan and Vijayaragavan^[15] in quail. The tissues of white pulp was diffusely scattered associated and was with parenchymal vasculature. The white pulp was predominantly constituted of small and medium size lymphocytes, reticular cells and reticular fibres and was subdivided in to periarterial lymphatic

tissue, perivenous lymphatic tissue, periellipsoidal lymphatic tissue and ellipsoids. These finding are similar to other avian species as reported by Hashimoto and Sugimura^[6] in duck, Song *et al.*^[12] in Ostrich chicks, Baishya and Bhattacharyya^[2] in Assam fowl, Liman and Bayram^[9] and Venkatesan *et al.*^[14] in Japanese quail (Fig. 1, 2, 5 & 6).

During present work, the periatrerial lymphatic tissue and perivenous lymphatic tissue was composed of aggregates of lymphocytes with few granulocytes and macrophages around the central arteries and collecting veins of splenic parenchyma obeyed the reports made by Hashimoto and Sugimura^[6] in duck. The size of periatrerial and perivenous lymphatic tissue was found to be increased with the advancement of the age.

The sections stained by Gomori's reticulin stain revealed dense network of reticular fibres around the parenchymal vasculature (Fig. 5 & 6).

The periellipsoidal lymphatic tissue was consisted of small, medium and large sized lymphocytes with macrophages around the ellipsoids (Fig. 1 & 2) is similar to reports made by Olah and Glick^[11] in chicken, Hashimoto and Sugimura^[6] in duck, Venkatesan *et al.*^[14] and Liman and Bayram^[9] in Japanese quail. The progressive increase in numbers of macrophages in the periellipsoidal lymphatic tissue was recorded with the advancement of age. The marginal zone was found absent in all age group of birds during present study is in accordance with the earlier reports of Olah and Glick^[11] in chicken and Baishya and Bhattacharyya^[2] in Assam fowl.

No germinal centre was observed in white pulp in all age group of birds during the present study. In agreement to this observation Venkatesan *et al.*^[14] reported appearance of germinal centre in spleen of Japanese quail of above four weeks of age. However, well developed white pulp as circumscribed lymphatic tissue in the form of follicles in four week old Japanese quail birds earlier reported by Venkatesan *et al.*^[14] was not recorded during the present study.

The ellipsoids were lightly stained round to oval structures around the penicillar capillaries. The ellipsoids were chiefly composed of concentric layers of reticular cells with few lymphocytes in the network of reticular and collagen fibres in white pulp and at junction with red pulp (Fig. 1, 2, 5 & 6). The ellipsoids were composed of one to five concentric layers of reticular cells with euchromatic nucleus in all age group of birds. The confluence of two to four ellipsoids was commonly observed in all age groups of birds. These observations are in agreement with the reports of Olah and Glick^[11] in chicken, Baishya and Bhattacharyya^[2] in Assam fowl, Venkatesan *et al.*^[14] and Liman and Bayram^[9] in Japanese quail. The ellipsoids surrounded by periellipsoid lymphatic tissue with progressive increase in number of macrophages with the advancement of age recorded in present study might be correspond to mammalian spleen marginal zone as opined by Jeurissen^[7] in chicken.

The red pulp was found less capacious in all age group of birds in present study is in agreement with Liman and Bayram^[9] in Japanese quail. The tissue of red pulp was composed of cords and sinuses in the network of reticular cells and reticular fibres (Fig. 1, 2, 5 & 6). The sinuses were narrow irregular spaces lined by flattened endothelial cells. The cords were composed of lymphocytes, macrophages and granulocytes. These observations are in line with earlier reports made by Venkatesan *et al.*^[14] in Japanese quail, Baishya and Bhattacharyya^[2] in Assam fowl, Song *et al.*^[12] in Ostrich Chicks and Khan *et al.*^[8] in chicken.

4. Conclusion

Spleen in post-hatched Japanese quail was encapsulated by fibromuscular tissue capsule of uneven thickness. The capsular component varied with the advancement of age. Thin muscular layer appeared from third week of age in inner aspect of capsule. The trabeculae were poorly developed in the all age groups of birds studied. The thickness of capsule, size of periarterial and perivenous lymphatic tissue and number of macrophages in the periellipsoidal lymphatic tissue progressively increases with the advancement of age. The well defined white pulp in form of lymphatic follicles was not observed during present work indicated growing period and structural rearrangement of splenic tissue up to four weeks of age. The red pulp was less capacious in all groups of birds, composed of cords and sinuses. The result of present study showed that, histologically spleen of Japanese quail was very close to spleen of other avian species. The present study findings might be useful to provide baseline data for ensuing and comparative studies with other avian species.

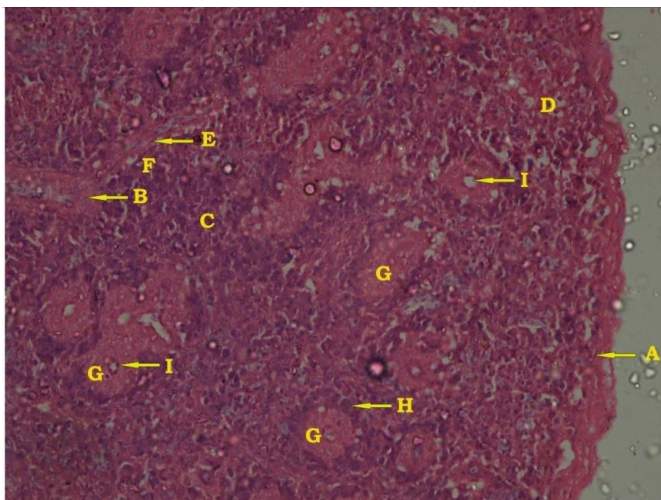


Fig 1: Photomicrograph of spleen at one week of age **A.** Capsule **B.** Trabecula **C.** White pulp **D.** Red pulp **E.** Central artery **F.** Periarterial lymphatic tissue **G.** Ellipsoid **H.** Periellipsoidal lymphatic tissue **I.** Penicillar capillary (H&E, x400)

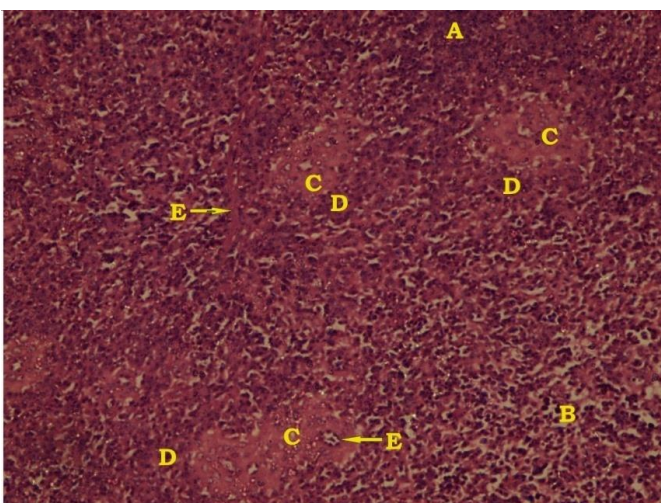


Fig 2: Photomicrograph of spleen at four week of age **A.** White pulp **B.** Red pulp **C.** Ellipsoid **D.** Periellipsoidal tissue **E.** Penicillar capillary (H&E, x400)

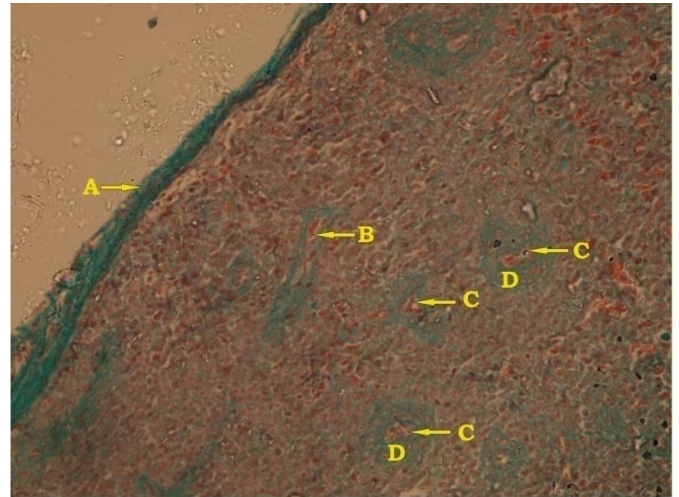


Fig 3: Photomicrograph of spleen at one week of age **A.** Capsule **B.** Central artery **C.** Penicillar capillary **D.** Ellipsoid (Masson's Trichrome, x400)

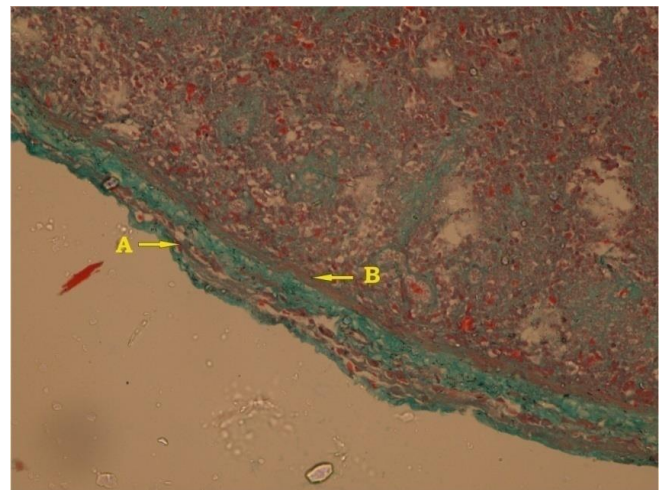


Fig 4: Photomicrograph of spleen at three week of age **A.** Outer layer of capsule **B.** Inner muscular layer of capsule (Masson's Trichrome, x400)

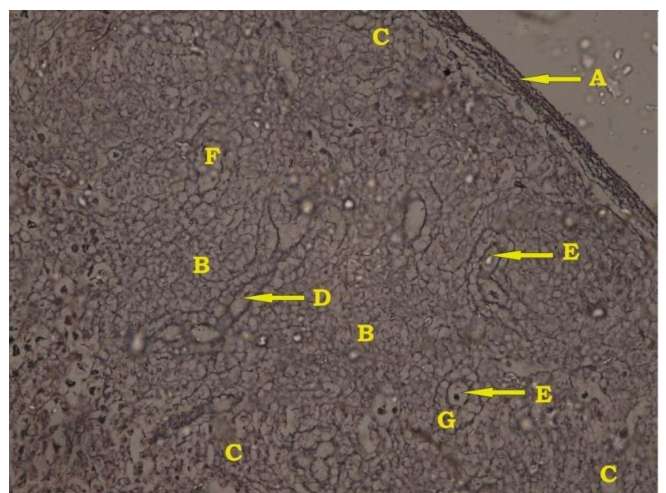


Fig 5: Photomicrograph of spleen at one week of age **A.** Capsule **B.** White pulp **C.** Red pulp **D.** Central artery **E.** Penicillar capillary **F.** Pulp vein **G.** Ellipsoid (Gomori's Reticulin, x400)

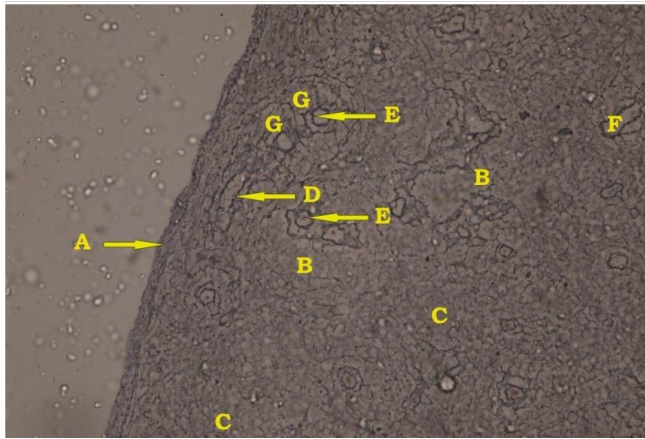


Fig 6: Photomicrograph of spleen at four week of age. A. Capsule B. White pulp C. Red pulp D. Central artery E. Penicillar capillary F. Pulp vein G. Ellipsoid (Gomori's Reticulin, x400)

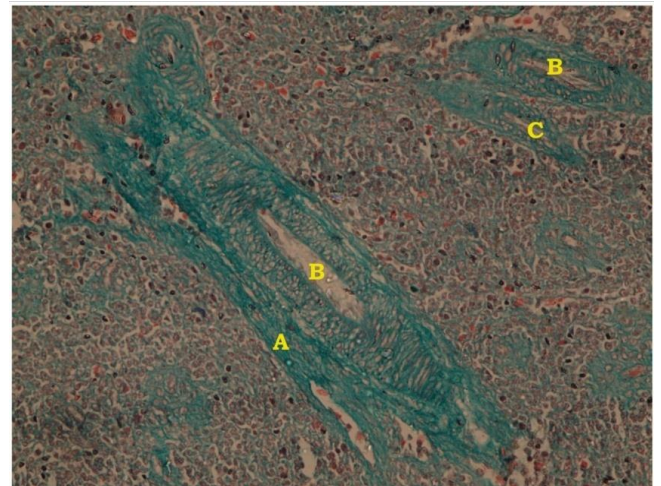


Fig 9: Photomicrograph of spleen at two week of age. A. Trabecula B. Trabecular artery C. Trabecular vein Masson's Trichrome, x400)

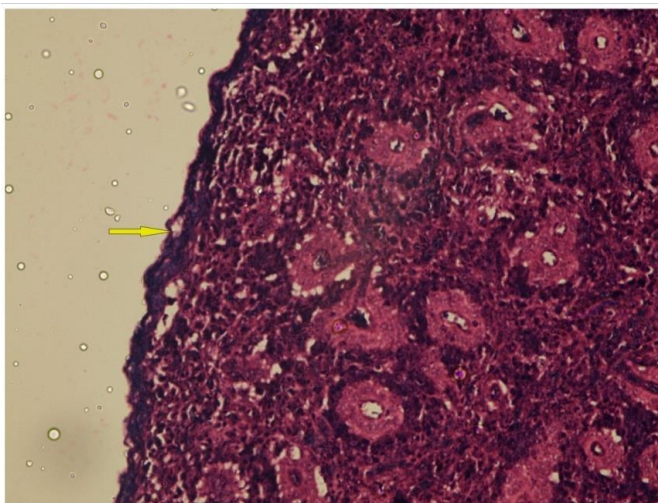


Fig 7: Photomicrograph of spleen at one week of age. A. Capsule (Arrow) (Verhoeff's elastic stain, x400)

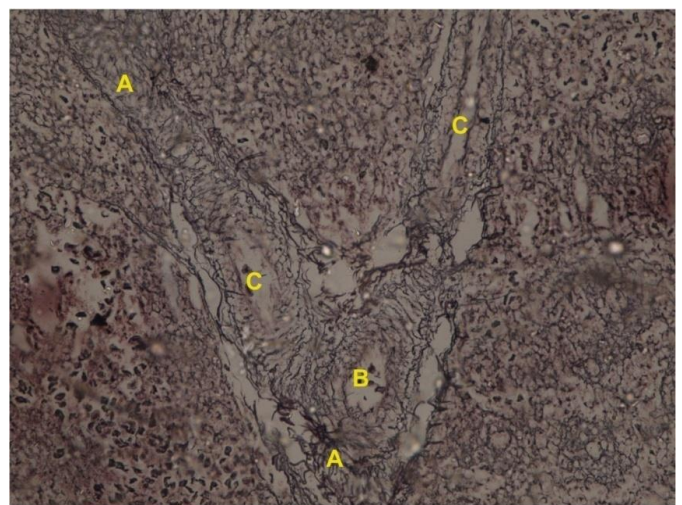


Fig 10: Photomicrograph of spleen at three week of age A. Trabeculae B. Trabecular artery C. Trabecular vein (Gomori's Reticulin stain, x400)

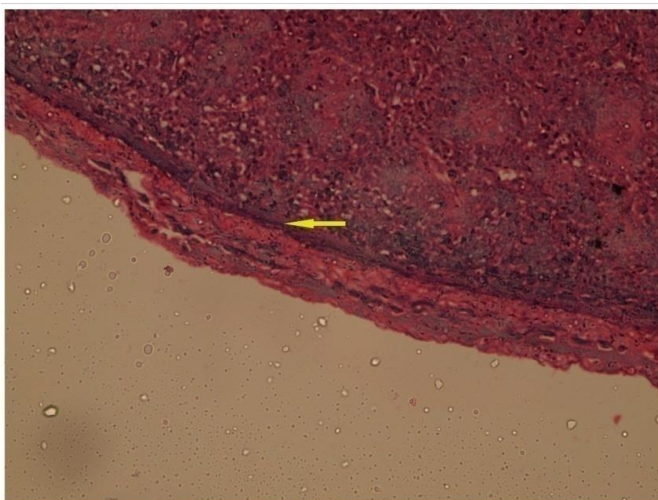


Fig 8: Photomicrograph of spleen at three week of age showing elastic fibers in inner muscular layer of capsule (Arrow) (Verhoeff's elastic stain, x400)



Fig 11: Photomicrograph of spleen at four week of age A. Trabecula B. Trabecular artery C. Trabecular vein D. Lattice of elastic fiber (Verhoeff's Elastic stain, x400)

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