



## HISTOLOGICAL STUDY OF THE SPLEEN IN GUINEAFOWL (*NUMIDA MELEAGRIS*)

Lamees Ezaldeen Mohammed, Nuha Ibrahim Mohammed and Abdulkarim Jafar Karim

Department of Anatomy, Histology and Embryology, College of Veterinary Medicine, University of Baghdad, Baghdad - Iraq.  
Corresponding author; karinjafar59@yahoo.com

### Abstract

Spleen plays a crucial role in immunity. The current investigation was performed on the spleen of guineafowl to study its histoarchitectural format. Ten adult clinically healthy female guineafowl were used in this study. Histological views showed that the spleen was covered by connective tissue capsule in which there was a thin layer of mesothelium on its external surface. The spleen was distinct by white pulp and red pulp with presence of trabeculi. The white pulp comprises by diffuse lymphatic tissue and lymphatic nodule with more germinal centers and presence of arteries derived from trabecular arteries. These arteries were branching, giving rise to arterioles and capillaries that enclosed by sheath of lymphatic and reticular cells with reticular fibers called schweigger-seidel sheath or ellipsoid. The red pulp that enclosed the white pulp composed of meshwork of cellular cords surrounding the venous sinuses that lined by endothelial cells and filled with erythrocytes, lymphocytes and granulocytes. It was concluded that spleen of guineafowl resembles the criteria of spleen in other birds with no any variation.

**Keywords :** Histological, spleen, Guinea fowl.

### Introduction

Spleen is the principal and largest organ of immun system. It is the primary site for immune cell proliferation and differentiation, specialised to filterate blood and its importance in disease resistance is accentuated by the scarcity of avian lymph nodes (Zhang *et al.*, 2015). The striking intraspecific variation in size partly reflect seasonal changes in spleen morphology and activity (John, 1994).

The splenic paranchyma plays a crucial role in immune responses such as exposure to blood-born antigen (Brendolan, 2007; Zhang *et al.*, 2015). Following an antigen penetration, an array of functional and morphological changes occurs in the white pulp. Conspicuous changes in the germinal centers are among the most variable structures in the spleen (Graczyk *et al.*, 2003). The ellipsoids may act as an antigen trapping zone of the spleen (Biro *et al.*, 2011). The ellipsoid, or schweigger-seidel sheath, is a specialised capillary segment in the spleen of chicken (Kasai *et al.*, 1995). These ellipsoids may act as an antigen trapping zone of the spleen (Biro *et al.*, 2011). The presence of reticular cells and macrophage helps the ellipsoids to regulate the movement of cells and antigens between the blood vessels and the white pulp (Colombatti *et al.*, 1989).

Immunity of birds varies towards various diseases, e.g. Salmonellosis (Al-Khatib and Al-Qutbey, 2005). Lymphocyte depletion accompanying stress is a predisposing factor for many diseases (Pope 1991). Spleen plays a crucial role in humoral and cellular immunity. Spleen histo-architectiure is the first view for the availability of lymphocytes that are mainly responsible for immunity (Yabe *et al.*, 2017). Therefore, the present study aimed to investigate the cytoarchitecture of spleen in Gunea fowl.

### Materials and Methods

Ten adult clinically healthy female guineafowl (12-15) months, obtained from Baghdad local markets were used in this investigation. The study was carried out during April 2019 in the Department of Anatomy and Histology, Faculty of Veterinary Medicine, University of Baghdad, and approved by the Animal Care and Use Committee (ACUC

approval no. 1901/26 March 2019). The birds were euthanized by slaughtering and the spleen was examined and collected. Around 1 cm<sup>3</sup> of spleen was fixed in 10% neutral buffered formaldehyde solution, dehydrated in graded series of alcohols, cleared in xylene and emdbded in paraffin. The tissues were sectioned at 6 µm with rotary microtome, mounted on glass slides and stained with Haematoxylin and Eosin, Masson Trichrome and Van Gieson stains. Slides were examined by light microscope (Bancroft and Stevens, 2007).

### Result and Discussion

The present study revealed that spleen of guineafowl was surrounded by a thick connective tissue capsule that was composed of collagen bundles and few elastic fibers with muscle cells at the inner most part of the capsule. The external surface of the capsule was covered by a thin layer of flattened mesothelium. Trabeculae extended from the capsule through paranchyma (Fig. 1, 2, 3). Similar findings were reported in chicken (Kannan *et al.*, 2015a; Reshag and Hamza, 2017) and in guineafowl (Onyeanusu (2006)).

The paranchyma of the spleen was observed to have white pulp and red pulp. The white pulp appeared as islands enclosed by red pulp (Fig. 2, 3, 4). Similar finding was reported by Zhang *et al.* (2015) in cicken and Baishya and Bhattacharyya (2012) in adult indigenou fowl. The white pulp was made up of network of reticular cells and reticular fibers within which small, medium and large lymphocytes and plasma cells were diffusely distributed. It contained sheathed arteries and lymphatic nodules (Fig. 2, 4). The lymphoid tissue surrounded the arteries and arterioles as sheaths of small lymphocytes or as clusters of large and small lymphocytes, lymphoblasts and reticular cells. Round or oval lymphoid nodules were also observed (Fig. 4, 5). This finding concurs with Kannan *et al.* (2012) in chicken and Hamza and Balash (2005) in quail.

The periarterial lymphatic sheath (PALS) was observed as a diffuse lymphatic sheath adjacent to the central artery (Fig. 5, 6). This finding is similar to that stated by Kannan *et al.* (2015 b) in chicken. It consists of closely packed small lymphocytes with reticular cells. The arterioles and capillaries form the peripheral of the white pulp. The red

pulp were found to be surrounded by a meshwork of reticular cells, muscle cells and macrophages with reticular fiber which continued into the red pulp and formed the ellipsoids or sheathed capillaries (Fig. 6, 7). The sleeve sheath, schweigger-seidel sheath, run over the entire length of the penicillar capillary from the central artery including the branching area at the red pulp. Similar observation was reported by Biro *et al.* (2011) in chicken.

The red pulp of the spleen composed of anastomosing sinuses lined by endothelial cells. These sinuses were found to be separated from each other by the pulp cords. The network of the sinuses filled with erythrocytes, lymphocytes and granulocytes (Fig. 4, 5). Similar finding was recorded by Kasai *et al.* (1995) in chicken. The pulp cords consisted of reticular cells, lymphocytes of various size, erythrocytes, macrophages, granulocytes with plasma and mast cells. This finding is similar with that of Onyeausi (2006) in guineafowl, Hamza and Balash (2005) in European quail, and Venkatesan *et al.* (2005) and Kadam *et al.* (2019) in Japanese quail.

### Conclusions

It was concluded that spleen of guineafowl resembles the criteria of spleen in other birds with no any variation.

**Author Contributions:** All authors have been involved in Conceptualization, methodology, developing, writing, and commenting on the manuscript. All authors read and approved the final manuscript.

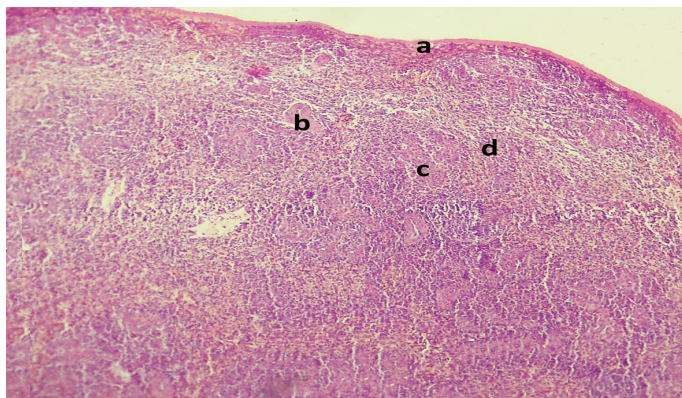
**Funding:** This research was funded by the College of Veterinary Medicine, University of Baghdad.

**Acknowledgments:** The authors are grateful to Dr Dhiya A. Abood for the technical support.

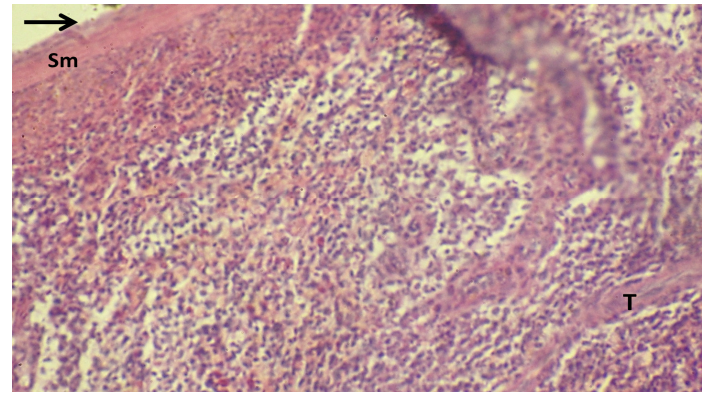
**Conflicts of Interest:** The authors declare no conflict of interest.



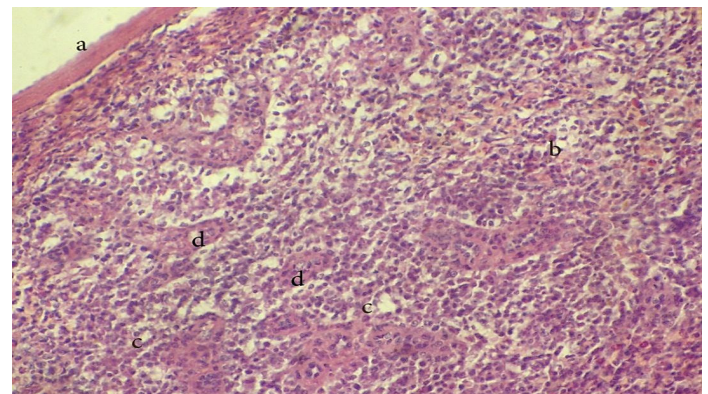
**Fig. 1:** Photomicrograph of the spleen (Guineafowl) showing: (a) Capsule, (b) Connective tissue trabeculae (Van Gieson, 40x).



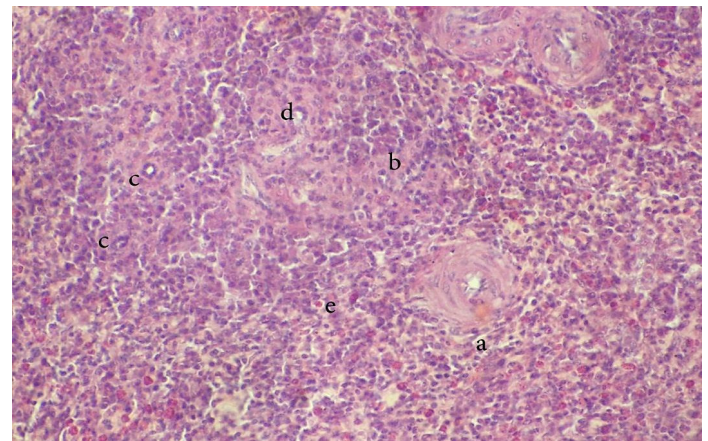
**Fig. 2:** Photomicrograph of the spleen (Guineafowl) showing: (a) Capsule, (b) Trabeculae, (c) White pulp, (d) Red pulp, (e) Lymphatic nodule (H&E, x40).



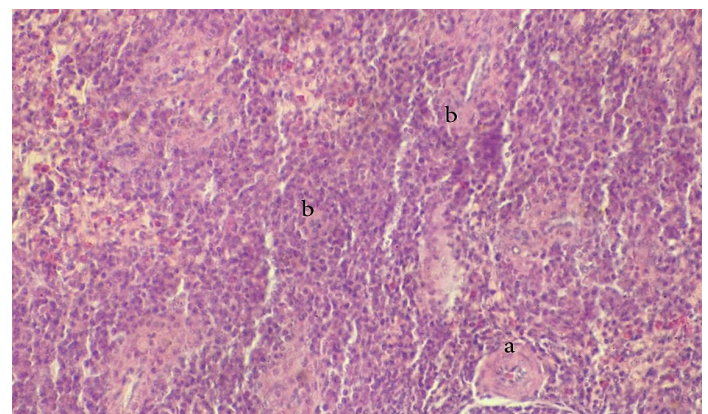
**Fig. 3:** Photomicrograph of the spleen (Guineafowl) showing: (Sm) smooth muscle fibers, (T) trabeculae, (arrow) mesothelial cells (Masson Trichrome, x100)



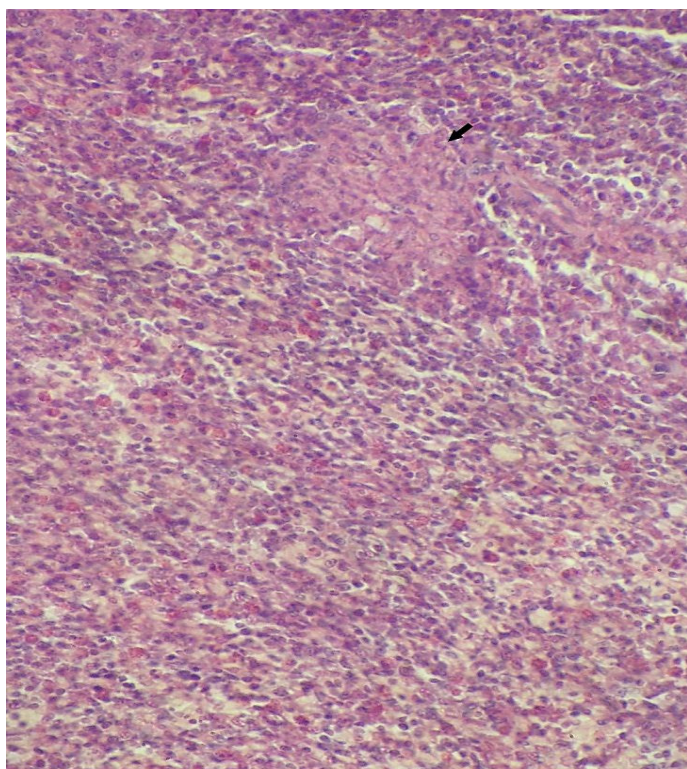
**Fig. 4:** Photomicrograph of the spleen (Guineafowl) showing: (a) Capsule, (b) red pulp & venous sinus, (c) white pulp, (d) arterioles (H&E, x100).



**Fig. 5:** Photomicrograph of the spleen (Guineafowl) showing: (a) White pulp, (b) Red pulp, (c) Sheathed artery, (d) erythrocyte, (e) granulocyte (H&E, x400).



**Fig. 6:** Photomicrograph of the spleen shows: (a) Central artery, (b) Ellipsoidal lymphatic tissue (H&E, x400).



**Fig. 7:** Photomicrograph of the spleen shows; Red pulp and ellipsoidal lymphatic tissue with arterial vessel inside lined with endothelium (Arrow) (H&E, x400).

### References

- Al-Khatib, G.M. and Al-Qutbey, S.H. (2005). Isolation and Identification of *Salmonella spp.* which contaminated Poultry slaughter houses. The Iraqi Journal of Veterinary Medicine, 29(1): 92-97.
- Baishya, G. and Bhattacharyya, R. (2012). Gross and Micro-Anatomy of the spleen of Adult Indigenous Fowl of Assam. Indian Journal of Veterinary Antomy, 24(2): 84-86.
- Bancroft, J.D. and Stevens, A. (2007). Theory and practice of histological techniques. Churchill Livingstone. London.
- Biro, E.; Kocsis, K.; Nagy, N.; Molnar, D. and Palya, V. (2011). Origin of the chicken splenic reticular cells influences the effect of the infectious bursal disease virus on the extracellular matrix. Avian Pathology, 40: 199-206.
- Brendolan, A.; Rosado, M.M.; Carsetti, R.; Selleri, L. and Dear, T.N. (2007). Development and function of the mammalian spleen. Bioassays. News and Review in Molecular, Cellular and Developmental Biology 29: 166-177.
- Colombatti, A.; Poletti, A.; Carbone, A.; Volpin, D. and Bressan, G.M. (1989). Extracellular matrix of lymphoid tissues in the chick. Journal of Histochemistry and Cytochemistry, 37: 757-763.
- Graczyk, S.; Kuryszko, J. and Madej, J. (2003). Reactivity of Spleen Germinal Centres in Immunized and ACTH-treated Chickens. Acta Vet. Brno., 72: 523-531.
- Hamza, L.O. and Balash, K.J. (2005). Histological study of the quail spleen (*Coturnix coturnix coturnix*). The Iraqi Journal of Veterinary Medicine, 29(1): 98-110.
- John, J.L. (1994). The avian spleen: a neglected organ. Quarterly Review of Biology, 69: 327-351.
- Kadam, S.D.; Waghaye, J.Y. and Thakur, P.N. (2019). Histomorphological study of spleen in post-hatched Japanese quail (*Coturnix coturnix Japonica*). Journal of Entomology and Zoology Studies 7(1): 1581-1585.
- Kannan, T.A.; Ramesh, G.; Ushakumari, S.; Dhinakarraj, G. and Vairamuthu, S. (2012). Light microscopic studies on Spleen of Chicken (*Gallus domesticus*). Haryana Vet., 51(12): 114-115.
- Kannan, T.A.; Ramesh, G.; Ushakumari, S.; Dhinakarraj, G. and Vairamuthu, S. (2015a). Histological and Ultrastructural studies of Caecal tonsil in Chicken (*Gallus domesticus*). Int. J. Curr. Microbiol. App. Sci., 4(6): 63-68.
- Kannan, T.A.; Ramesh, G.; Venkatesan, S.; Ushakumari, S. and Basha, S.H. (2015b). Cytoarchitecture of Periarterial lymphatic sheath (PALS) in Chicken spleen-Light and Transmission electronmicroscopic study. International Journal of Advance Research, 3(11): 1167-1172.
- Kasai, K.; Nakayma, A.; Ohbayashi, M.; Nakagawa, A.; Saga, A.; Ito, M.; Saga, S. and Asai, J. (1995). Immunohistochemical characteristic of chicken spleen ellipsoids using newly established monoclonal antibodies. Cell and Tissue Research, 281: 135-141.
- Onyeanusu, B.I. (2006). The guineafowl spleen at embryonic and post-hatch periods. Anat. Histol. Embryol. 35(3): 140-143.
- Pope, C.R. (1991). Pathology of lymphoid organs with emphasis on immunosuppression. Vet Immunol Immunopathol. 30: 31-44.
- Reshag, A.F. and Hamza, A.H. (2017). Anatomical and histological changes in the spleen of post hatching indigenous chicken in Iraq. The Iraqi Journal of Veterinary Medicine, 41(1): 174-178.
- Venkatesan, C.; Ramesh, G. and Vijayaragavan, C. (2005). Age related changes in Histomorphology of the spleen of the Japanese Quail (*Coturnix coturnix japonica*). Indian Journal of Veterinary Anatomy 17(1,2): 19-23.
- Yabe, M.; Medeiros, L.; Wang, S.; Tang, G.; Bueso-Ramos, C.; Jorgensen, J.; Bhagat, G.; Chen, W.; Li, S.; Young, K. and Miranda, R. (2017). Distinguishing Between Hepatosplenic T-cell Lymphoma and  $\gamma\delta$  T-cell Large Granular Lymphocytic Leukemia: A Clinicopathologic, Immunophenotypic, and Molecular Analysis. The American Journal of Surgical Pathology, 41(1): 82-93.
- Zhang, Q.; Chen, B.; Yang, P.L.; Zhang, Y.; Liu, S.; Wu, Y.; Waqas, Y.; Le, W. and Chen, Q. (2015). Identification and structural composition of the blood-spleen barrier in chicken. The Veterinary Journal 204: 110-116.