

Cairo University Faculty of Veterinary Medicine



Diagnostic Histological, Ultrastructural and Immunohistochemical Studies on the Normal Spleen of Water Buffalo (*Bubalus bubalis*)

A Thesis Submitted By

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ABSTRACT

In the current study, we assessed the immune status of water buffalo spleen, described the macro and micro components of the splenic tissue, and investigated the distribution of different immunocompetent cells in the splenic parenchyma. This study was performed on 20 spleen samples gathered from apparently healthy water buffaloes of both males and females. Techniques that were applied covered the anatomical, histological, histochemical, ultrastructural, immunohistochemical, and immunofluorescent branches. The current thesis showed that the spleen of water buffalo had a thick fibromuscular connective tissue capsule. Stromal trabeculae emerged in two forms: avascular trabeculae and vascular trabeculae. The splenic parenchyma was formed of white pulp, marginal zone, and red pulp. These areas were examined by light and electron microscopy. Regarding the histochemical stains, a high number of ferric ions, and hemosiderin pigment were highlighted occupying the MZ. The distribution of immune cells was obtained using various antibodies. The expression of CD3+, CD4+, and CD8+ on T lymphocyte was high in PALS, RP, and MZ with a significant area % difference than in lymph nodule, while CD45RO+ showed insignificant area % between the splenic compartments. Regarding the expression of CD5, CD19, CD20, and IgM on splenic cells, insignificant area % was obtained. However, CD21+, CD79A+, and IgG+ exhibited significant area %. Significant area % in the cytoplasmic reaction of NKCs, MQ, FDCs was highlighted by using CD56, CD68 and CD1A, respectively. ADAM17, TGF\u03b3, Fibrin, ENOS, and NRF2 were represented in few amounts inside LN, contrary to MZ and RP. Meanwhile, EGFR, MMP2, and TF showed a strong reaction in all parts of the splenic tissue. Trabeculae of the red pulp recorded their positivity to MMP2 and Fibrin. This research reflects a valuable opportunity to describe the components of the water buffalo spleen on different levels of study, and to identify the distribution of different immunocompetent cells all over the splenic tissue. Consequently, we hope that this study will be useful for immunologists and pathologists to distinguish, diagnose, and vaccinate hematopoietic and immunological disorders in water buffalo spleen.

(**Keywords:** Water Buffalo spleen, T Lymphocyte, B Lymphocytes, CD68, CD138, CD56, CD1A, TGFβ, MMP2, ENOS, NRF2, Fibrin).

DEDICATION

I dedicate this thesis to **God** almighty my creator, my strong pillar, my source of inspiration, strength, wisdom, knowledge and understanding.

A special thanks to my family. Words cannot express how grateful I am to my mother, father, sister, and brother for all of the sacrifices that you've made on my behalf. You always loved me unconditionally and gave me good examples that taught me to work hard for the things that I aspire to achieve. You have been a constant source of support in the moments when there was no one to answer my queries. Your prayer for me was what sustained me thus far.

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	Photomicrograph showed the IF expression of ADAM17 antibody inside water buffalo spleen: (A , B) Few positive expression (arrows) in the splenic cells inside the lymph nodule (LN). Notice: the reaction (arrowhead) in the marginal zone (MZ). (ADAM17 X 20). (B) Mostly cytoplasmic reaction (arrow) was observed inside (L.N). (C , D) Marginal zone (MZ) remarks the reaction of many cells as high positive expression (arrow) (ADAM17 X40). (D) Mainly cytoplasmic (arrow) with few nuclear ones (arrowhead). (E , F) Splenic cords (SC) and splenic sinuses (SS) showed the highest reaction (arrows). (ADAM17 X20). (F) Most cells showed cytoplasmic expression (arrow) with few nuclear (arrowhead) immunopositivity.	
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	(arrowhead). Notice the reaction along the marginal sinus (MS). (C,	
	D) Marginal zone (MZ) highlighted high immunofluorescence	
	reactivity (arrow). (EGFR X20). (D) The positive cytoplasmic reaction	
	was expressed in most cells (arrow) with few nuclear reactions in some	
	cells (arrowhead) close to the lymph nodule (LN). (E, F) High	
	expression (arrow) along with Splenic cords (SC) and the splenic	
	sinuses (SS) (EGFR X40). (F) Cytoplasmic (arrow) and nuclear	
	(arrowhead) immunopositivity expression were noticed.	
3	Photomicrograph highlighted TGFβ antibody IF expression in water	215
	buffalo spleen: (A, B) Lymph nodule (LN) area with few expression	
	(arrow) of TGFβ. Notice the reaction along the marginal zone (MZ)	
	and marginal sinus (MS). (TGFβ X 10). (B) Both cytoplasmic (arrow)	
	and nuclear (arrowhead) expressions were noticed (C, D) Marginal	
	zone (MZ) remark highest immunofluorescence reactivity (arrow).	
	Notice expression inside the lymph nodule (arrowhead). (TGFβ X40).	
	(D) It is presented mainly as a nuclear reaction (arrow), in addition to	
	the positive nuclear reaction of the central arteriole (arrowhead) inside	
	the lymph nodule (LN). (E, F) Red pulp recognized with high	
	immunopositivity expression (arrows) along with splenic cords (SC)	
	and lining cells of the splenic sinuses (SS). (TGF β X20). (F) Both	
	cytoplasmic (arrow) and nuclear (arrowhead) expression were noticed	
	in red pulp.	
4	Photomicrograph revealed the IF reaction of water buffalo spleen to	217
	MMP2 antibody: (A, B) The expression is moderate (arrow) in the	
	lymph nodule (LN). Notice the reaction that is surrounding the	
	marginal sinus (MS) in the marginal zone (MZ). (MMP2 X 20). (B)	
	Mainly nuclear (arrowhead) expression was seen in L.N. while the	
	central arteriole showed cytoplasmic (arrow) one. (C, D) Marginal	
	zone points moderate immunofluorescence reactivity (arrow). (MMP2	
	X40). (D) Either cytoplasmic (long arrow) or nuclear (short arrow).	
	Notice the cytoplasmic expression of the central arteriole (arrowhead)	
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