

# **HISTOMORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON PIG SPLEEN**

**Thesis**

**Submitted to Guru Angad Dev Veterinary and Animal Sciences University  
in partial fulfillment of the requirements for the degree of**

**MASTER OF VETERINARY SCIENCE  
in  
VETERINARY ANATOMY  
(Minor Subject: Veterinary Physiology)**

**By**

**Harmanpreet Kaur  
(L-2017-V-20-M)**



**Department of Veterinary Anatomy  
College of Veterinary Science  
©Guru Angad Dev Veterinary and Animal Sciences University  
Ludhiana-141 004**

**2019**

## **CERTIFICATE – I**

This is to certify that the thesis entitled, “**HISTOMORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON PIG SPLEEN**” submitted for the degree of **M.V.Sc.**, in the subject of **Veterinary Anatomy** (Minor subject: **Veterinary Physiology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Harmanpreet Kaur (L-2017-V-20-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

---

**(Dr. Opinder Singh)**  
**Major Advisor**  
Professor  
Department of Veterinary Anatomy  
Guru Angad Dev Veterinary  
and Animal Sciences University  
Ludhiana – 141 004 (India)

## **CERTIFICATE – II**

This is to certify that the thesis entitled, “**HISTOMORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON PIG SPLEEN**” submitted by **Harmanpreet Kaur (L-2017-V-20-M)** to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.V.Sc.**, in the subject of **Veterinary Anatomy** (Minor subject: **Veterinary Physiology**) has been approved by the student’s Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

---

**(Dr. Opinder Singh)**  
**Major Advisor**

---

**(External Examiner)**  
**Dr. Pawan Kumar**  
**Professor-cum-Head**  
**Department of Veterinary Anatomy**  
**College of Veterinary Sciences**  
**LUVAS, Hisar – 125 004**

---

**(Dr. Varinder Uppal)**  
**Head of the Department**

---

**(Dr. Sanjeev Kumar Uppal)**  
**Dean, Postgraduate Studies**  
**Guru Angad Dev Veterinary and**  
**Animal Sciences University,**  
**Ludhiana, Punjab**

## ACKNOWLEDGEMENT

*First and foremost, I express my deepest gratitude to **The Almighty** for being with me right from the beginning, until the end.*

*I express my sincere heartfelt gratitude to my major advisor, **Dr. Opinder Singh**, Professor, Department of Veterinary Anatomy, College of Veterinary Science, GADVASU, Ludhiana for his non-compromised, constant motivation and ready help rendered in research. I will be ever grateful and obligated to him for all his cooperation and timely advice. I feel extremely honored for the opportunity bestowed upon me to work under his versatile guidance.*

*I owe my heartiest and sincere thanks to **Dr. (Mrs.) Varinder Uppal**, Professor-cum-head, Department of Veterinary Anatomy for their luminous guidance, inspiration, effective counseling and providing necessary facilities to execute my research work.*

*I feel privileged to express my gracious thanks to the respected members of my advisory committee **Dr. Rajesh Jindal** (Minor Advisor) Professor, Department of Veterinary Physiology, **Dr. Devender Pathak** (Assistant Scientist), Department of Veterinary Anatomy, for their invaluable suggestions and everlasting guidance.*

*It is my pleasure to acknowledge the valuable advice and encouragement from **Dr. (Mrs.) Anuradha Gupta**, Dean PGS Nominee (Associate Professor), Department of Veterinary Anatomy during my work.*

*I would like to thank **Dr. Devendra Pathak** (Assistant Scientist), Department of Veterinary Anatomy for his support and motivation during my studies.*

*I express my thanks to my seniors **Dr. Shweta Raghav**, **Dr. Amit Poonia** and **Dr. Samikshya Sarangi***

*I am thankful to my department colleagues **Dr. Kirpal Singh**, **Dr. Harmandeep Singh**, **Dr. Jasleen Kaur** and my junior **Dr. Dilmanpreet Singh**. With my whole heart I would like to thank them for their excellent company, wonderful moments shared, advice and constant inspiration.*

*No words can adequately express my indebted feelings to the most affectionate and caring mother **Mrs. Manjeet Kaur** for her selfless love and contribution, father **Mr. Mohan Singh** and brother **Sharandeep Singh** for their constructive ideas, valuable guidance and incessant encouragement and altruistic help throughout the period of research.*

*I also express my sincere gratitude to all the supporting field and laboratory staff **Smt. Harjeet Kaur**, **Shri Neeraj**, **Balwinder Singh** for their help. I am very thankful to the office staff **Shri Satyam** and **Mrs. Sarika Singh** for providing all the necessary and timely help.*

*Financial assistance in the form of **University Merit Scholarship** by GADVASU, Ludhiana is duly acknowledged. Lastly, I thank all those who care for me. All might have not been mentioned but none is forgotten.*

Place: Ludhiana

Date:

(Harmanpreet Kaur)

**Title of the Thesis** : **Histomorphological and histochemical studies on pig spleen**

**Name of the student** : Harmanpreet Kaur

**Admission No.** : *L-2017-V-20-M*

**Major Subject** : *Veterinary Anatomy*

**Minor Subject** : *Veterinary Physiology*

**Name and Designation of Major Advisor** : Dr. Opinder Singh  
Professor

**Degree to be Awarded** : M.V.Sc.

**Year of award of Degree** : 2019

**Total Pages of Thesis** : 57 + VITA

**Name of University** : Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana – 141 004 (Punjab), India

#### **ABSTRACT**

The present study was conducted on the pig spleen (n=12) to elucidate its histomorphological, histochemical, histoenzymic and ultrastructural features. The gross morphological observations revealed that the pig spleen was elongated tongue shaped structure and was reddish-brown in colour. Histomorphological observations revealed that the spleen was surrounded by double layered connective tissue capsule, made up of outer fibrous and inner smooth muscle layer. The connective tissue trabeculae extended from the inner muscular layer of capsule into the parenchyma. The parenchyma of spleen was divided into red pulp and white pulp. The red pulp was composed of narrow splenic sinuses and splenic cords whereas white pulp consisted of lymphatic nodules and well developed peri-arteriolar lymphatic sheath. The ellipsoids were present abundantly in the marginal zone. Erythrophagocytosis characterized by presence of hemosiderin pigment was observed in red pulp. Histochemical study revealed varying amount of mucopolysaccharides, total lipids, basic proteins and phospholipids localized in entire spleen. The activity of phosphatases and oxidoreductases was moderate to strong in lymphoid follicles. The presence of B and T-lymphocytes was localized in white pulp using immunohistochemical methods. The presence of large number of T-lymphocytes in white pulp and around ellipsoids led to the conclusion that T-lymphocytes possibly acted as phagocytic cells in spleen. Scanning electron micrography showed variety of nucleated cells in white pulp. The distinguished area of grouped non-nucleated and grouped nucleated cells were seen with different shape, size and nuclear morphology in transmission electron micrography.

**Keywords:** Histochemistry, histomorphology, pig, spleen, ultrastructure.

---

**Signature of Major Advisor**

---

**Signature of the Student**

## CONTENTS

---

<b>CHAPTER</b>	<b>TOPIC</b>	<b>PAGE NO.</b>
I	INTRODUCTION	1 – 2
II	REVIEW OF LITERATURE	3 – 21
III	MATERIALS AND METHODS	21 – 27
IV	RESULTS AND DISCUSSION	28 – 46
V	SUMMARY AND CONCLUSIONS	43 – 50
	REFERENCES	51 – 57
	VITA	

---

## LIST OF TABLES

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
1	Histomorphological stains used on paraffin sections of spleen of pig	26
2	Histochemical stains used on paraffin/cryostat sections of spleen of pig	26
3	Histoenzymic methods used on cryostat sections of spleen of pig	27
4	Biometrical data showing average length (cm), width (cm), volume(ml) and weight (g) (Mean±SE) of pig spleen	43
5	Micrometrical data showing thickness of capsule, trabeculae, diameter of lymphoid follicles and central artery (Mean±SE) of pig spleen	43
6	Histochemical distribution of mucopolysaccharides, lipids and proteins in pig spleen	45
7	Histoenzymic localization of phosphatases and esterases in pig spleen	45
8	Histoenzymic localization of oxidoreductases in pig spleen	46

## LIST OF FIGURES

<b>Fig. No.</b>	<b>Title</b>	<b>Page No.</b>
1	Biometrical data showing mean length (cm) and width (cm) of pig spleen	44
2	Micrometrical data showing thickness of capsule ( $\mu\text{m}$ ) and trabeculae ( $\mu\text{m}$ ) and diameter of lymphoid follicle ( $\mu\text{m}$ ) and central artery ( $\mu\text{m}$ ) of pig spleen.	44

## LIST OF PLATES

Plate No.	Description
1a	Photograph of spleen of pig showing parietal surface
1b	Photograph of spleen of pig showing visceral surface
2a	Section of the spleen of pig showing the connective tissue capsule (C), trabeculae (T), white pulp (WP), red pulp (RP) and lymphoid follicle (LF) (Arrow). Haematoxylin and Eosin X 100
2b	Section of the spleen of pig showing the two layered connective tissue capsule (C), trabeculae (T), and lymphoid follicle (LF) (arrow). Haematoxylin and Eosin X 100
2c	Section of spleen of pig showing two layered capsule (C), trabeculae (T). The outer layer of capsule made up of collagen fibers (CF) (single arrow) and inner layer was smooth muscle layer (SM) (double arrows) (Arrow). Masson's trichrome X 100
2d	Section of the pig spleen showing the connective tissue capsule (C) surrounded by adipose tissue (A) (arrow). Masson's trichrome X 400
3a	Section of pig spleen showing reticular fibers (RF) in the capsule (C), trabeculae (T) and white pulp (WP). (Arrow). Gridley's X40
3b	Section of pig spleen showing reticular fibers (RF) in capsule (C), trabeculae (T), lymphoid follicle (LF) and ellipsoid (E) (arrow). Gridley's X 100
3c	Section of spleen of pig showing elastic fibers (EF) in the blood vessel (BV), ellipsoid (E) and lymphoid follicle (LF) (arrow). Gridley's X 100
3d	Section of pig spleen showing elastic fibers (EF) in capsule (C), trabeculae (T), white pulp (WP), red pulp (RP) and lymphoid follicle (LF) (arrow). Verhoeff's X 40
4a	Section of pig spleen showing branching trabeculae (T) extending into parenchyma, lymphoid follicle (LF) and red pulp (RP) (arrow). Hemotoxylin and Eosin X 100
4b	Section of pig spleen showing distribution of trabeculae (T) in parenchyma and lymphoid follicle (LF) with central artery (CA) (arrow). Picrosirius red X 40
4c	Section of pig spleen showing smooth muscle fibers (SM) and collagen fibers (CF) in capsule (C) and trabeculae (T) (arrow). Masson Tricome X 400

<b>Plate No.</b>	<b>Description</b>
4d	Section of pig spleen showing reticular fibers (RF) distribution in the trabeculae (T) (arrow). Gridley's X 400
5a	Section of pig spleen showing trabeculae (T), lymphoid follicle (LF) and red pulp (RP). Hemotoxylin and Eosin X 40
5b	Section of pig spleen showing aggregation of lymphoid follicle (LF) and red pulp (RP) (arrow). Hemotoxylin and Eosin X100
5c	Section of pig spleen showing collagen fibers in the marginal zone (MZ) in lymphoid follicle (LF) (arrow). Picosirius Red X 100
5d	Section of spleen of pig showing germinal center (GC) in lymphoid follicle (LF) and ellipsoids (E) (arrow). Hemotoxylin and Eosin X100
6a	Section of pig spleen showing lymphoid follicle (LF) with lightly stained germinal center (GC), ellipsoid (E) and marginal zone (MZ) dividing red and white pulp. AB/PAS X 100
6b	Section of pig spleen showing central artery (CA) surrounded by PALS (Peri-arteriolar lymphatic sheath) (arrow). Hemotoxylin and Eosin X 1000
6c	Section of spleen of pig showing marginal zone (MZ) dividing red pulp (RP) and white pulp (WP) (arrow). Hemotoxylin and Eosin X1000
6d	Section of pig spleen showing reticular fibers in the marginal zone (MZ) and around central artery (CA) (arrow). Gridley's X 400
7a	Section of pig spleen showing macrophages (M), plasma cells (PC), reticular cells (RC) and lymphocytes (L) (arrow). Hemotoxylin and Eosin X 1000
7b	Section of pig spleen showing T-lymphocytes with cytoplasmic reaction in spleen present around ellipsoid (E) (arrow). HRP X 400
7c	Section of pig spleen showing B-lymphocytes with cytoplasmic reaction in spleen (arrow). HRP X 400
7d	Section of spleen of pig showing B-lymphocytes with cytoplasmic reaction in spleen (arrow). HRP X 1000
8a	Section of spleen of pig showing area of red pulp (RP) and white pulp (WP) Haemotoxylin and Eosin X100
8b	Section of pig spleen showing differential area of white pulp (WP) with nucleated cells and red pulp (RP) with splenic sinuses and erythrocyte Haemotoxylin and Eosin X1000

<b>Plate No.</b>	<b>Description</b>
8c	Section of spleen of pig showing ellipsoid (E) around the lymphoid follicle (LF). Haemotoxylin and Eosin X100
8d	Section of pig of spleen showing central artery (CA) with erythrocytes and ellipsoids (E). Haemotoxylin and Eosin X1000
9a	Section of spleen of pig showing macrophages (M) present in ellipsoid (E) (arrow) in spleen. Haemotoxylin and Eosin X 1000
9b	Section of pig spleen showing macrophages (M) with cytoplasmic reaction around ellipsoid (E) in spleen (arrow). HRP X 1000
9c	Section of pig spleen showing erythrophagocytosis in the red pulp (RP) with deposition of hemosiderin pigment and white pulp (WP) in pig spleen. Perl's Prussian blue X 40
9d	Section of pig spleen showing accumulation of bluish granules of hemosiderin pigment in red pulp macrophages (arrow). Perl's Prussian blue X 400
10a	Section of pig spleen showing moderate to strong activity for neutral mucopolysaccharides in capsule (C), trabeculae (T) and lymphoid follicles (LF). Moderate activity for neutral mucopolysaccharide is also present in red pulp (RP). PAS X 100
10b	Section of spleen of pig showing strong acidic mucopolysaccharides activity in outer fibrous layer of capsule (c). Trabeculae (T) showed mixed activity for acidic mucopolysaccharides. AB/PAS X 100
10c	Section of pig spleen showing activity for acidic mucopolysaccharides around central artery (CA), and ellipsoids (E). Lymphocytes in white pulp are strongly positive for neutral mucopolysaccharides. AB/PAS X 100
10d	Section of pig spleen showing activity for acidic mucopolysaccharides around central artery (CA) AB/PAS X 1000
11a	Section of pig spleen showing weak to moderate protein activity in the trabeculae (T), lymphoid follicle (LF) and red pulp (RP). Bromphenol Blue X 100
11b	Section showing weak activity in red pulp (RP) and weak to moderate in trabeculae (T) and weak in ellipsoids (E). Bromphenol Blue X 100
11c	Cryosection of pig spleen showing strong activity of phospholipids in capsule (C), trabeculae (T) and parenchyma (P). Acid hematin X 100
11d	Cryosection of spleen showing reaction of lipids in trabeculae (T), lymphoid follicle (LF) and central artery (CA). Sudan black X 100

<b>Plate No.</b>	<b>Description</b>
12a	Cryosection of pig spleen showing activity for AKPase in lymphoid follicle (arrow)AKPase X100
12b	Cryosection of spleen of pig showing weak activity for G-6-phosphate in parenchyma (P) and blood vessels (BV)Lead nitrate method X400
12c	Cryosection of spleen of pig showing moderate to strong activity for ATPase in lymphoid follicle (arrow).ATPase X400
12d	Cryosection of pig spleen showing moderate activity for lactic dehydrogenase (LDH) in capsule Nitro BT method X100
13a	Cryosection of spleen of pig showing weak MDH activity in the capsule (C) and parenchyma (P). Nitro BT method X 100
13b	Cryosection of pig spleen showing weak activity for LDH in capsule (C), lymphoid follicle (LF) and blood vessel (BV). Nitro BT method X 100
13c	Cryosection of pig spleen showing moderate activity of NADPH in trabeculae (T), germinal enter (GC) and strong reaction at periphery in lymphoid follicle (LF). Nitro BT method X 100
13d	Cryosection of spleen of pig showing moderate activity of NADPH in capsule (C) and trabeculae (T). Nitro BT method X 100
14a	Cryosection of spleen of pig showing weak ALC activity in capsule (C), trabeculae (T) and parenchyma (P). Nitro BT method X 100
14b	Cryosection of pig spleen showing moderate activity for G-6-PD in the capsule (C), lymphoid follicle (LF) and strong activity in blood vessel (BV). Nitro BT method X 100
14c	Cryosection of spleen of pig showing moderate to strong activity for G-6-PD in the trabeculae (T). Nitro BT method X 100
14d	Cryosection of pig spleen showing moderate activity for GLD in capsule (C) and lymphoid follicle (LF). Weak activity in parenchyma (P) (arrow). Nitro BT method X 100
15a	Cryosection of spleen showing moderate to strong activity for NADH in trabeculae (T), lymphoid follicle (LF) and small blood vessels. Nitro BT method X 100
15b	Cryosection of spleen showing positive NSE activity in trabeculae (T) and blood vessel (BV). Napthol acetate method X100
15c	Cryosection of spleen showing moderate to strong activity for NADH in blood vessels (BV). Nitro BT method X 100

<b>Plate No.</b>	<b>Description</b>
15d	Cryosection of spleen showing moderate to strong activity in blood vessels (BV) and weak to moderate in lymphoid follicle (LF). Naphthol acetate method X100
16a	Scanning electron micrograph showing uneven cut surface of splenic tissue. X 41
16b	Scanning electron micrograph showing trabeculae (T) extending into the parenchyma and opening of blood vessels (BV). X 1180
16c	Scanning electron micrograph showing opening of blood vessels (BV) and macrophages (M) around blood vessels. X 6520
16d	Scanning electron micrograph showing multiple nucleated cells of white pulp. X 2400
17a	Transmission electron micrograph (TEM) of spleen showing area of white pulp showing nucleated cells (NC) and few non-nucleated erythrocyte (E) (arrow). X 1180
17b	Transmission electron micrograph (TEM) of spleen showing different nucleated cell with different morphology in white pulp. X 2300
17c	Transmission electron micrograph (TEM) of spleen showing nucleated lymphocyte (L) of white pulp and non-nucleated erythrocytes (E) in parenchyma (arrow). X 2550
17d	Transmission electron micrograph (TEM) of spleen showing nucleated macrophage (M) cell in white pulp. X 2550

## LIST OF ABBREVIATIONS

%	:	Percentage
µm	:	micrometer
A	:	Adipose Tissue
AKPase	:	Alkaline Phosphatase
ALC	:	Alcoholic Dehydrogenase
ATPase	:	Adenosine Triphosphatases
CA	:	Central Artery
CF	:	Collagen Fiber
cm	:	centimeter
CO <sub>2</sub>	:	Carbon dioxide
CPD	:	Critical Point Drying
E	:	Erythrocyte
EF	:	Elastic Fiber
ER	:	Endoplasmic Reticulum
<i>et al</i>	:	<i>et alia</i> (and others)
Fig.	:	Figure (s)
g	:	Gram
G-6-Pase	:	Glucose-6-Phosphatase
G-6-PD	:	Glucose-6-Phosphate Dehydrogenase
GC	:	Germinal Centre
H <sub>2</sub> O <sub>2</sub>	:	Hydrogen peroxide
HRP	:	Horse
IL	:	Interleukins
LDH	:	Lactic Dehydrogenase
LF	:	Lymphoid follicle
ml	:	milliliter
MZ	:	Marginal Zone
NADH-d	:	Nicotinamide Adenine Dinucleotide-Diaphorase
NADPH-d	:	Nicotinamide Adenine Dinucleotide Phosphate Diaphorase
NBF	:	Neutral Buffered Formalin
NC	:	Nucleated Cell
NSE	:	Non Specific Esterases
PALS	:	Periarteriolar Lymphoid Sheaths
PAS	:	Periodic Acid Schiff
PAS-AB	:	Periodic Acid Schiff-Alcian Blue
SDH	:	Succinic Dehydrogenase
SE	:	Standard Error

## CHAPTER I

### INTRODUCTION

Meat production has registered a healthy growth in India from 5.95 million in Twelfth Five Year Plan which has been further increased to 7.0 million in 2016 -17. The total pig population is 10.30 million and contributed 5.5 per cent of total meat production in the country, (DAHDF, GOI, Annual report 2017 – 18). Meat production in India registered a steady growth and increased from 5.4 million tones in 2012-13 to 7.4 million tones. Among the various livestock species, piggery is potential source of meat production and more efficient feed converter after the broilers. In India, presently pig farming provides livelihood to poor belonging to the lowest socio-economic strata. Apart from providing meat, it is also source of bristles, manure. The other advantages of pig farming are shorter generation interval, utilizes wide variety of feed stuff, higher dressing percentage, good demand for domestic and export market for its by-products such as bacon, ham, sausages etc. Pig farming, therefore, has a considerable potential to fulfil the requirement of meat as well as employment opportunities to seasonally employed farmers and supplementary income to improve their livelihood. Pig farming is turning out to be a good alternative for the farmers in Punjab with the state government giving a boost by a number of measures and incentives. Further swine share a number of anatomic and physiologic characteristics with humans that make swine potentially a better model for some procedures and studies compared with other large animal species

Pigs are domesticated animals, of the genus *Sus* and of the family *Suidae*, that are raised for food all over the world. Spleen as the largest lymphoid organ and involved in immune cell proliferation and differentiation and phagocytosis of aged erythrocytes. Spleen also acts as erythropoietic organ besides being involved in lymphopoiesis during embryonic stage. It is involved in haemopoiesis and phagocytosis of aged erythrocytes (Das *et al* 2005). The spleen is classified into defensive type, storage type and intermediate type (Sinha *et al* 2013). Defensive type has few trabeculae and smooth muscle fibers but abundant lymphatic tissue and occurs in human beings and rabbit. Storage type has many trabeculae and smooth

muscles and contains less white pulp e.g. horse, dog and cat. In ruminants and swine intermediate type of spleen is present. Dellmann and Brown (2006) classified mammalian spleens depending on the type of post-capillary vessels into sinusal and non-sinusal type. In majority of domestic animals except dog non-sinusal type of spleen is present. The architectural design of spleen varies due to functional reasons (Alim *et al* 2012).

Spleen is comprised of two functionally and morphologically distinct compartments, the red pulp and the white pulp. The red pulp is involved in blood storage and phagocytosis whereas the white pulp is the primary region for immune response and B-lymphocyte maturation (Ikpegbu *et al* 2014). The spleen is also the largest secondary lymphoid organ containing about one-fourth of the body's lymphocytes and initiates immune responses to blood-borne antigens (Kuper *et al* 2002; Nolte *et al* 2002; Balogh *et al* 2004). This function is attributed to the white pulp which surrounds the central arterioles. The white pulp is composed of three sub compartments: the periarteriolar lymphoid sheath (PALS), the follicles and the marginal zone.

A thorough knowledge of histomorphology and histochemistry will be helpful in explaining the immune response of pigs to varying clinical conditions and also in understanding peculiarities of porcine immune system for applied experimental studies. In the available literature most of the work has been carried out on the histology of spleen of human (Alex *et al* 2015), dog (Das *et al* 2005), goat (Devi *et al* 2016) and camel (Maina *et al* 2014), however scanty literature is available on histomorphology and especially histochemistry of pig spleen. So keeping in view the paucity of literature, the present research proposal was proposed with following objectives:

1. To study the gross and histomorphological features of pig spleen.
2. To characterize histochemical and histoenzymic features of pig spleen.

## CHAPTER II

### REVIEW OF LITERATURE

#### 2.1. Gross Morphology

Mishra *et al* (2001) studied the biometry of the spleen of 10 developing chicks from day 1 to 60. The spleen weight increased with advancement of age. The percent increment of the spleen weight in relation to the previous age of the chick was highly fluctuating and it was maximum between the ages of 21st to 25th day. The spleen diameter was 0.363 cm and 1.875 cm on 1st and 60th days, respectively. The spleen weight index was 0.091 on day one which increased to 0.253 by day 30 and thereafter revealed a fluctuating decline.

Khan *et al* (2003) recorded the biometrical observations of spleen in cattle. The mean length of the spleen of male cattle at the age of 2, 3, 4 and 5 years was  $39.09 \pm 0.05$ ,  $44.75 \pm 1.65$ ,  $45.5 \pm 4.26$  and  $51.5 \pm 0.85$  cm, respectively while females was  $38.87 \pm 0.21$ ,  $43.25 \pm 2.91$ ,  $43.34 \pm 1.43$  and  $45.30 \pm 0.68$  cm, respectively. The mean width of the spleen of male cattle at the age of 2, 3, 4 and 5 years was  $10.25 \pm 0.22$ ,  $10.85 \pm 0.11$ ,  $11.92 \pm 0.41$  and  $12.66 \pm 0.27$  cm, respectively, while in female it was  $10.58 \pm 0.14$ ,  $10.82 \pm 0.31$ ,  $10.88 \pm 0.24$  and  $10.96 \pm 0.24$  cm, respectively. The mean thickness of the spleen of male cattle at the age of 2, 3, 4 and 5 years was  $1.85 \pm 0.02$ ,  $1.9 \pm 0.14$ ,  $2.2 \pm 0.35$  and  $2.26 \pm 0.07$  cm, respectively, while in female it was  $1.86 \pm 0.14$ ,  $1.92 \pm 0.11$ ,  $1.93 \pm 0.73$  and  $2.23 \pm 0.10$  cm, respectively. The mean weight of the spleen of male cattle at the age of 2, 3, 4 and 5 years was  $395.05 \pm 9.41$ ,  $401.5 \pm 42.91$ ,  $571.75 \pm 37.16$  and  $734.19 \pm 29.47$  gm, respectively, while in female it was  $377.05 \pm 6.75$ ,  $455.87 \pm 5.76$ ,  $518.81 \pm 15.93$  and  $572.73 \pm 5.87$  gm, respectively.

Alam *et al* (2005) described the morphometry of spleen with special emphasis on its arterial circulation of indigenous horse in Bangladesh. They stated that the spleen of horse of Bangladesh was creamy–white colored, triangular, comma shaped. The mean length, width and thickness of horse spleen recorded by them was  $19.187 \pm 0.394$  cm,  $6.287 \pm 1.156$  cm and  $1.437 \pm 0.260$  cm, respectively. The spleen of horse was supplied by single splenic artery, which was the largest branched of the celiac

artery. The main stem of the splenic artery traverses from the hilus to the apex and gave of seventeen prominent branches that entered into the parenchyma of spleen.

Khan *et al* (2006) studied the spleen of musk deer and goat. It was reddish brown in colour, compact oval in shape with well marked pointed ventral extremity whereas it was rectangular in goat. The average weight was 50-55 gm in musk deer and it was approximately 65-70 gm in goat. The length of spleen in musk deer was 9.5-10.5 cm from dorsal to ventral extremity and in goat 9.4-12.4 cm. It was 6.0-6.5 cm wide at the broadest point in musk deer and in goat 6.5-7.0 cm. The thickness of spleen at the thickest point at cranial border was 2.5 cm. Spleen was well marked by two surfaces, two borders and two extremities. Parietal surface was dome shaped, convex and lying against diaphragm. Visceral surface was deeper, concave and was firmly attached to the rumen.

The proportional analysis of the pig spleen arterial segments was carried out by Pereira-Sampaio and Marques-Sampaio (2006). The splenic artery was divided close to the surface of hilus in to two primary branches, one to the dorsal extremity and other to the ventral extremity. The dorsal branch was smaller than the ventral branch. The two segments of splenic artery were separated by an avascular plane and there was no anastomosis between them. They observed strong difference between the two segmental areas, which was 83.19% of the total splenic area for the ventral segment and 16.18% for the dorsal segment. They concluded that pig arterial segments were quite similar to dog, buffalo, goat and sheep spleen where they consisted of small dorsal segment and large ventral segment.

Dyce *et al* (2010) described the anatomy of pig spleen. They stated that pig spleen was bright red, elongated and strap like present on the left side and was attached by gastrosplenic ligament. The parietal surface remained in contact with diaphragm. The long hilus divided visceral surface in to two narrow stripes. The cranial stripe remained related to the stomach and caudal to the intestine. The dorsal extremity extended in to the space between the stomach, left kidney and pancreas whereas, ventral extremity may extended below the left costal arch and exceptionally even cross the abdomen to the right side.

Ibe *et al* (2010) found that the spleen of the African giant pouched rat was long slipper-shaped and dorso-ventrally flattened organ lying on the left lateral surface of the abdominal cavity, medial to the lateral abdominal wall and lateral to the greater curvature of the stomach. They recorded an average spleen weight of  $3.31 \pm 0.54$  gm and length of  $6.44 \pm 0.48$ mm. The spleen constituted 0.313% of the body weight and 2.069% of the-rump length.

The gross morphology of the healthy adult Awasi sheep was studied by Khalel (2010). The average length, width and thickness of spleen was  $9.93+0.342$  cm,  $6.48+0.233$  cm and  $2.48+0.142$  cm, respectively whereas weight was  $69+6.663$  gm.

Ellenport (2012) stated that spleen was soft, highly vascular and bright red to dark purple in colour located in the left hypogastric region in pig. The size and weight varied throughout life and under different conditions.

Bhupender *et al* (2013) observed spleen of two year old black Bengal goat and found that the mean weight of spleen was  $47.0\pm 2.0$  g. The mean length and width were  $10.30\pm 0.21$  and  $7.90\pm 0.15$  cm, respectively.

Maina *et al* (2014) investigated the gross morphological features of spleen of one-humped camel. The spleen of camel was C-shaped, dark brown in colour with blunt and rough edges.

Abed Muslih and Mirhish (2017) investigated the gross anatomical features of spleen in gazelle. The study revealed that the spleen of gazelle appeared dark brown in color with elliptical shape. The average length and width of the spleen was  $7.94 \pm 0.1$  cm and  $5.88 \pm 0.2$  cm, respectively. Three ligaments connected the spleen with adjacent structures, namely splenogastric, splenorenal and splenophrenic. The splenic artery was divided into three primary branches, each one further subdivided into two secondary branches.

Reshag and Hamza (2017) observed anatomical changes in the spleen of post hatching indigenous chick. The spleen of one day old chicks was round in shape with pink colour and in two weeks old chicks the spleen was pale brown in colour with triangular shape.

Shringi *et al* (2017) studied the morphometry of the spleen in White Yorkshire pig (*Sus scrofa*). The average weight of spleen in White Yorkshire pig was  $128.55 \pm 4.764$  g with average volume of 131.87 ml. They stated that the spleen was widest at the middle aspect than the dorsal and ventral aspect. The average width of spleen was  $1.386 \pm 0.139$  cm,  $6.250 \pm 0.107$  cm and  $2.701 \pm 0.107$  cm at dorsal, middle and ventral aspect, respectively. The average thickness of spleen at dorsal, middle and ventral aspect was  $0.595 \pm 0.0301$  cm,  $1.213 \pm 0.063$  cm and  $0.448 \pm 0.0151$  cm respectively.

Sivagnanam *et al* (2017) conducted an age related study on the length, width, weight, volume and thickness of spleen in non-descript goats of different age groups ranging from 3-5 months to 5 years and above irrespective of sex. A parallel increase in length and width of spleen was noticed with advancement of age. There was a gradual increase in weight of the spleen up to 4 years of age. The weight of the spleen beyond four years of age increased rapidly. There was increase in the volume of spleen with increase in age of goats. The rate of increase in volume was higher when compared with the rate of increase in weight with age, which implied that the density of the organ decreased beyond 4 years of age. Thickness of spleen also showed a progressive increase with age.

Suri *et al* (2017) studied the gross and biometry of the spleen of sheep and goat of Jammu region. The spleen of goat was quadrangular whereas that of sheep was triangular. The biometrical measurements of the spleens of sheep and goat revealed that the weight of spleen of sheep was  $81.39 \pm 12.79$  gm while that of goat was  $64.48 \pm 7.82$  gm. The length of the spleen was  $12.70 \pm 0.81$  cm and  $11.48 \pm 0.73$  cm in sheep and goat, respectively. The width of spleen of sheep was recorded to be  $9.26 \pm 0.38$  cm and that of goat was measured as  $9.37 \pm 0.79$  cm and the thickness of spleen was found to be  $2.69 \pm 0.2$  cm and  $2.37 \pm 0.21$  cm in sheep and goat, respectively.

Gnanadevi *et al* (2019) reported that the spleen of sheep was triangular, whereas, in goat it was roughly quadrangular in outline. In both the species, parenchyma was covered by a thick capsule, predominantly made up of collagen, elastic and smooth muscle fibres.

## 2.2. Histomorphology

Bacha and Bacha (2000) reported that in pig, horse and ruminants splenic capsule was rich in elastic fibers and smooth muscle cells. In horses and cow, two or three layers of muscles were oriented perpendicular to each other while pig, sheep and goat the muscle fibers were interwoven. The trabeculae were found projected from the capsule in to the interior of spleen. They mentioned that splenic parenchyma was divided into white and red pulp. Dense accumulation of lymphocytes around the central arteries formed the periarterial lymphatic sheath. The red pulp contained large numbers of erythrocytes in the reticular meshwork and blood vessels. The marginal zone was the region between white and red pulp. The red pulp consisted of wide splenic sinuses, lined by elongated, longitudinally oriented endothelial cells. They reported numerous wisps of smooth muscle fibers in the pig and ruminants. The central arteries on leaving white pulp branched in to two or three arteriole called as penicillar arteries.

Zidan *et al* (2000) reported that camel spleen was surrounded by thick connective tissue capsule. The capsule was  $292 \pm 106 \mu\text{m}$  thick and divided into clearly demarcated outer and inner layers. The outer layer consisted of collagen, elastic and reticular fibers with few smooth muscle cells and inner layer composed predominantly of smooth muscle cells supported by reticular, collagen and elastic fibers.

Geetha *et al* (2001) observed splenic nodules in the peripheral area in mice, rat and guinea pig. The splenic capsule was composed of collagen, reticular, elastic and smooth muscle fibers. In all species, fibers increased with the advancement of age. The lymphatic nodule with central artery surrounded by small and medium size lymphocytes were observed in white pulp in one week old guinea pig. However, in mice and rat, lymphatic nodules were observed at three weeks of age. Isolated smooth muscle fibers were noticed in red pulp of spleen in rats however these were absent in mice. The venous sinuses were not observed in mice, whereas these were present in guinea pig and rats. The peri-arterial macrophage sheath was not observed in rat and mice, whereas it was present in guinea pig.

Xue *et al* (2001) observed the erythrocyte shapes in splenic cords of living mouse spleens using *in vivo* cryotechnique followed by scanning (SEM) or transmission (TEM) electron microscopy. Erythrocytes in living mouse spleens showed a variety of shapes with narrow spaces between them, trapped among reticular fiber tissues. Similar shapes of erythrocytes were kept in the red pulp even after blocking normal blood circulation, as prepared by the *in vitro* freezing method. Some erythrocytes changed to biconcave discoid shapes by the conventional immersion fixation with chemical fixative and showed wide spaces between each other among reticular fiber tissues. The various shapes of erythrocytes in the functioning spleens were demonstrated by *in vivo* cryotechnique. It was suggested that most erythrocytes congesting in spleen kept their original configuration in spite of microenvironmental alteration in splenic blood circulation.

Hande *et al* (2002) observed the microscopic structure of spleen in rat. They stated that white pulp showed central artery in centre and consisted of densely packed lymphocytes, whereas diffused lymphocytes and erythrocytes were observed in the red pulp. They observed marked deposition of haemosiderin pigment in spleen. There was no variation in male and female rat spleen.

Karad *et al* (2002) reported the microanatomical structure of spleen of goat. The splenic capsule consisted of elastic fibers, collagen fibers and smooth muscle fibers. The composition of trabeculae was similar to capsule with addition of arteries and veins. The splenic parenchyma was composed of red and white pulp. The framework of red pulp was formed by reticular fibers and smooth muscle cells and consisted of pulp arterioles, venous sinuses and splenic sinuses and white pulp was made up of lymphatic nodules and peri-arteriolar sheath.

Nolte *et al* (2002) stated that the splenic marginal zone compartment separated the lymphoid white pulp and red pulp in mice. They demonstrated that both development and maintenance of this marginal zone was highly dependent on the presence of B cells. Spleens from B cell-deficient mice were found to lack both metallophilic and marginal zone macrophages. They concluded that B cells were not only important for the development, but also for maintenance, of the marginal zone.

Mishra *et al* (2003) reported the histological and immunohistochemical development of chicken spleen. They mentioned that there was gradual increase in the thickness of splenic capsule up to 30 days of age and RBC's were scattered in the small groups or in isolated manner bounded by focal lymphoid patches of primitive white pulp. They stated that venous tributaries were conspicuous by day first. By day fifth, arterioles and venules were prominent in the parenchyma and lymphoid cell masses bathed freely in haemopoietic system. By 25th day, differentiation of red pulp and white pulp was difficult. They also stated that on day first, white pulp appeared as small ovoid cell mass. It mostly consisted of reticular cells and few lymphocytes

Raju *et al* (2003) studied the histological structure of the spleen of adult Indian goat. The capsule was covered by loosely arranged serosa. The outer fibrous layer of the capsule consisted of equal amount of collagen, reticular and elastic fibers which were interwoven with each other. The areas of white pulp were more and the central arteries were noted in large number in splenic corpuscles. The germinal centers were completely devoid of reticular fibers. A layer of reticular fibers was observed between the marginal zone and the nodule proper. Sheathed arteries were observed in the red pulp.

Das *et al* (2005) studied the histology of the spleen of indigenous dog of Bangladesh. The spleen was encircled by capsule composed of collagen, elastic, reticular, and smooth muscle fibers. The hilus contained major vessel but was poor in smooth muscle fibers. The white pulp was composed of fine meshwork of reticular connective tissue containing lymphocytes of various sizes. Meshwork of fine reticular fibers was observed throughout the red pulp. Sinusoids of spleen of indigenous dog were different in shapes and sizes and widely distributed within the red pulp and showed both arterial venous communications.

Mebius and Kraal (2005) reported that the spleen accommodated the efficient phagocytosis of erythrocytes and recycling of iron, the capture and destruction of pathogens and the induction of adaptive immune responses through its compartmentalization into different regions. They observed that the conduit system transport molecules in the splenic white pulp; however, in the red pulp, there is an extensive extracellular-matrix network that allowed the rapid transport of small

molecules. They failed to outline pathways that lymphocytes used to exit from the white pulp and the processes that controlled dependence of marginal-zone B cells and marginal-zone macrophages on each other and the implications of this dependence for immune responses.

Cesta (2006) described that the capsule of spleen in different species was composed of dense fibrous tissue, elastic fibers and smooth muscle. The outermost layer of the splenic capsule was composed of smooth muscle cells which were oriented perpendicular to each other. The capsule in horse consisted of an outer thick connective tissue and an inner thinner smooth muscle layer. In the pig, the capsule was mainly composed of smooth muscle, while in the dog and cat smooth muscle cells formed about 2/3 of the capsule thickness.

Press and Landsverk (2006) reported species difference in histology of spleen. They stated that spleen of horses, dogs and pigs consisted of abundant lymphatic nodules and periarterial lymphatic sheaths, whereas in cat and ruminants spleen consisted of less lymphatic tissue which occurred as lymphatic nodules with short periarterial lymphatic sheath. The pericapillary macrophage sheaths were large, abundant and particularly numerous near the white pulp in pig spleen

Alshamarry (2010) conducted the histological and histometric study on spleen of Iraqi camel. They observed thick capsule of camel spleen which was composed of mesothelial cells. The average splenic white pulp diameter and lymphoid follicle diameter of spleen was  $592.35 \pm 5.23 \mu\text{m}$  and  $279.19 \pm 4.85 \mu\text{m}$ , respectively whereas average thickness of splenic capsule and splenic trabeculae recorded by them was  $147.43 \pm 3.64 \mu\text{m}$  and  $106.5 \pm 6.29 \mu\text{m}$ , respectively. The white pulp area was large and surrounded by wide marginal zone. The diameter of white pulp was  $592.35 \pm 5.23 \mu\text{m}$ . The lymphoid follicles were spherical in shape with diameter of  $279.19 \pm 4.85 \mu\text{m}$ . The cross section of the PALS contained 3-4 arteries which were tortuous and branched in PALS. They stated that the white pulp was subdivided into the PALS, the follicles and the marginal zone. The cellular elements were lymphocytes, macrophages, dendritic cells, plasma cells, arterioles, and capillaries in a reticular framework.

The histology of healthy adult Awasi sheep was studied by Khalel (2010). The histological study included the thickness of capsule and trabeculae, splenic white pulp

diameter and lymphoid nodule diameter. The average measurements were  $140.5 \pm 13.712 \mu\text{m}$ ;  $82.75 \pm 9.7 \mu\text{m}$ ;  $505 \pm 34.245 \mu\text{m}$ ;  $315.25 \pm 23.185 \mu\text{m}$ , respectively. The periarterial lymphatic sheath (PALS) contained one central artery that branched into 2-3 arteries.

Birjandi *et al* (2011) reported that the marginal zones were architecturally organized for clearance and rapid response against blood-borne antigens entering the spleen. They observed gross architectural changes in the marginal zone of old mice, including reduction in the abundance of marginal zone macrophages surrounding the marginal zone sinus. A majority of old mice also showed reduced frequency of marginal zone B cells, which correlated with decreased abundance of marginal zone macrophages in old individual. The phagocytic ability of individual marginal zone macrophages was examined and no differences in phagocytosis were found between macrophages from young or old spleens. They concluded that an anatomical breakdown of the marginal zone occurred in advanced age, and a reduction in frequency of marginal zone macrophages might affect the ability of the marginal zone macrophages compartment to clear blood-borne antigens and mount proper T-independent immune response.

Ellenport (2012) reported that the collagenous capsule was thickened at hillus from where vessels enter and leave the spleen. Capsule penetrated the organ in the form of trabeculae. The reticular framework filled the spaces between capsule, hillus and trabeculae. The splenic tissue was composed of white pulp and red pulp. The arteries were closely connected with the white pulp and vein with the red pulp. The pig spleen had an open type of circulation.

Alim *et al* (2012) compared the micrometrical observations in spleen of human, cow and goat. The thickness of the splenic capsule was  $111.56 \pm 21.45 \mu\text{m}$  in human,  $196.88 \pm 11.91 \mu\text{m}$  in cow and  $251.44 \pm 12.56 \mu\text{m}$  in goat. The difference in thickness of the splenic capsule was statistically significant among the human, cow and goat ( $p < 0.001$ ). However, no difference was found in diameter, number and percentage of the white pulp of the spleen among these species.

According to Radhika *et al* (2012) the aggregation of lymphocytes was present at 11th week of gestation in human foetal spleen but no differentiation between red

and white pulp was found. The development of lymphoid follicles began at age of 20th week. The mature lymphoid follicles with large central arteriole were clearly seen at 32nd week of gestation. At 36th week of gestation arterioles shifted to the periphery of lymphatic aggregation.

Bhupender *et al* (2013) in the spleen of two year old black Bengal goat found that the capsule was formed by thick connective tissue which had mean thickness  $251.44 \pm 12.56 \mu\text{m}$ .

Onkar and Goverdhan (2013) compared dog and human spleen and reported that capsule and trabeculae were comprised of collagen and elastic fibers with few smooth muscle fibers in human spleen while in dog spleen more smooth muscle fibers and few elastic fibers were present. White pulp was more in human than dog. Red pulp and marginal zone of dog spleen showed ellipsoids and penicillar arteries which were poorly defined in human spleen. Red pulp showed prominent venous sinuses in dog spleen than human spleen.

Pantic *et al* (2013) studied age related changes in splenic hematopoietic tissue architecture in mice. The study concluded that complexity of spleen hematopoietic tissue decreased with age

The histomorphological observations on spleen of one humped camel were made by Maina *et al* (2014). The thick capsule was divided into an outer and inner layer. Both vascular and avascular trabeculae extended from the capsule into the splenic parenchyma. The presence of aggregation of the lymphatic nodules was unique feature of camel spleen.

Usende *et al* (2014) studied the spleen of Nigerian indigenous pig and West African Dwarf goat. The study revealed that splenic capsule of West African Dwarf goat showed abundant smooth muscle fibers giving it more contractile ability. The lymphoid tissue in West African Dwarf goat was evenly distributed and compact indicating better adaptability for immunological functions.

Alex *et al* (2015) reported that the microscopic structure of spleen varied depending on the developmental stage of the organ and the age and immune status of the human. The white pulp of spleen showed peri-arteriolar lymphatic sheath (PALS) and lymphatic follicles. The corona or mantle zone and the germinal centre were

discernible in many of the Malpighian bodies. The marginal zone separating the red pulp from the white pulp also could be clearly demarcated. The marginal sinus and peri-follicular zone could be seen in some sections only. The capsule thickness, trabecular network, cellularity of white pulp and red pulp, the connective tissue framework seen in the red pulp etc., showed variations in the different age groups. The PALS and the white pulp showed scanty cellularity in the six month foetus, and almost uniform cellularity in all areas of spleen at full term. Thereafter the follicles showed increase in its cellularity up to the third decade, and then seemed to become progressively atrophic.

Gautam and Mishra (2015) reported that the APUD cells and mast cells were scattered in the splenic cords. Small and oval argentaffin cells appeared in white pulp in day old neonates. A reticular cell syncytium carrying sequestered lymphocytes at central parenchyma of spleen of goat aged 106 day of gestation and with advancing age it revealed entrapment of more sequestered lymphocytes at different stages of gestation.

According to Souza *et al* (2015) in the spleen of human foetus stated that the splenic capsule of human foetus was formed by the connective tissue, which was cellular and had fibroblasts between 10th to 12th week of gestation. At this stage the parenchyma of spleen appeared to be incompletely subdivided by trabeculae. The lymphocytes were scattered throughout spleen and few aggregations were observed at some places by 10th week of gestation. The Central arterioles were not observed by these investigators at this stage. The lymphoid aggregation forming the PALS began to differentiating around the central arteriole by 20th week. By 23rd week of gestation, red pulp contained RBC and sinusoids, whereas, the white pulp had lymphoid follicles.

Aw *et al* (2016) investigated the integrity of the splenic microarchitecture in mice of increasing age and its reorganization following immune challenge in young and old mice. Several differences in the anatomy of the spleen with age in both the immune and stromal cells were observed. There was an age-related increase in the overall size of the white pulp, which occurred primarily within the T-cell zone and was mirrored by the enlargement of the T-cell stromal area, boundary between T cells and

B cells became less defined in older mice. There was loss of marginal zone macrophages, which was accompanied by an accumulation of fibroblasts in the spleens from older animals. Further the reorganization of the white pulp was resolved after several days following antigenic challenge in young animals, it remained perturbed in older subjects. All these age-related changes within the spleen could potentially contribute to the age-dependent deficiencies in functional immunity.

The histological features of spleen of Marwari goat was reported by Devi *et al* (2016). The capsule was composed of smooth muscle, collagen and elastic fibres. Trabeculae emerged from the capsule and entered into smaller compartments by forming a net like framework. Splenic parenchyma was composed of white and red pulp. White pulp was composed of lymphoreticular tissue of lymphatic nodule and peri-arterial lymphatic sheath. Peri-arterial lymphatic sheath was less abundant. Red pulp consisted of pulp arterioles, sheathed capillaries, terminal capillaries, splenic sinusoids and splenic cords. The splenic sinusoids were less abundant and poorly developed.

Mukhia *et al* (2016) in foetal human spleen reported that at 12th to 14th gestational week the parenchyma was comprised of irregular network of mesenchymal cells with thin capsule, reticular cells, lymphoblasts and blood vessels. Reticular cells forming network in the parenchyma were present at 16th week of gestation but size of cells was small. The lymphocytes aggregation started earlier but well defined dense lymphocytic aggregations could not be established up to the age of 20th week. The well differentiating aggregations were observed around central arteriole with development of PALS. The splenic capsule having bundles of collagen fibers with fibroblast cell as well as fibrocytes were observed and increased up to the age of 24th and 25th week. The red pulp and white pulp were observed on 18th week of gestation but germinal center was observed at 38th week of gestation.

Rahman *et al* (2016) compared the capsular thickness, trabecular thickness, lymphoid follicle diameter and central arteriolar diameter in human, goat, buffalo, rabbit and rat. Capsule of spleen of rat was too thin to be measured. Rabbit got the thinnest capsule of 33.33  $\mu\text{m}$  thickness and measurements of capsular thickness in goat and buffalo are nearly same i.e., 146.25  $\mu\text{m}$  and 145.50  $\mu\text{m}$ , respectively. Value

of human capsular thickness (108.00  $\mu\text{m}$ ) was less than those of goat and buffalo and more than that of rabbit. Trabeculae of spleen were found to be thickest (109.50  $\mu\text{m}$ ) in buffalo and thinnest (30.00  $\mu\text{m}$ ) in rabbit. In human and Goat these were nearly of same thickness i.e., 75.00  $\mu\text{m}$  and 76.36  $\mu\text{m}$ , respectively. Diameters of lymphoid follicles in buffalo and goat were close to each other i.e. 456.00  $\mu\text{m}$  and 503.18  $\mu\text{m}$ , respectively. Human, rabbit and rat have diameters near to each other i.e., 320.63 $\mu\text{m}$ , 324.64 $\mu\text{m}$  and 295.00  $\mu\text{m}$ , respectively.

Ramesh *et al* (2016) observed the histochemical changes in the thymus, spleen and mesenteric lymph nodes in prenatal and postnatal age groups of mice, rat and guinea pigs. In spleen, the capsule, trabeculae and vascular network were PAS positive. Reticular cells and macrophages showed and Acid phosphatase reaction. In mesenteric lymph nodes, the macrophages were positive for PAS and Acid phosphatase activity in all the age groups studied.

Basir (2017) reported that trabeculae extended from the capsule and branched and divided the spleen into many parts in Iranian camel. The white pulp area was large, irregular shape, and the periarticular lymphocyte sheet, lymph follicles and the marginal zone were very clear. The average thickness of splenic capsule, splenic trabeculae, white pulp and lymphoid follicles was (131.43 $\pm$ 6.24 $\mu\text{m}$ ), (113.5 $\pm$ 6.33  $\mu\text{m}$ ), (691.33 $\pm$ 5.21  $\mu\text{m}$ ), (245.19 $\pm$ 3.82  $\mu\text{m}$ ), respectively.

Gupta *et al* (2017) recorded the development of goat spleen at various stages of gestation. The primordium of spleen was observed on 46th day of gestation in close proximity to the developing rumen. All the parameters viz. length, width, thickness and weight increased with advancement of age. Differentiating capsule and trabeculae were noticed on 69th day and 102th days of gestation, respectively. Reticular, collagen and elastic fibers were noticed at 60, 55 and 124 days of gestation, respectively. Red and white pulp was not fully differentiated till term.

Muslih and Mirhish (2017) reported that the capsule of spleen in gazelle was composed from of two layers, the outer one made of mainly of dense connective tissue; whereas the inner layer of capsule consisted of connective tissue fibers in addition to abundance of smooth muscle fibers interweaving among them. The splenic parenchyma consisted of white pulp represented by lymphoid follicles with their

marginal zones and periarterial lymphatic sheath while the red pulp was represented by splenic cords and sinusoids. Red pulp was composed mainly of connective tissue cords and small sinuses or sinusoids filled with blood cellular element and lined by flattened endothelial cells.

Suri *et al* (2017) reported that spleen was covered by thick capsule with thickness  $282.27 \pm 14.88 \mu\text{m}$  in goat and  $150.13 \pm 8.14 \mu\text{m}$  in sheep. The thickness of trabeculae in goat was  $224.67 \pm 67 \mu\text{m}$  and in sheep was  $104.35 \pm 8.92 \mu\text{m}$ . Average diameter of white pulp was  $478.20 \pm 26.88 \mu\text{m}$  in sheep and  $412.22 \pm 47.85 \mu\text{m}$  in goat. Red pulp consisted of splenic cords and sinusoids. Sinusoids were lined by endothelial cells with large nuclei bulging into the sinusoidal lumen.

Turner and Mabbott (2017) observed structural changes in spleen and lymph node of aged mice and human. These structural changes affected the functioning of the immune cells within which ultimate resulted in less effective or decreased immune response. The elderly have a decreased response to vaccination and an increased susceptibility to infectious diseases. In aged mice and humans structural changes occurred in both the spleen and lymph nodes. These structural changes affect the functioning of the immune cells within, which may ultimate result in less effective or decreased immune responses

Waghaye *et al* (2017) conducted study on age related histomorphological changes of splenic connective tissue in goat. They observed that the spleen of goat was composed of capsule, trabeculae, red pulp and white pulp. The capsule showed two types of layers outer fibrous and inner muscular layer. The inner smooth muscular layer of splenic capsule was very thin in early gestation period which later on increased in thickness with advancement of age and became two layered during late prenatal period. The inner muscular layer of the capsule had collagen, elastic and reticular fibers interposed between the smooth muscle cells. The amount of collagen and elastic fibers increased and reticular fibers decreased with the advancement of age. The connective tissue trabeculae radiated from capsule into the splenic pulp and supported the blood vessels. The trabeculae were few in early prenatal age, but their amount and size increased and branching was observed advancement of age. They observed that the smooth muscle cells were the major component of splenic

trabeculae. The vessels entered in the splenic pulp and were supported by the trabeculae in all age groups. The largest vessels were observed near the hillus.

Nolte *et al* (2018) reported that the splenic marginal zone was unique compartment that separates the lymphoid white pulp from the surrounding red pulp. Macrophages and B cells played an important role in uptake of blood-borne antigens and it gave the spleen its specialized function in antibacterial immunity. They demonstrated that both development and maintenance of this marginal zone was highly dependent on the presence of B cells. They also showed that the integrity and function of an established marginal zone was also dependent on the presence of B cells.

Piao *et al* (2018) studied the morphology, growth, histological structure and development of the spleen of the Chinese yellow quail at different ages. The yellow Quail spleen was spherical, brownish red. The length and width of the spleen gradually increased with age, reaching a maximum for each of these measurements at the age of 6 weeks and then remained unchanged between 10 and 26 weeks of age. There was no significant difference at 30 weeks of age compared to 34 to 38 weeks ( $P>0.05$ ). The average area and diameter of the splenic nodules, the white pulp ellipsoid and the periarterial lymphatic sheath gradually increased, reaching maximum sizes at the age of 26 weeks. It was seen that a sustained developmental period occurred at 0-6 weeks of age and a maturation period occurred at 10-38 weeks during the development of quail spleen.

Shringi *et al* (2018) reported that the capsule was composed of smooth muscle, collagenous and elastic fibers in pig spleen. Trabeculae emerged from the capsule and entered into parenchyma and subdivided it into smaller compartments by forming a net like framework. Splenic parenchyma was composed of white and red pulps. White pulp was lymphoreticular tissue and consisted of lymphatic nodule and peri-arterial lymphatic sheath. Peri-arterial lymphatic sheath and Splenic nodules were abundant in pig spleen. Ellipsoids (pericapillary macrophage sheath) were especially large and abundant in the marginal zone, the region between the red and white pulp. Red pulp consisted of pulp arterioles, sheathed capillaries, terminal capillaries, splenic sinusoids and splenic cords. The splenic sinusoids were less abundant and poorly developed.

Gnanadevi *et al* (2019) reported that thickness of capsule in sheep was  $150\pm 10.33\mu\text{m}$  and in goat it was  $141\pm 11.5\mu\text{m}$ . Trabeculae originated from inner side of the capsule and extended in to parenchyma in both the species. Parenchyma was made up of white pulp and red pulp and the proportion of these were almost equal. The white pulp was composed of splenic nodule and peri-arterial lymphatic sheath. Red pulp was composed of irregular splenic cords separated by splenic sinusoids. Immunohistochemical localization of T-lymphocytes revealed the distribution of T-lymphocytes around the PALS and also scattered in the red pulp.

### **2.3 Histochemistry**

Geetha and Vijayaragavan (1999) in rat, mice and guinea pig reported periodic acid schiff (PAS) positive reaction in capsule, trabeculae and vascular network.

Venkatesan and Vijayaragavan (1999) in Japanese quail spleen reported PAS positive activity in capsule, reticular network, macrophages and vascular structure.

Venkatesan *et al* (2004) in Japanese quail reported that capsule of spleen showed strong PAS positive reaction. They stated that, strong PAS positive reaction in splenic capsule might be due to predominance of collagen fibers. They also observed PAS positive reaction in reticular network, macrophages and erythrocytes of the red pulp.

Gautam and Mishra (2015) conducted the histochemical and ultrastructural study of spleen in prenatal stages of goat and observed strong PAS reaction in capsule, trabeculae, trabecular arteriole and nodular arteriole and in RBCs of the red pulp.

Geetha *et al* (2016) studied the age related changes in mice, rat and guinea pig. They stated that capsule and trabeculae were rich in glycogen in all species. The reticular cells, macrophages and erythrocytes in mice of all age groups were PAS positive, whereas in rat and guinea pig, the splenic erythrocytes didn't showed the PAS reaction.

### **2.4 Enzyme histochemistry**

Wilson *et al* (1972) recorded the alteration for alkaline phosphatases and their pattern of splenic activity in mice. They observed that the alkaline phosphatase enzyme was found higher in lymphaceous condition of spleen.

Anagnostou *et al* (2007) observed non specific esterases (NSE) positive activity in foetal spleen whereas weak reactivity was seen in adult human spleen.

Kaur *et al* (2018) reported the histoenzymic distributions of phosphatases and oxidoreductases in hemal nodes of buffalo. They observed the activity of different enzymes in capsule and lymphoid follicle and showed that variable activity of different enzymes was related with maturation of lymphocytes and different metabolic pathways.

Ramesh *et al* (2016) observed the histochemical changes in thymus, spleen and mesenteric lymph nodes in pre natal and post age groups of mice, rat and guinea pig. They observed that alkaline phosphatases activity was absent in these species whereas reticular cells and macrophages showed activity for Acid phosphatases.

## **2.5 Scanning and Transmission Electron Microscopy**

Hataba *et al* (1981) observed three-dimensional fine architecture of the red pulp, especially the relationships between the splenic sinuses and the cordal capillaries in mouse spleens. They reported that in comparison with other mammals such as human, dog and rat, stomata were rather few and distributed irregularly. It was observed that a large number of cells, most likely macrophages, were present fixed to the network of the cordal reticulum. These observations strongly supported the concept of "open blood circulation" in the red pulp of the mouse spleen.

The vascular and stromal arrangements of the red pulp in congested and contracted dog spleens were studied by transmission electron microscopy by Blue and Weiss (1981). They observed that the proximal portion of each arterial capillary was surrounded by a "periarterial macrophage sheath (PAMS) and these PAMS were composed of a fine meshwork of reticular cells and reticular fibers which held tightly-packed macrophages and interspersed blood cells. The reticular meshwork was formed by reticular cells which appeared to be specialized for contraction. Venous sinuses formed an anastomosing system of vessels draining into pulp veins which then joined trabecular veins. The reticular meshwork may contract to assist trabecular and capsular smooth muscle in expelling stored red cells and effecting hemoconcentration.

Kimura *et al* (2001) observed the fine structures of contractile trabecules in the splenic red pulp by electron microscopy to elucidate their participation in the active contraction of the spleen in rat. The fine thread trabecules were developed in the red pulp and enveloped with a cytoplasmic layer of reticular cells and consisted of elongated smooth muscle cells, fascicles of collagenous fibrils and elastic fibers. Close associations were seen between the smooth muscle cells and elastic fibers which terminated to the fascicles of collagenous fibers. Cell-to-cell connections were expressed by fibrous connections between spiny processes and a small number of punctate intermediate junctions and nexuses. They reported that in the rat spleen, the fine trabecules in the red pulp were muscular contractiles which were responsible for the active contraction due to sympathetic stimuli and the administration of  $\alpha$ 1-adrenoceptor agonists, while the elastic lamina in the capsule plays a role in the comprehensive contraction of the subcapsular vascular bed.+

Xue *et al* (2001) observed erythrocyte shapes in splenic cords of living mouse spleens, using scanning (SEM) and transmission (TEM) electron microscopy. Erythrocytes in living mouse spleens showed a variety of shapes with narrow spaces between them, trapped among reticular fiber tissues. They reported that erythrocytes of various shapes were present in the red pulp even after blocking normal blood circulation and some erythrocytes changed to biconcave discoid shapes. They also showed wide spaces between each other among reticular fiber tissues and various shapes of erythrocytes in the functioning spleens were demonstrated.

Polak *et al* (2009) observed that the Billroth's (splenic) cords were formed from three-dimensional network of fibroblastic reticular cells located among branched sinuses in human. Arterial terminals were observed in the Billroth's cords. The wall of sinuses was a sieve like and lined with a special type of endothelium. In electron microscope, endothelial cells appeared like rods oriented parallel to the longitudinal axis of sinuses. The open and the closed blood circulation were present in the human spleen simultaneously. Blood flowing in the closed circulation helped "absorption" of extra-vascular liquid and the blood elements into the vascular lumen.

Song *et al* (2012) described the anatomical, light microscopic, and ultrastructural characterization of the immune organs in ostrich. There were several

round or elliptical structures, similar to ellipsoids, in the parenchyma of the spleen. Homogeneous acidophilic material was found between the ellipsoid and the lymph tissue, and intermittent transverse striations were detected by transmission electron microscopy. They reported that the immune organs of ostrich chicks, especially the spleen and cloacal bursa, have numerous distinct, conformational, and structural features. The acidophilic material between the ellipsoid analog and circum-lymph tissue of the spleen, was identified as collagen fiber which has not been reported in other animals.

Kannan and Ramesh (2013) reported light and electron microscopic studies of blood-spleen barrier in various age groups of chicken from day-old to forty weeks. They described spleen as the largest secondary lymphoid organ which performed filtration of blood and also the primary site for immunocyte proliferation and differentiation. The results showed that the parenchyma was made up of white and red pulp. The white pulp appeared as islands enclosed by red pulp and there was no distinct marginal zone between the red and white pulp and trabeculae were not present in all ages. The arterioles from the periphery of the white pulp to entered the red pulp as sheathed capillaries and were surrounded by reticular cells and macrophages which formed the ellipsoids or sheathed capillaries. Under transmission electron microscope, the ellipsoids were found to have a meshwork of stellate reticular cells, reticular fibres and a few macrophages.

Kumaravel *et al* (2019) reported the epithelial reticular cells, T lymphocytes, B lymphocytes, plasma cells, dendritic cells and interdigitating cells by both deparaffinised and conventional method for SEM in goat.

## **CHAPTER III**

### **MATERIALS AND METHODS**

#### **3.1 Tissue collection**

The present study was conducted on the spleen of total 12 pigs (n=12). All the samples used in the present study were collected from local slaughter house.

#### **3.2 Gross Anatomical Studies**

The gross anatomical features of pig spleen were recorded which included length, weight and volume of spleen and measurement was taken in cm. The weight of the spleen was measured in grams.

#### **3.3 Fixations and Processing of Tissue**

The tissue sample from pig spleen were fixed in 10% neutral buffered formalin immediately after collection. After achieving the complete fixation, the tissue samples were processed for paraffin block preparation by acetone-benzene schedule (Luna 1968). The paraffin blocks were prepared and sections of 4-5  $\mu\text{m}$  were obtained on clean glass slides with the help of rotary microtome. These paraffin sections were stained with various stains to study the histomorphological and histochemical details (Table 2).

#### **3.4 Cryostat sectioning**

In addition to paraffin sections, fresh unfixed postnatal pig spleen samples were collected and stored in liquid nitrogen. The cryostat section of 10-12  $\mu\text{m}$  were obtained on clean glass slide at  $-20^{\circ}\text{C}$ . The cryostat sections were utilized for demonstration of various enzymes (Table 3). The intensity of staining provided a qualitative indication of the amount of enzyme activity. In addition control sections were also incubated in media devoid of substrate to check the specificity of the enzyme activity. The cryostat sections were also utilized for demonstration of lipids and phospholipids (Table 2).

#### **3.5 Micrometry**

The micrometrical observations were recorded on hematoxylin and eosin stained sections with the help of image J software. The micrometrical observation

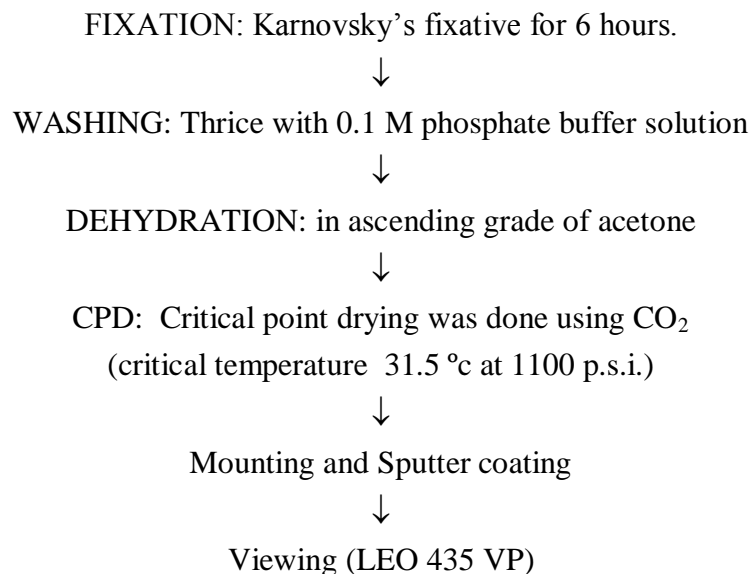
included the thickness of the capsule, trabeculae, diameter of lymphoid follicle and central artery of spleen.

### 3.6 Scanning electron microscopy

Scanning electron microscopic studies were performed as procedure described earlier by Pathak and Bansal (2008). The tissue samples were collected and washed in chilled 0.1M phosphate buffer (pH 7.2) and were fixed in Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer solution at pH 7.2) for 4-6 hours. Fixed samples were washed in 0.1M phosphate buffer with 3 changes of 15 minutes each at 4°C. Thereafter, the samples were dehydrated in ascending grade of acetone solutions i.e. 30%, 50%, 70%, 80%, 90%, 95% and 100% acetone (dry acetone) at 4°C.

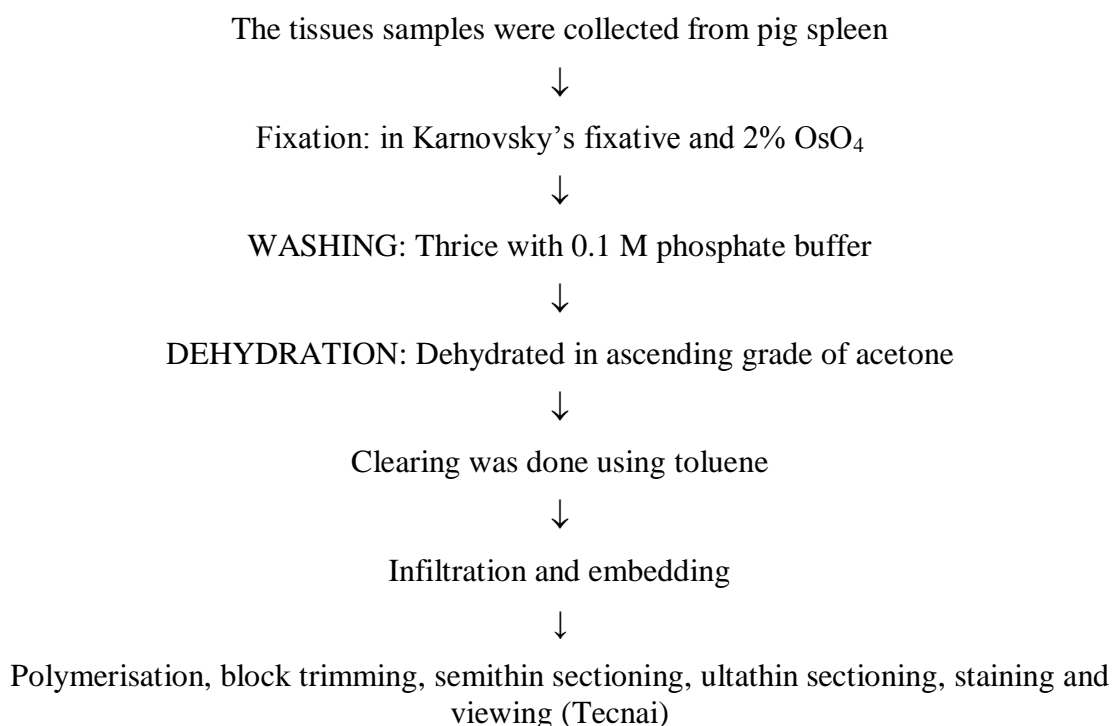
The dehydrated specimens were then placed in the small stainless steel mesh baskets, which were placed in boat of critical point drying apparatus containing acetone and critical point drying was done at critical temperature (31.5°C) at 1100 p.s.i. There after the tissues were placed in desiccators, mounted on aluminum stubs, sputters coated with 35nm thick layer of gold and were viewed under scanning electron microscope.

The tissue samples were collected from pig spleen



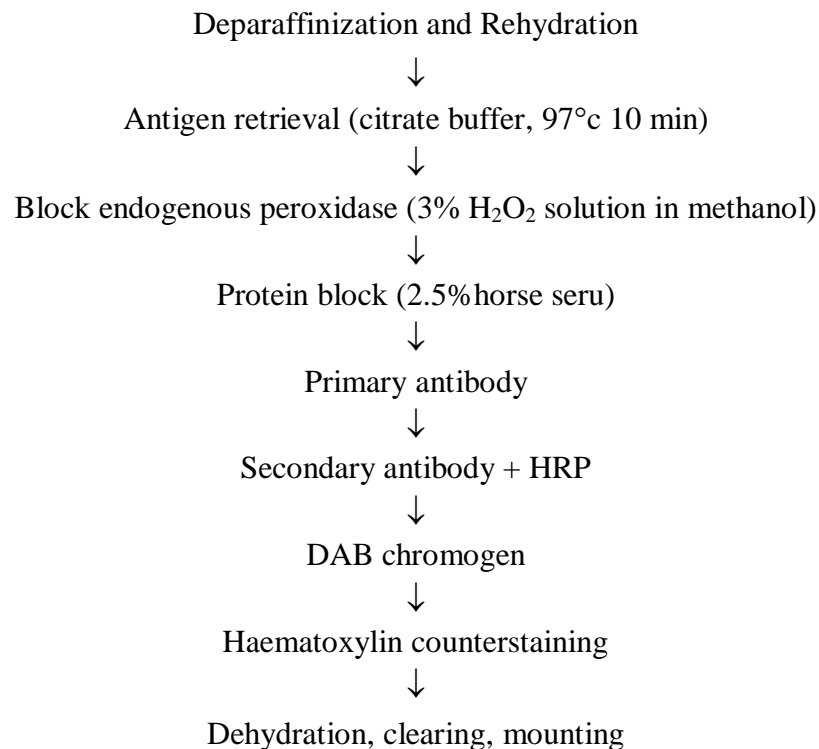
### 3.7 Transmission electron microscopy

Transmission electron microscopic studies were performed as procedure described earlier by Pathak *et al* (2011). The tissues from spleen of pigs were thoroughly washed in phosphate buffer saline (pH 7.4) solution and subsequently trimmed of 1 mm<sup>3</sup> size. These samples were fixed for 2 hours in Karnovsky's fixative and then secondary fixation was done for 2 hours in 2% OsO<sub>4</sub>. Subsequently tissue samples were subjected to dehydration in ascending grades of acetone (30% to absolute). The dehydration in dry acetone was done at room temperature. The clearing of the samples was accomplished by treatment with toluene. Subsequently infiltration was carried out. The tissues were then embedded in pure embedding media using beam capsule. Subsequently, polymerization was undertaken. The blocks thus prepared were trimmed by block trimmer (Reichert TM 60) and semi thin sections (.5-2.0 µm) were cut and stained with IB to scan the tissues under optical microscope for selection of area for ultrathin sectioning. After scanning, the ultrathin sections (70-90 nm) were cut and lifted on copper grids (100 mesh size) and stabilized by coating with carbon film of 50Å thickness. The grids were then stained with uranyl acetate (15 min) followed by lead citrate (10 min). The grids thus prepared were examined under TEM (Tecnai) for detailed study and required photographs were taken.



### 3.8 Immunohistochemistry

Polymer-based Horseradish peroxidase method was used for immunostaining as described by Pathak *et al* (2019). The sections in duplicate were mounted on super frost positively charged slides (Fisher Scientific). After dewaxing and rehydration, heat-induced antigen retrieval was done in citrate buffer (AR 3 solution, Biogenex) and heating in the microwave at 95°C for 10 minutes and 98°C for 5 minutes. Slides were then left for 30 min in the hot buffer and washed in 0.1M phosphate buffered saline (at pH 7.4). The endogenous peroxidase activity was blocked by immersing the sections in 3% (v/v) H<sub>2</sub>O<sub>2</sub> in methanol for 20 min followed by washing in 0.1M phosphate buffered saline (at pH 7.4). To prevent non-specific binding of antibodies, the sections were blocked with normal horse serum (Vector's Laboratories USA). The sections were incubated with primary antibodies (Anti-CD 20 (B cell), Anti-T Cell and Anti-macrophage) by Biogenex (laboratory) at 4°C for overnight incubation chamber. After washing in 0.1M phosphate buffered saline (at pH 7.4), the sections were incubated with universal secondary antibody (Vector Laboratories, USA). The chromogen used was 3, 3'-diaminobenzidine tetrahydrochloride (DAB) (Vector Laboratories, USA) with Gill's III hematoxylin counterstaining. The sections were washed in running tap water, dehydrated, cleared and mounted with DPX.



**Table 1: Histomorphological stains used on paraffin sections of spleen of pig**

<b>Stain</b>	<b>Reference</b>	<b>Purpose</b>
<b>1. Hematoxylin and Eosin</b>	Luna (1968)	Routine morphology
<b>2. Masson's trichrome</b>	Luna (1968)	Collagen fibers
<b>3. Gridley's</b>	Sheehan and Hrapchak (1973)	Reticular fibers
<b>4. Verhoeff's</b>	Sheehan and Hrapchak (1973)	Elastic fibers
<b>5. Holme's</b>	Luna (1968)	Neuronal elements

**Table 2: Histochemical stains used on paraffin/cryostat sections of spleen of pig**

<b>Stain</b>	<b>Reference</b>	<b>Histochemical moiety</b>
<b>1. Periodic Acid Schiff</b>	Sheehan and Hrapchak (1973)	Neutral mucopolysaccharides
<b>2. Alcian Blue (at pH 1.0 &amp; 2.5)</b>	Luna (1968)	Acid mucopolysaccharides
<b>3. Bromphenol Blue</b>	Chayen <i>et al</i> (1969)	Basic proteins
<b>4. Sudan Black B</b>	Chayen <i>et al</i> (1969)	Lipids*
<b>5. Acid Haematin</b>	Chayen <i>et al</i> (1969)	Phospholipids*
<b>6. Perl's PrussianBlue</b>	Sheehan and Hrapchak (1973)	Hemosidrin

\*Lipids and Phoshplipids were demonstrated on fresh cryostat sections.

**Table 3: Histoenzymic methods used on cryostat sections of pig spleen**

S. No.	Enzyme	Substrate	Method	Reference	Incubation time
<b>A. Phosphatases</b>					
i.	Alkaline phosphatase (AKPase)	Naphthol AS-MX phosphate disodium salt in combination with Fast Blue RR	Simultaneous coupling azo dye method using substituted naphthols	Barka and Anderson (1963)	30 minutes
ii.	Adenosine Triphosphatase (ATPase)	Naphthol ASTR phosphate with paraosanilin	Simultaneous coupling azo dye method using substituted naphthols	Barka and Anderson (1963)	60 minutes
iii.	Glucose 6- Phosphatase (G-6-Pase)	Glucose-6-phosphate and lead nitrate	Lead nitrate method	Barka and Anderson (1963)	20 minutes
<b>B. Oxidoreductases and Esterases</b>					
i)	Alcoholic dehydrogenase (ALC)	Ethanol	Nitro BT method	Pearse (1972)	15 minutes
ii)	Glucose-6 phosphate dehydrogenase (G-6-PD)	Di-Na glucose-6-phosphate	-do-	-do-	30 minutes
iii)	Glutamic dehydrogenase (GLD)	Na-L glutamate	-do-	-do-	30 minutes
iv)	Lactic dehydrogenase (LDH)	Na-DL lactate	-do-	-do-	30 minutes
v)	Malic dehydrogenase (MDH)	L-Malic acid	-do-	-do-	30 minutes
vi)	Succinic dehydrogenase (SDH)	Di-Na succinate	-do-	-do-	15 minutes
vii)	Nicotinamide adenine dinucleotide phosphate diphorase (NADPH-diphorase)	Co-enzyme (NADPH)	-do-	-do-	30 minutes
viii)	Reduced Nicotinamide adenine dinucleotide diphorase (NADH-diphorase)	Co-enzyme (NADH)	-do-	-do-	30 minutes
ix)	Non specific Esterases	Alpha naphthol acetate	Naphthol acetate method	Barka and Anderson (1963)	10 min

## **CHAPTER IV**

### **RESULTS AND DISCUSSION**

The present study was undertaken to elucidate gross, histomorphological, histochemical and histoenzymological changes in the spleen of pig.

#### **4.1 GROSS MORPHOLOGY**

##### **4.1.1 Shape and colour of spleen**

The spleen of pig in the present study was elongated tongue like structure and reddish-brown in colour (Plate 1a). Similar observations were made by Shringi *et al* (2017) in spleen of pig was tongue like triangular in structure. Khan *et al* (2006) observed that the spleen was reddish brown in colour and compact oval in shape in musk deer and goat whereas it was rectangular in goat and triangular in sheep. Khalel (2010) observed that the spleen of the Iraqi sheep was triangular in shape with rounded angles. The spleen of camel was 'C' shaped and the colour of spleen was dark brown as observed by Maina *et al* (2014). Suri *et al* (2017) reported triangular spleen in sheep and roughly quadrangular in goat. The colour of spleen in goat was reddish grey and it was reddish in sheep. Similar observations were recorded by Sivagnanam *et al* (2017) in goat. Gnanadevi *et al* (2019) also reported triangular spleen in sheep and roughly quadrangular in goat.

##### **4.1.2 Surfaces**

The spleen of pig in the present study consisted of two surfaces i.e. parietal surface and visceral surface (Plate 1a and 1b). The parietal surface of the spleen was convex and the visceral surface was concave in pig. Similar observation is made by Shringi *et al* (2017) in pig. Parietal surface was dome shaped, convex and visceral surface was deeper and concave in musk deer and goat (Khan *et al* 2006). Khalel (2010) also reported convex parietal surface and concave visceral surface in Iraqi sheep. Devi *et al* (2012) reported that the visceral surface was concave and its anterior half was firmly attached to dorsal curvature of rumen and parietal surface was convex and adherent to peritoneum in goat. Suri *et al* (2017) also observed that parietal surface was convex and visceral surface was concave in case of sheep and goat. Similar observations were made by Gnanadevi *et al* (2019) in sheep and goat.

### **4.1.3 Hilus**

The hilus in present study was located at the upper middle of visceral surface. Shringi *et al* (2017) described that hilus was present as longitudinal ridge on the visceral surface at the upper part of spleen. Gnanadevi *et al* (2019) observed that the hilus was present at the dorsal end of the cranial border of spleen in case of sheep and goat. Similar observations were made by Khan *et al* (2006) in musk deer and goat.

### **4.1.2 Biometry of spleen**

The biometrical observations of different parameters of healthy spleen of 12 pigs were recorded.

#### **4.1.2.1 Weight of spleen (g)**

The weight of the spleen in the present study varied between 49.28 - 131.48 gm with mean weight of  $86.83 \pm 7.47$  g. Shringi *et al* (2017) reported the average weight of pig spleen to be  $128.55 \pm 4.76$  g. Khalel (2010) reported the mean weight of spleen in Iraqi sheep was  $69 \pm 6.66$  g. Maina *et al* (2014) observed that the mean weight of one-humped camel is found to be  $425 \pm 0.04$  g. Suri *et al* (2017) reported that the weight of spleen in case of sheep was  $81.39 \pm 12.7$  g and in case of goat it was  $64.48 \pm 7.82$  g. Svignanam *et al* (2017) observed that mean weight of spleen in goat was  $135.83 \pm 7.60$  g above 5 years of age. The average weight was 50-55 g in musk deer and 65-70 g in goat, reported by Khan *et al* (2006).

#### **4.1.2.2 Length of spleen (cm)**

The length of spleen in pig in the present study varied between 26 to 32.5 cm with mean length of  $29.88 \pm 0.61$  cm. Shringi *et al* (2017) recorded that mean length in case of pig spleen varied between 23.1 to 36 cm with mean of 29.15 cm which is in close conformity with present findings. The length of spleen in musk deer was 9.5 to 10.5 cm and in goat it was 9.4 to 12.4 cm as reported by Khan *et al* (2006). Khalel (2010) reported that the mean length of spleen in case of Iraqi sheep was found to be  $9.93 \pm 0.34$  cm. The mean length of spleen in case of one-humped camel was  $35.5 \pm 0.71$  cm, (Maina *et al* 2014). Suri *et al* (2017) reported that, the mean length of spleen in sheep was  $12.70 \pm 0.81$  cm and in case of goat it was  $11.48 \pm 0.73$  cm. Svignanam

*et al* (2017) reported that mean length of spleen in goats above 5 years of age was found to be  $14.45 \pm 0.48$  cm.

#### **4.1.2.3 Width of spleen (cm)**

The maximum width of spleen varied from 2.5 to 5.5 cm in the present study in pig with average width of  $4.11 \pm 0.24$  cm. Shringi *et al* (2017) observed that the average maximum width of pig spleen was  $6.25 \pm 0.23$  cm. Khalel (2010) reported that the average width of spleen was  $6.48 \pm 0.23$ cm in Iraqi sheep and in case of one-humped camel it was  $8.15 \pm 0.49$  cm, (Maina *et al* 2014). Suri *et al* (2017) reported that the mean width of spleen in sheep was  $9.26 \pm 0.38$  cm and in goat it was  $9.37 \pm 0.79$  cm. Svignanam *et al* (2017) reported that the mean width of spleen in goat above 5 years of age to be  $13.43 \pm 0.37$  cm.

#### **4.1.2.4 Volume of spleen (ml)**

The volume of spleen in present study ranged between 45 to 100 ml with mean volume of  $72.66 \pm 5.59$  ml. Shringi *et al* (2017) observed that the volume of spleen in pig varied from 119.8 ml to 150 ml with average volume of 131.87 ml. The average volume of spleen in case of goat above 5 years of age was  $75.83 \pm 1.66$  ml, as observed by Svignanam *et al* (2017).

#### **4.1.3 Micrometry of spleen**

In the present study, the micrometrical observations of different parameters were recorded on spleen of 12 healthy pigs.

##### **4.1.3.1 Thickness of splenic capsule ( $\mu\text{m}$ )**

The thickness of the splenic capsule in pig in the present study ranged from 53.55 to 371.19  $\mu\text{m}$  with mean splenic capsule thickness of  $151.14 \pm 8.82$   $\mu\text{m}$ . Usende *et al* (2014) compared the thickness of splenic capsule in Nigerian indigenous pig and goat and reported that it was  $1173 \pm 57.2$   $\mu\text{m}$  in goat and  $271.8 \pm 17.08$   $\mu\text{m}$  in pig. Alshamarry (2010) reported that the thickness of splenic capsule in case of Iraqi camel was  $147.43 \pm 3.64$   $\mu\text{m}$  and it was  $140.5 \pm 13.712$   $\mu\text{m}$  in Iraqi sheep (Khalel 2010). Alim *et al* (2012) compared thickness of splenic capsule in different species viz; human, cow and goat. It was  $111.56 \pm 21.45$   $\mu\text{m}$ ,  $196.88 \pm 11.91$   $\mu\text{m}$  and  $251.44$

$\pm 12.56 \mu\text{m}$  in human, cow and goat, respectively. Maina *et al* (2014) observed that the splenic capsule was  $171.37 \pm 7.72 \mu\text{m}$  thick in one-humped camel. According to Usende *et al* (2014) the thickness of splenic capsule in goat was  $1173 \pm 57.2 \mu\text{m}$  and in Nigerian indigenous pig it was  $271.8 \pm 17.08 \mu\text{m}$ . Suri *et al* (2017) compared the splenic capsule thickness of spleen in sheep and goat reported that it was  $150.13 \pm 8.14 \mu\text{m}$  in sheep and  $282.27 \pm 14.88 \mu\text{m}$  in goat. Rahman *et al* (2017) compared the thickness of splenic capsule in human, goat, buffalo and rabbit and were 108.88, 146.25, 145.50 and  $33.33 \mu\text{m}$ , respectively. Gnanadevi *et al* (2019) compared thickness of splenic capsule in sheep and goat and recorded thickness of  $150 \pm 10.33 \mu\text{m}$  in sheep and  $141 \pm 11.5 \mu\text{m}$  in goat.

#### **4.1.3.2 Thickness of trabeculae ( $\mu\text{m}$ )**

The average thickness of splenic trabeculae in present study varied from 28.273 to  $310.042 \mu\text{m}$  with mean thickness of  $144.71 \pm 9.38 \mu\text{m}$  in the present study. The thickness of trabeculae in Nigerian indigenous pig and goat was compared by Usende *et al* (2014) which were found to be  $71.8 \pm 17.18 \mu\text{m}$  in indigenous pig and  $52.17 \pm 3.07 \mu\text{m}$  in goat. Alshamarry (2010) measured the splenic trabeculae thickness in Iraqi camel which was  $106.5 \pm 6.29 \mu\text{m}$ . It was  $82.75 \pm 9.7 \mu\text{m}$  in Iraqi sheep, (Khalel 2010). Maina *et al* (2014) stated that the trabeculae were  $148.43 \pm 3.98 \mu\text{m}$  thick in one-humped camel. Rahman *et al* (2017) compared the thickness of trabeculae in different species like human, goat, buffalo and rabbit which were found to be 75.00, 76.36, 109.50 and  $30.00 \mu\text{m}$ , respectively. Suri *et al* (2017) compared the thickness of trabeculae in spleen of sheep and goat and it was,  $104.35 \pm 8.92 \mu\text{m}$  in sheep and  $224.67 \pm 67 \mu\text{m}$  in goat. Gnanadevi *et al* (2019) compared the trabeculae thickness in sheep and goat and reported that it was  $134 \pm 12.06 \mu\text{m}$  in sheep and  $105 \pm 9.79 \mu\text{m}$  in goat.

#### **4.1.3.3 Diameter of lymphoid follicle ( $\mu\text{m}$ )**

The diameter of lymphoid follicle in the present study in spleen of pig varied from 172.034 to  $611.827 \mu\text{m}$  with mean diameter of  $395.68 \pm 15.97 \mu\text{m}$ . Alshamarry (2010) recorded the diameter of lymphoid follicle in Iraqi camel which was  $279.19 \pm 4.85 \mu\text{m}$ . Khalel (2010) recorded the diameter of lymphoid follicle in Iraqi sheep and it was found as  $315.25 \pm 23.185 \mu\text{m}$ . The diameter of lymphoid follicle in different

species was compared by Rahman *et al* (2017). It was 320.63  $\mu\text{m}$  in human, 503.18  $\mu\text{m}$  in goat, 456.10  $\mu\text{m}$  in buffalo and 324.64  $\mu\text{m}$  in rabbit.

#### **4.1.3.4 Diameter of central artery ( $\mu\text{m}$ )**

The central artery diameter in present study in pig ranged from 23.499 to 84.55  $\mu\text{m}$  with mean diameter of  $46.12 \pm 3.48 \mu\text{m}$ . Usende *et al* (2014) compared the mean diameter of central artery in Nigerian indigenous pig and goat which were  $35.10 \pm 2.002 \mu\text{m}$  and  $45.91 \pm 1.49 \mu\text{m}$  which is in conformity with present finding. Rahman *et al* (2017) compared the mean diameter of central artery in human 48.33  $\mu\text{m}$ , 48.75  $\mu\text{m}$  in goat, 55.71  $\mu\text{m}$  in buffalo and 35  $\mu\text{m}$  in rabbit.

## **4.2 HISTOMORPHOLOGY**

The structure of spleen of pig consisted of capsule, trabeculae, white pulp and red pulp.

### **4.2.1 Capsule and trabeculae**

In the present study, pig spleen was enclosed by connective tissue capsule (Plate 2a). Capsule was composed of collagen, elastic and reticular fibers along with smooth muscle fibers (Plate 2b, 2c, 3a and 3b). The capsule was made up of two layers, outer fibrous connective tissue layer and inner muscular layer of smooth muscle fibers (Plate 2b). Adipose tissue was also observed in capsule (Plate 2d). Shringi *et al* (2018) in Large White Yorkshire pig, also reported that splenic capsule was composed of collagen, elastic and reticular fibers along with smooth muscle fibers. Similar observations were made by Raju *et al* (2003) and Waghaye *et al* (2017) in goat. Maina *et al* (2014) reported that the Nigerian camel spleen was surrounded by connective tissue capsule which was divided into outer layer of connective tissue with few smooth muscles whereas the inner layer was composed mainly of smooth muscles. Alshamarry (2010) made similar observations in Iraqi camel.

The collagen fibers were found abundantly in fibrous layer and elastic fibers were more in between inner muscular layer of smooth muscle in the present study (Plate 2c and 4c). Similar observations were made by Waghaye *et al* (2017) in goat. The elastic fibers in capsule were arranged parallel to the direction of muscle fibers

which might help in distention and contraction of smooth muscles (Plate 3c and 3d). The collagen fibers in the outer layer provided firmness. Similar observations were made by Das *et al* (2005) in dog, Devi *et al* (2016) in Marwari goat, Geetha *et al* (2001) in mice, rat, and guinea pig. Alshamarry *et al* (2010) in camel, Khalel (2010) in Iraqi sheep and Onkar and Govardhan (2013) in human and dog reported interwoven collagen and elastic fibers with smooth muscle fibers in splenic capsule.

The thickness of capsule was uneven in pig spleen and ranged from 53.548 to 371.196  $\mu\text{m}$ . In agreement with present observations, Usende *et al* (2014) in Nigerian indigenous pig, Alshamarry (2010) in camel reported variation in thickness of splenic capsule at different regions. Waghaye *et al* (2017) in goat reported uneven thickness of splenic capsule.

The branching connective tissue trabeculae extended from the inner muscular layer of capsule into splenic parenchyma and divided it into smaller compartments by forming a net like framework (Plate 4a). These observations were in agreement with Devi *et al* (2016) in Marwari goat. The trabeculae were scattered throughout splenic tissue and were composed predominantly of reticular fibers and smooth muscle fibers (Plate 4b and 4d). Reticular fibers were arranged parallel to direction of smooth muscle fibers of trabeculae. The collagen fibers were also present in the capsule and trabeculae of the pig spleen in the present study (Plate 4c).

#### **4.2.2 Parenchyma**

The splenic parenchyma in the present study was composed of red pulp and white pulp (Plate 5a). Similar observations were made by Shringi *et al* (2018) in large White Yorkshire pig. Khalel (2010) revealed that the spleen of sheep have typical composition, a parenchymatous consisting of white and red splenic pulp that was enclosed by a fibrous capsule and subdivided by trabecule. Onkar and Goverdhan (2013) made similar observations in human and dog spleen. Usende *et al* (2014) reported that the trabeculae were composed of predominantly smooth muscles that serve as a rigid framework of the pulp.

### 4.2.3 White pulp

White pulp in the present study consisted of lymphocytes, plasma cells, reticular cells and macrophages which were distributed throughout splenic parenchyma (Plate 7a). The white pulp was composed of two components, which were splenic nodules or Malphigian corpuscles or lymphatic nodules and peri-arterial lymphatic sheath (Plate 5b, 6a and 6b). The observations of present study were in line with reports made by Devi *et al* (2016) in Marwari goat and Shringi *et al* (2018) in pig.

The peri-arteriolar lymphatic sheath was mainly made up of lymphocytes with reticular cells, macrophages and plasma cells with central artery placed eccentrically (Plate 5c and 6b). The peri-arteriolar sheath was abundant in pig spleen. The peri-arterial lymphatic sheaths were less abundant in the spleen of goat as reported by Devi *et al* (2016) in Marwari goat.

Splenic nodules in the present study were composed of aggregation of the lymphatic tissue which occurred as aggregations of two or three nodules (Plate 5a and 5b). Similar observations were made in the spleen of one-humped camel by Maina *et al* (2014). The splenic nodules showed germinal center which appeared as lightly stained area with loosely aggregated large lymphocytes and few plasma cells and eccentrically placed central arteries (Plate 5d and 6a). Similar observations were recorded by Khaleel (2010) in Iraqi sheep, Maina *et al* (2014) in camel. They reported the presence of germinal center in splenic nodule. In agreement with present observations, Devi *et al* (2016) in Marwari goat reported that the germinal center of the nodule was light stained area consisted of loosely arranged lymphocytes.

The present study revealed that ellipsoids (pericapillary macrophage sheath) were large and abundant in the marginal zone (Plate 6a, 8c and 8d). The shape of periarterial macrophage sheaths varied from oval to elongated and was composed of macrophages, reticular cells and lymphocytes around the arteries. In accordance with present observations, Press and Landsverk (2006) reported large and abundant pericapillary macrophages sheaths near white pulp in pig spleen. Alshamarry *et al* (2010) in camel and Onkar and Govardhan (2013) in dog observed that marginal zone of spleen contained sheathed artery.

The marginal zone was made up of two or more layers of reticular and collagen fibers in spleen of pig in the study. Similar observations were made by Shringi *et al* (2018) in pig. This marginal zone in the present study separated the red and white pulp (Plate 6c and 6d) as reported by Maina *et al* (2014) in one humped camel and Suri *et al* (2017) in sheep and goat. They reported presence of marginal zone between splenic white pulp and red pulp.

Two or three central arteries were observed in the splenic nodule in present study (Plate 6b), which was in agreement with the observations of Das *et al* (2005) in dog. The central artery extended out of splenic nodules and branched to form penicillar arteries in red pulp. These were further surrounded by macrophages, reticular cells and lymphocytes to form peri-arteriolar macrophages (Plate 6b).

#### **4.2.4 Lymphoid follicle diameter**

The diameter of lymphoid follicle in the present study in spleen of pig varied from 172.034 to 611.827  $\mu\text{m}$  with mean diameter of  $395.68 \pm 15.97 \mu\text{m}$ . Alshamarry (2010) observed the diameter of lymphoid follicle in Iraqi camel which was  $279.19 \pm 4.85 \mu\text{m}$ . Similar observations were made by Khalel (2010) in Iraqi sheep where diameter of lymphoid follicle was found as  $315.25 \pm 23.185 \mu\text{m}$ . The diameter of lymphoid follicle in different species was recorded by Rahman *et al* (2017). It was 320.63  $\mu\text{m}$  in human, 503.18  $\mu\text{m}$  in goat, 456.10  $\mu\text{m}$  in buffalo and 324.64  $\mu\text{m}$  in rabbit.

#### **4.2.5 Red pulp**

The red pulp occupied space between white pulp and trabeculae (Plate 8a). Red pulp consisted of splenic sinuses, splenic cords and blood vessels (Plate 8b). The splenic cords were composed of reticular cells, lymphocytes, macrophages and plasma cells. Similar observations in accordance with present findings were recorded by Geetha *et al* (2001) in mice, rat and guinea pig and Onkar and Govardhan (2013) in human and dog.

Reticular fibers formed the meshwork in the spleen of pig in accordance with Usende *et al* (2014) as they reported that red pulp cords were consisted of reticular network with megakaryocytes in Nigerian indigenous pig. In the present study, the red

pulp splenic sinuses were observed as irregular anastomosing channels between splenic cords. Within the spaces between the cords were erythrocytes, granulocytes and circulating mononuclear cells. In contrast to present observations, Shringi *et al* (2018) reported less and poorly developed splenic sinuses in spleen of Large white Yorkshire pig. Devi *et al* (2016) reported that the splenic sinusoids were less abundant and poorly developed in Marwari goat.

#### **4.2.6 Erythrophagocytosis**

In the present study, the hemosiderin pigment formed by the degradation process was found in the parenchyma of the spleen especially in red pulp (Plate 9c). Macrophages engulfing the erythrocytes were observed in the sinuses. These ingested erythrocytes undergo lysosomal degradation and lead to formation of hemosiderin pigment. Few bluish granules were observed in the splenic sinus as well as in cytoplasm of macrophages and white pulp of the spleen (Plate 9d). This indicated the active process of degeneration of erythrocytes by phagocytic cells, resulting into formation of hemosiderin pigment. The present study observed large quantities of hemosiderin pigment in both sinuses and cytoplasm of macrophages of pig and transmission electron microscopy images showed numerous macrophages and polymorphonuclear cells with erythrocyte debris in their cytoplasm (Plate 17a and 17d).

The ellipsoids in the present study were surrounded by cells having hemosiderin pigment in the cytoplasm. The presence of large number of macrophages around ellipsoids was confirmed by immunohistochemistry (Plate 9a and 9b).

### **4.3 HISTOCHEMISTRY**

The distribution of neutral and acidic mucopolysaccharides, lipids, phospholipids and proteins was studied in spleen of pig.

#### **4.3.1 Polysaccharides**

The distribution of acidic and neutral mucopolysaccharides was studied in spleen of pig.

##### **4.3.1.1 Acid Mucopolysaccharides**

The capsule covering the spleen showed the presence of acidic mucopolysaccharides. The outer fibrous layer was strongly positive for acidic

mucopolysaccharides and inner muscular layer was weakly positive for acidic mucopolysaccharides (Plate 10b). The trabeculae extending from the capsule also showed weak activity for presence of acidic mucopolysaccharides. The lymphocytes present in the lymphoid follicle of white pulp were devoid of acidic mucopolysaccharides activity (Plate 10c). However, some activity was observed around central artery and in connective tissue framework (Plate 10d). Ellipsoids were weakly positive for acidic mucopolysaccharide. The lymphocytes present in germinal centers were weakly positive for acid mucopolysaccharides indicating presence of immature lymphocytes. The connective tissue fibers showed weak to moderate positivity for the acid mucopolysaccharides.

However, the erythrocytes present in the sinuses were devoid of acid mucopolysaccharides but the periphery of the sinuses showed positive reaction for acid mucopolysaccharides. It may be due to presence of connective tissue framework.

#### **4.3.1.2 Neutral Mucopolysaccharides**

The inner muscular layer of capsule covering the spleen was moderately positive for neutral mucopolysaccharides indicating the presence of neutral mucopolysaccharides however outer fibrous layer was weakly positive (Plate 10a). The trabeculae extending from the capsule towards the parenchyma of spleen also showed moderate reaction for neutral mucopolysaccharides. The lymphoid follicles present in the white pulp of spleen contained strongly positive cells especially lymphocytes for neutral mucopolysaccharides (Plate 10a). The connective tissue framework was strongly positive for neutral mucopolysaccharides in the parenchyma of the spleen. The area around blood vessels were weakly positive for neutral mucopolysaccharides. Gautam and Mishra (2015) in prenatal goat spleen observed strong PAS reaction in capsule, trabeculae, trabecular arteriole and nodular arteriole and in erythrocytes of the red pulp.

#### **4.3.1.3 Lipids and Phospholipids**

Fine sudanophilic lipid droplets were observed inside the capsule and trabeculae of spleen (Plate 11d). These were present as fat lobules surrounding the capsule of the spleen. These lobules suggested that spleen was embedded in the white adipose tissue. The capsule was positive for phospholipids (Plate 11c). The trabeculae

extending from parenchyma showed positive reaction for phospholipids. The presence of phospholipids was also observed around blood vessels.

#### **4.3.4 Proteins**

In the spleen of pig, the bromphenol blue positive activity was observed throughout the spleen. However, the intensity of the activity varied in different structures present in the spleen. The capsule showed moderate to strong activity for proteins (Plate 11b). This might be due to presence of collagen and reticular fibers which contained glycoproteins. The trabeculae extending from the capsule showed moderate to strong activity for proteins. The lymphoid follicles present in pulp region showed moderate activity for proteins (Plate 11a). Erythrocytes present in the sinuses showed intense strong activity for proteins. The connective tissue present in the parenchyma also showed moderate to strong positive activity for proteins. In the parenchyma region, the diffused lymphocytes showed moderate protein content.

### **4.4 ENZYMEHISTOCHEMISTRY**

#### **4.4.1 Phosphatases**

##### **4.4.1.1 Alkaline Phosphatase (AKPase)**

In the present study, alkaline phosphatase activity was constantly weak in the capsule of the spleen. The alkaline phosphatase activity was moderate to strong in the lymphoid follicles of pig (Plate 12a). The activity was more prominent at the periphery of the lymphoid follicles. The activity to this enzyme was weak at germinal centers of the lymphoid follicles and more pronounced at the periphery of the lymphoid follicles in pig. Similar observations were made by Kaur *et al* (2018) in buffalo calves. The presence of AKPase activity indicated its involvement in maintenance of the inter-cellular concentration of phosphate ions and in the process of absorption and transport across membrane (Chayen *et al* 1969).

##### **4.4.1.2 Glucose-6-Phosphatase (G-6-Pase)**

Weak to mild activity for glucose-6-phosphatase was observed in entire spleen (Plate 12b). The capsule and trabeculae showed weak activity for glucose-6-phosphatase. Weak to mild activity was seen in lymphoid follicle of pig spleen. Glucose-6-phosphatase consisted of amino acids, anchored to the endoplasmic

reticulum (ER) and is involved in the release of glucose into the circulation. The capsule of the spleen show positive activity for glucose-6-phosphatase.

#### **4.4.1.3 Adenosine Triphosphatase (ATPase)**

The activity for ATPase in the capsule and trabeculae of pig spleen was weak. The activity for ATPase was moderately to strong in the lymphoid follicle of spleen in pig (Plate 12c).

#### **4.4.2 Oxidoreductases**

##### **4.4.2.1 Dehydrogenase**

###### **4.4.2.1.1 Succinic Dehydrogenase (SDH)**

In the present study in spleen of pig, weak SDH activity was noted in the capsule and trabeculae of the spleen. The parenchyma also showed weak activity for SDH, however moderate activity was observed in the lymphoid follicles (Plate 12d). The blood vessels showed positive activity for SDH. SDH is a mitochondrial enzyme that is involved in generation of energy by oxidation-reduction reaction in the cell (Smith 1969). This enzyme might be associated with oxidation of fatty acids that lead to formation of lipid pigment granules (Smith 1969).

###### **4.4.2.1.2 Lactate Dehydrogenase (LDH)**

A weak to moderate lactate dehydrogenase activity was observed in the capsule and trabeculae in pig spleen. However, the lymphoid follicles showed moderate activity for LDH (Plate 13b). The diffused lymphocytes arranged loosely in the parenchyma showed weak to moderate activity for LDH. The blood vessels also showed moderate activity for lactate dehydrogenase.

LDH is an NAD dependant enzyme found in cells in which glycolytic pathway is active. It catalyses the formation of lactate in anaerobic glycolysis and pyruvate in aerobic respiration. The presence of LDH enzyme in spleen suggested the presence of glycolytic pathway in cellular elements especially in the capsule and lymphoid follicles and their role in differentiation of lymphocytes.

###### **4.4.2.1.3 Malic dehydrogenase (MDH)**

The malic dehydrogenase activity was weak to moderately present in the capsule and trabeculae of pig spleen (Plate 13a). A weak to moderate activity was seen in the blood vessel area and lymphoid follicle. MDH is an enzyme that

reversibly catalyses the oxidation of malate to oxaloacetate using the reduction of  $\text{NAD}^+$  to NADH and is a part of many metabolic pathways.

#### **4.4.2.1.4 Glutamic dehydrogenase (GLD)**

In the present study, glutamic dehydrogenase activity was moderate in capsule and trabeculae of spleen of pig. Moderate reaction was seen in the blood vessels of pig spleen. The lymphoid follicles showed moderate activity for glutamic dehydrogenase (Plate 14d). Glutamic dehydrogenase (GLD) is a mitochondrial enzyme required for urea synthesis that converts glutamate to  $\alpha$  ketoglutarate and vice versa.

#### **4.4.2.1.5 Alcoholic dehydrogenase (ALC)**

Alcoholic dehydrogenase activity was weak to moderate in capsule (Plate 14a). The trabeculae of spleen showed positive activity for alcoholic dehydrogenase. Moderate activity was seen in blood vessels of pig spleen. Weak to moderate activity was present in lymphoid follicles. Alcoholic dehydrogenase (ALC) facilitate the interconversion between alcohols and aldehydes with the reduction of nicotine adenine dinucleotide to NADH.

#### **4.4.2.1.6 Glucose-6-Phosphate Dehydrogenase (G-6-PD)**

In the present study, glucose-6-phosphate dehydrogenase activity was moderate in the capsule and trabeculae. The parenchyma also showed weak activity. The white pulp including lymphoid follicles showed moderate activity for G-6-PD in pig spleen (Plate 14b). The G-6-PD activity was also seen strong in the trabeculae and blood vessels (Plate 14c). The glucose 6 phosphate dehydrogenase enzyme activity is associated with the pentose phosphate shunt (Fennel and Pearse 1961). These pentose phosphates might be utilized for nucleic acid synthesis during development. Turkoglu and Aldemir (2003) also reported the glucose 6-phosphate dehydrogenase (G-6-PD) enzyme activity in all mammalian tissues especially in cytosol and mitochondria.

### **4.4.3 Diaphorases**

#### **4.4.3.1 Reduced Nicotinamide Adenine Dinucleotide Diaphorase (NADH-D) and Reduced Nicotinamide Adenine Dinucleotide Phosphate Diaphorase (NADPH-D)**

In the present study, moderate activity of nicotinamide adenine dinucleotide diaphorase was observed in the capsule and trabeculae of the spleen (Plate 15a). The activity was weak to moderate in the parenchyma of spleen. Strong activity was observed in the lymphoid follicle. The periphery of the lymphoid follicle showed moderate activity for NADH-D and the germinal center of lymphoid follicle showed weak activity. The cellular elements showed moderate to strong activity. NADH-D activity. The activity was also seen in the blood vessels (Plate 15c).

Moderate activity of nicotinamide adenine dinucleotide phosphate diaphorase was present in the capsule and trabeculae of the spleen (Plate 13c and 13d). In parenchyma moderate activity of this enzyme was observed in lymphoid follicles and red pulp (Plate 1c). Similar observations were observed by Kaur *et al* (2018) in lymphoid follicles. NADH and NADPH diaphorase are co enzyme dehydrogenases and act in the cell as a part of hydrogen transport chain. The enzyme intensity is an indicator of metabolic activity of the cell.

#### **4.4.4 Non-specific Esterase (NSE)**

The non-specific esterases activity was seen in capsule, trabeculae and blood vessels of pig spleen (Plate 15b and 15d). The activity was moderate in the capsule of pig spleen. Moderate activity was seen in the trabeculae and blood vessels in pig spleen. Weak activity was also observed in the periphery of lymphoid follicles (Plate 15d). Similar observations were made by Anagnostou *et al* (2007) in fetal spleen where NSE positive activity was seen.

### **4.5 Scanning Electron Microscopy**

Scanning electron microscopy explained the 3-dimensional network of pig spleen in the present study (Plate 16a). Hataba *et al* (1981) also observed three dimensional network in mouse. The trabeculae arose from capsule divided the parenchyma into red pulp and white pulp (Plate 16b). Multiple opening of the blood

capillaries were seen in the white pulp in pig spleen (Plate 16c). These capillaries were surrounded by macrophages and reticular cells. Similar observations were reported by Kannan and Ramesh (2013) in chicken where capillaries were surrounded by macrophages and formed ellipsoids.

Ellipsoids were found to have a meshwork of reticular cells, reticular fibers and macrophages. These were found distributed throughout the marginal zone. Reticulum was arranged circumferentially about the central artery at the periphery of PALS. The variety of nucleated cells was observed in pig spleen. These cells were having microvilli and processes (Plate 16d). Similar observations were made by Kimura *et al* (2001).

Red pulp consisted of splenic sinuses and splenic cords. Splenic cords were rich in macrophages that helped in phagocytosis. Polak *et al* (2009) reported that the main functional element of red pulp cords were macrophages which were able to phagocytise in human spleen. Numerous RBC were seen in the red pulp. Erythrophagocytosis was observed in the splenic cord macrophages and was confirmed by two phagocytic vacuoles. Similar observations were recorded by Barnhart *et al* (1976) in human spleen and reported erythrophagocytosis in splenic cords with phagocytic vacuoles.

#### **4.6 TRANSMISSION ELECTRON MICROSCOPY**

Transmission electron microscopy revealed a distinguished area of grouped non-nucleated and grouped nucleated cells in the pig spleen in the present study (Plate 17a). These non-nucleated cells were presenting the area of red pulp and nucleated cells presented the area of white pulp.

The white pulp consisted of cells of different shape, sizes and nuclear morphology were observed in pig spleen (Plate 17b). Intercellular junctions and connections were meshed with collagen and elastic fibers. Similar observations were made by Kimura *et al* (2001) in rat where intercellular connections and intermediate junctions were found with evenly enmeshed collagen and elastic fibers.

Transmission electron microscopy revealed that the strongly acidophilic material between the ellipsoids and circum-lymph tissue contained collagen fibers.

Song *et al* (2012) reported similar observations in the spleen of ostrich chick. Different cell types seen in white pulp were plasma cells, fibroblast, monocytes, lymphocytes and macrophages (Plate 17c and 17d). Macrophages were seen frequently in white pulp among lymphoid cells. Similar report was made by Barnhart *et al* (1976) in human spleen and found macrophages in the red pulp of spleen.

#### 4.7 IMMUNOHISTOCHEMISTRY

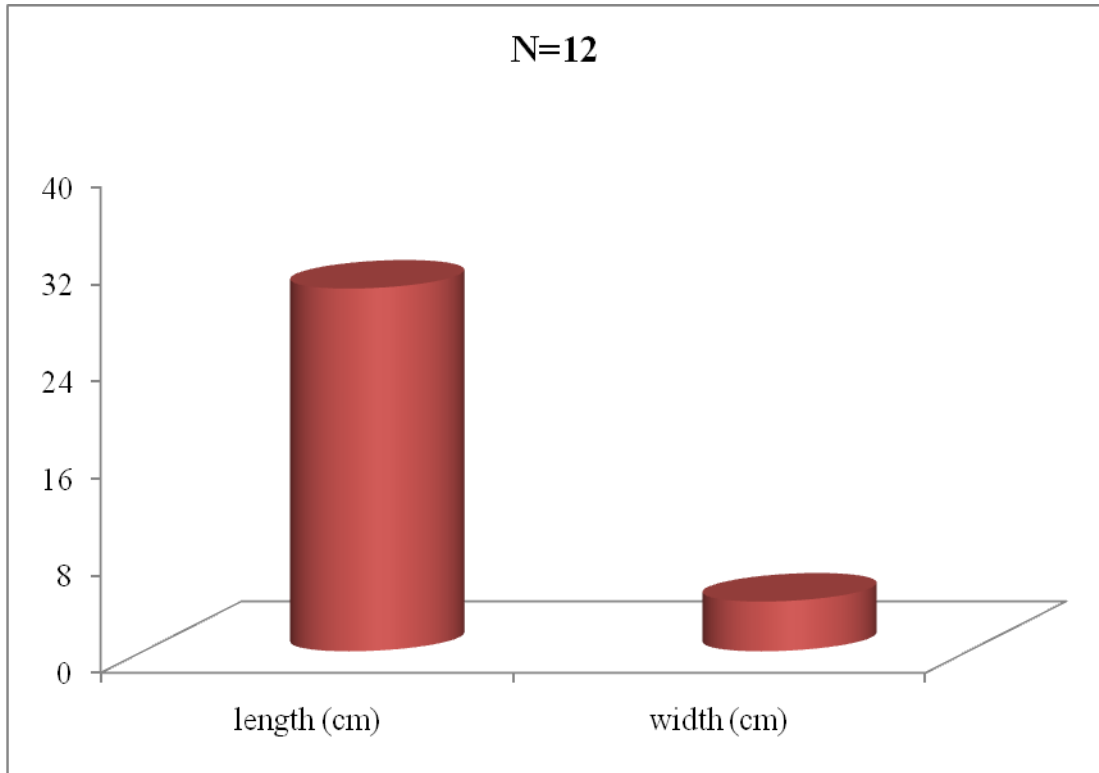
Immunohistochemical studies on the pig spleen revealed that numerous B-lymphocytes and T-lymphocytes were present in the area of white pulp of spleen (Plate 7b and 7c). More T-lymphocytes were present around the ellipsoids (Plate 7b). Localisation of macrophages were seen more in white pulp and ellipsoids (Plate 9b). So, it was concluded that T-lymphocytes possibly act as phagocytic cells in pig spleen. Similar observations were made by Cesta (2006) in the lab animals. Sheathed capillaries were found to be surrounded by reticular cells and macrophages and formed the ellipsoids. These observations were in line with the observations of Gnanadevi *et al* (2019) in sheep and goat which reported that macrophages were found mostly in ellipsoid area.

**Table 4: Biometrical data showing average length (cm), width (cm), volume (ml) and weight (g) (Mean±SE) of pig spleen.**

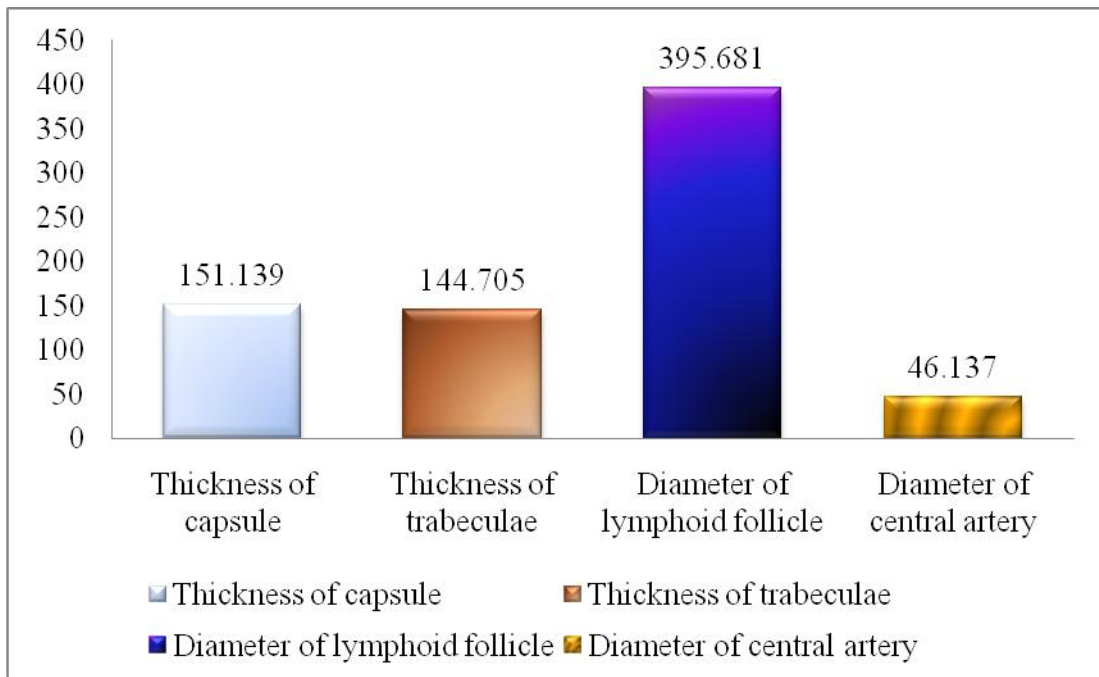
Spleen	Length	Width	Volume	Weight
N=12	29.88 ± 0.61	4.11 ± 0.24	72.66 ± 5.59	86.83 ± 7.47

**Tables 5: Micrometrical data showing thickness (µm) of capsule, trabeculae, diameter of lymphoid follicles and central artery (Mean±SE) of pig spleen**

Spleen	Micrometry
Thickness of capsule	151.14 ± 8.82 µm
Thickness of trabeculae	144.71 ± 9.38 µm
Diameter of lymphoid follicle	395.68 ± 15.97 µm
Diameter of central artery	46.12 ± 3.48 µm



**Fig. 1: Biometrical data showing mean length (cm) and width (cm) of pig spleen.**



**Fig 2: Micrometrical data showing thickness of capsule (μm) and trabeculae (μm) and diameter of lymphoid follicle (μm) and central artery (μm) of pig spleen.**

**Table 6: Histochemical distribution of mucopolysaccharides, lipids and proteins in pig spleen**

	<b>Capsule</b>	<b>Trabeculae</b>	<b>White pulp</b>	<b>Lymphoid follicle</b>	<b>Red pulp</b>	<b>Blood vessels</b>
Neutral Mucopolysaccharides	+ /+++	++	+++	+++	+ /+++	+ /+++
Acid Mucopolysaccharides	+ /+++	+	+	+	+ /+++	++
Basic Proteins	+ /+++	+ /+++	+ /+++	+ /+++	+ /+++	+ /+++
Total Lipids	++	++	+ /+++	+ /+++	+	++
Phospholipids	++	++	+ /+++	+ /+++	+ /+++	++

+Weak, ++ moderate, +++ strong.

**Table 7: Histoenzymic localization of phosphatases and esterases in pig spleen**

<b>Enzymes</b>	<b>capsule</b>	<b>Trabeculae</b>	<b>White pulp</b>	<b>Lymphoid follicle</b>	<b>Red pulp</b>	<b>Blood vessels</b>
Phosphatases						
AKPase	+	+ /+++	+++ /++++	+++ /++++	+ /+++	++
ATPase	+	+	+++ /++++	+++ /++++	+ /+++	+ /+++
G-6-Pase	+ /+++	+ /+++	+ /+++	+ /+++	+	+ /+++
Non specific esterases	++	++	+	+	+ /+++	+ /+++

+Weak, ++ moderate, +++ strong.

**Table 7: Histo enzymic localization of oxidoreductases in pig spleen**

<b>Enzyme</b>	<b>Capsule</b>	<b>Trabeculae</b>	<b>White pulp</b>	<b>Lymphoid follicle</b>	<b>Red pulp</b>	<b>Blood vessels</b>
SDH	+	+	++	++	+	+/++
MDH	+/+++	+/+++	+/+++	+/+++	+	+/+++
LDH	++	++	++	++	+/+++	++
ALC	+/+++	+/+++	+/+++	+/+++	+/+++	++
GLD	++	++	++	++	+/+++	++
NADH Diaphorase	++	++	+++	+++	+/+++	+++
NADPH Diaphorase	++	++	++	++	+/+++	++
G-6-PD	++	++	++	++	++	+++

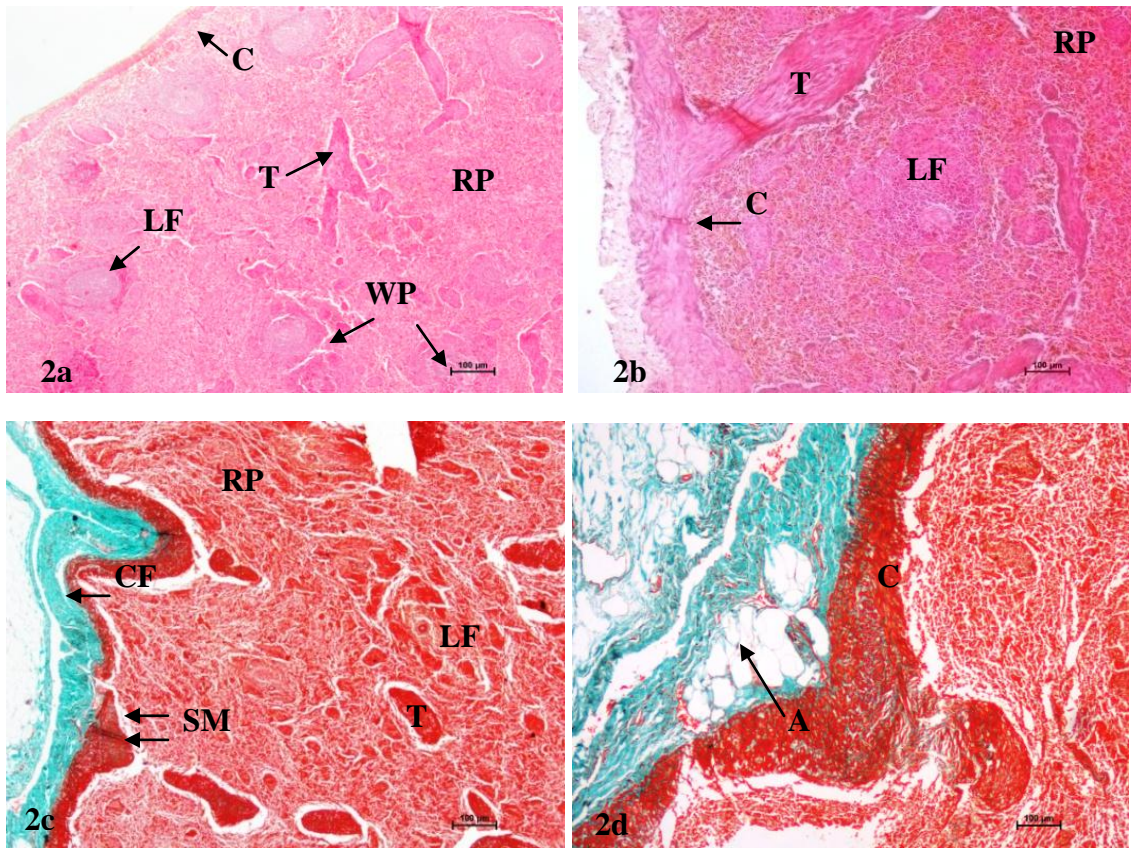
+Weak, ++ moderate, +++ strong.



**1a) Photograph of spleen of pig showing parietal surface**



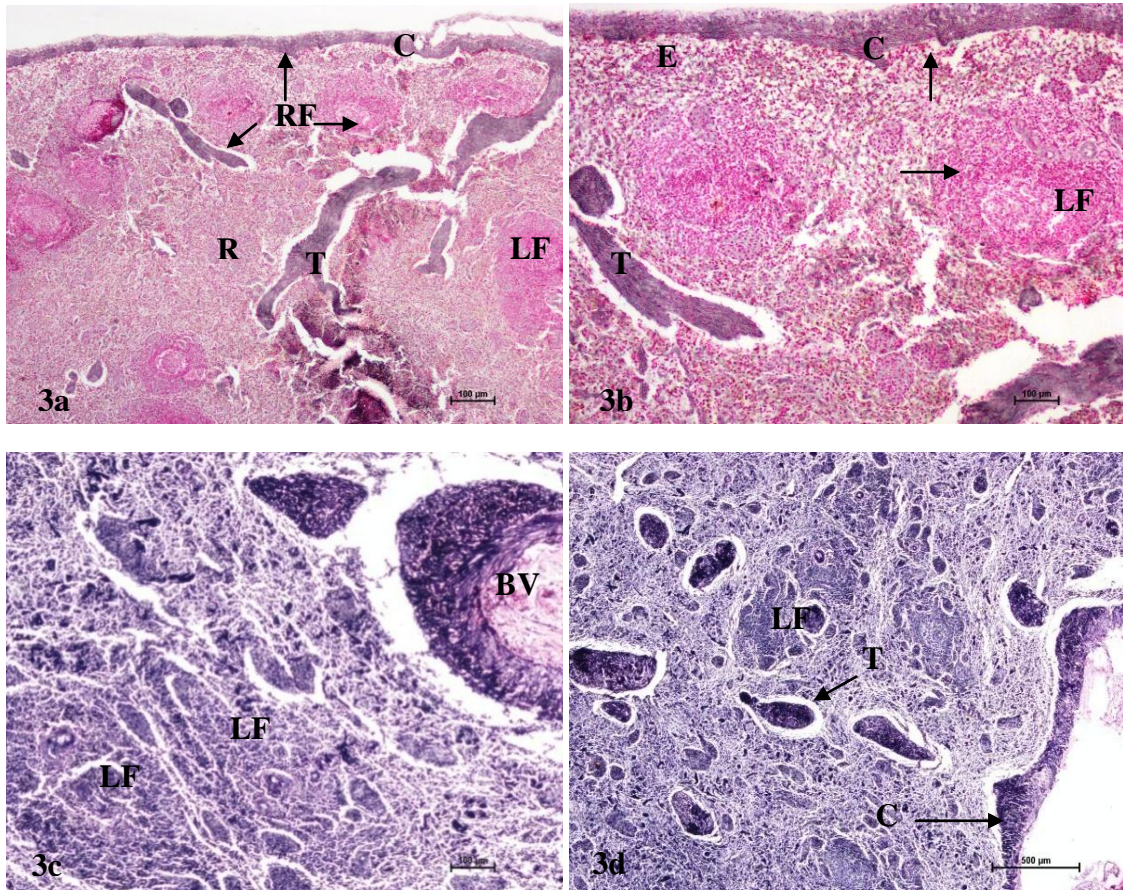
**1b) Photograph of spleen of pig showing visceral surface**



**Plate 2**

**Photomicrograph of**

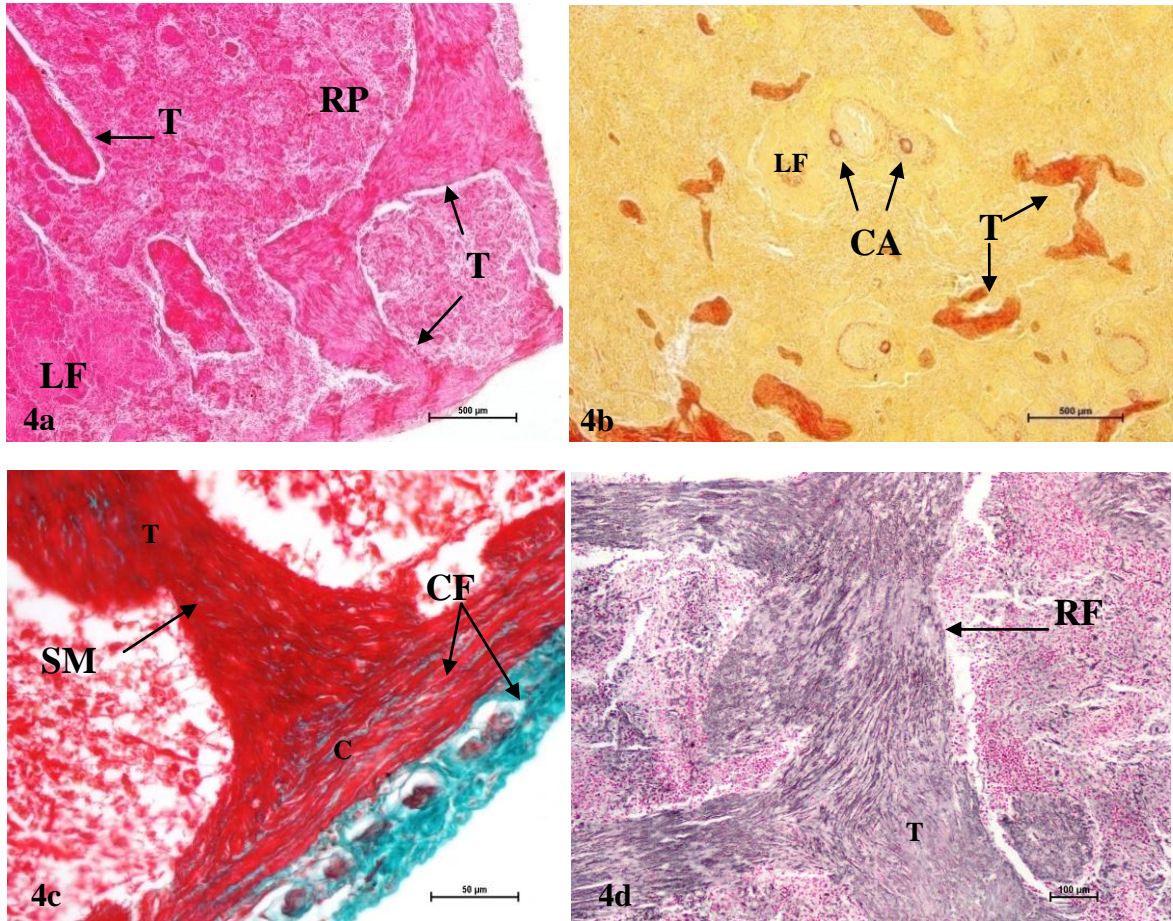
- 2a)** Section of the spleen of pig showing the connective tissue capsule (C), trabeculae (T), white pulp (WP), red pulp (RP) and lymphoid follicle (LF) (Arrow). Haematoxylin and Eosin X 100.
- 2b)** Section of the spleen of pig showing the two layered connective tissue capsule (C), trabeculae (T), and lymphoid follicle (LF) (arrow). Haematoxylin and Eosin X 100.
- 2c)** Section of spleen of pig showing two layered capsule (C), trabeculae (T). The outer layer of capsule made up of collagen fibers (CF) (single arrow) and inner layer was smooth muscle layer (SM) (double arrows) (Arrow). Masson's trichrome X 100.
- 2d)** Section of the pig spleen showing the connective tissue capsule (C) surrounded by adipose tissue (A) (arrow). Masson's trichrome X 400.



**Plate 3**

**Photomicrograph of**

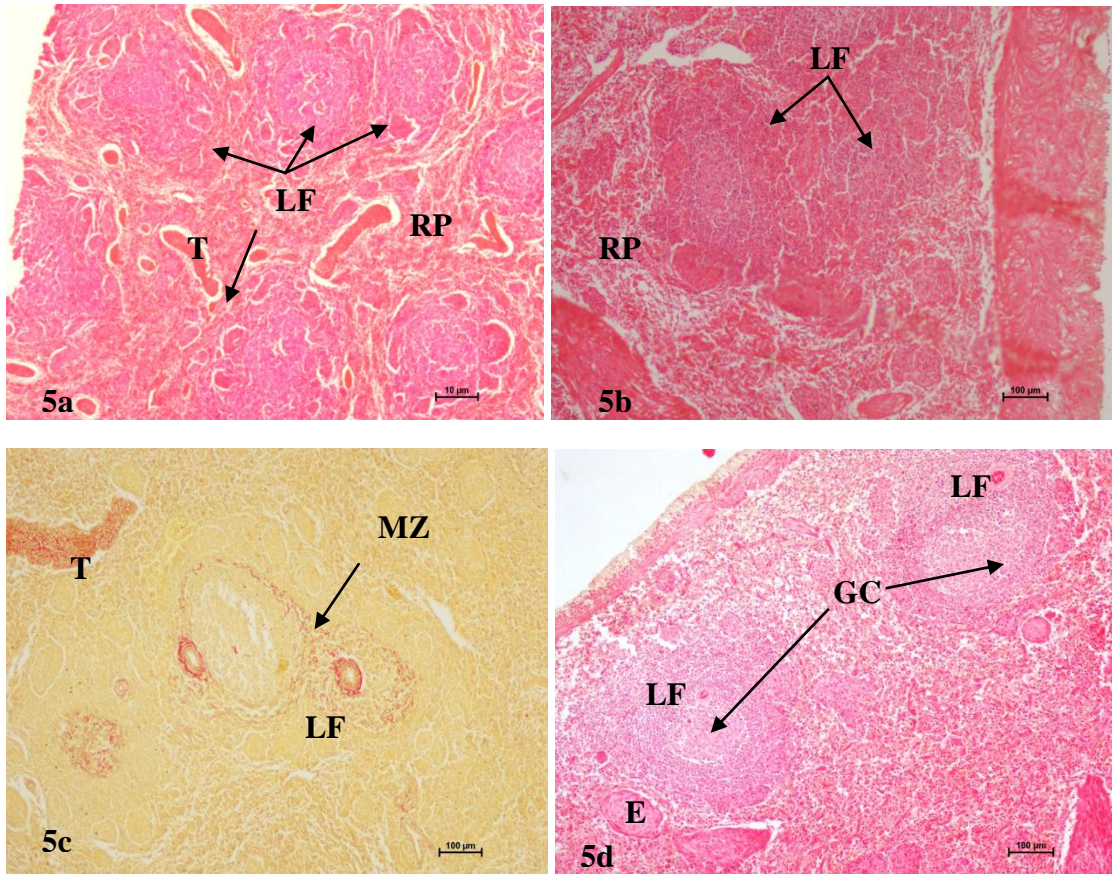
- 3a) Section of pig spleen showing reticular fibers (RF) in the capsule (C), trabeculae (T) and white pulp (WP). (Arrow).Gridley's X40**
- 3b) Section of pig spleen showing reticular fibers (RF) in capsule (C), trabeculae (T), lymphoid follicle (LF) and ellipsoid (E) Gridley's X 100**
- 3c) Section of pig spleen showing elastic fibers (EF) in the blood vessel (BV). Gridley's X 100**
- 3d) Section of spleen of pig showing elastic fibers (EF) in capsule (C), trabeculae (T). Verhoeff's X 40**



**Plate 4**

**Photomicrograph of**

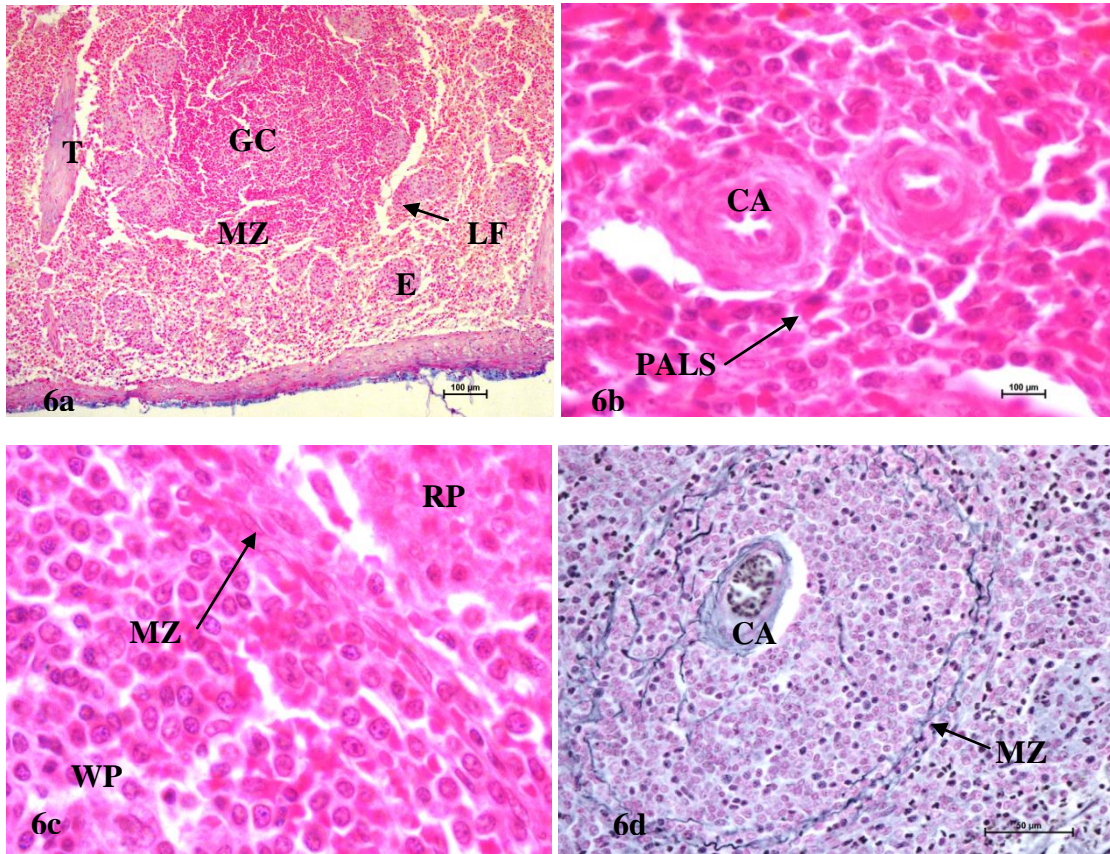
- 4a) Section of pig spleen showing branching trabeculae (T) extending into parenchyma, lymphoid follicle (LF) and red pulp (RP) (arrow). Haematoxylin and Eosin X 100**
- 4b) Section of spleen of pig showing distribution of trabeculae (T) in parenchyma, lymphoid follicle (LF) with central artery (CA) (arrow). Picrosirius Red X 40**
- 4c) Section of pig spleen showing smooth muscle fibers (SM) and collagen fibers (CF) in capsule (C) and trabeculae (T) (arrow). Masson Tricome X 400**
- 4d) Section of pig spleen showing reticular fibers (RF) distribution in the trabeculae (T). Gridley's X 400**



**Plate 5**

**Photomicrograph of**

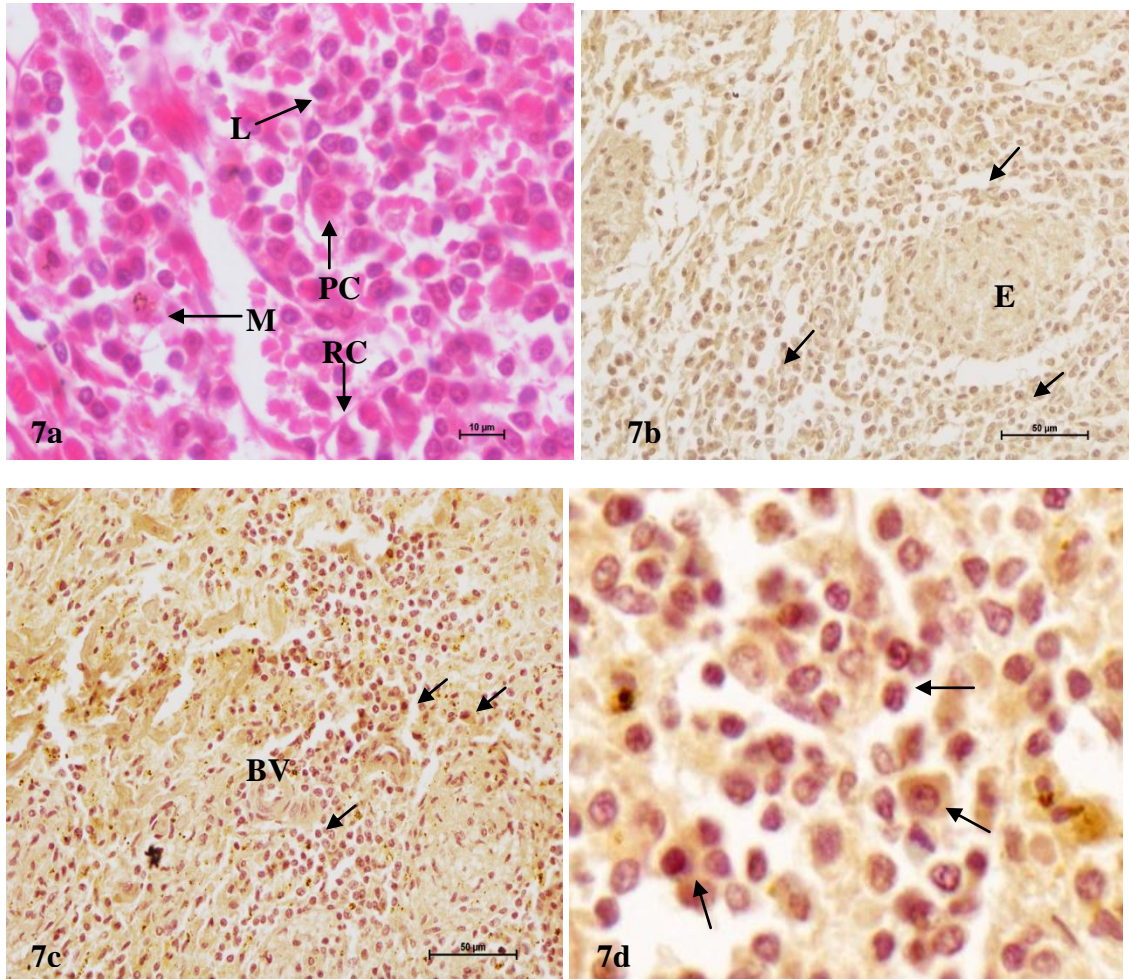
- 5a) Section of pig spleen showing trabeculae (T), lymphoid follicle (LF) and red pulp (RP). Haematoxylin and Eosin X 40**
- 5b) Section of spleen of pig showing aggregation of lymphoid follicle (LF) and red pulp (RP) (arrow). Haematoxylin and Eosin X100**
- 5c) Section of spleen of pig showing collagen fibers in the marginal zone (MZ) in lymphoid follicle (LF). Picosirius Red X 100**
- 5d) Section of pig spleen showing germinal center (GC) in lymphoid follicle (LF) and ellipsoids (E). Haematoxylin and Eosin X100**



**Plate 6**

**Photomicrograph of**

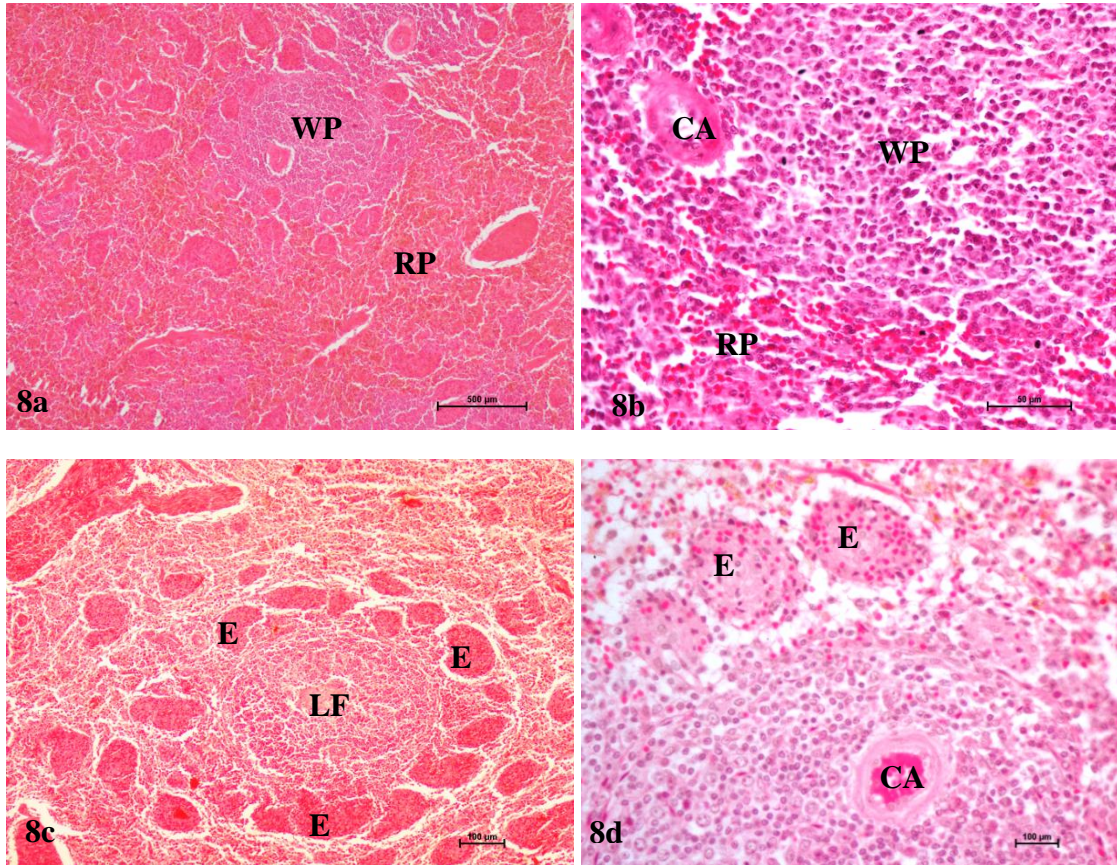
- 6a) Section of pig spleen showing lymphoid follicle (LF) with lightly stained germinal center (GC), ellipsoid (E) and marginal zone (MZ) dividing red and white pulp. AB/PAS X 100**
- 6b) Section of spleen of pig showing central artery (CA) surrounded by PALS (Peri-arteriolar lymphatic sheath) (arrow). Haematoxylin and Eosin X 1000**
- 6c) Section of pig spleen showing marginal zone (MZ) dividing red pulp (RP) and white pulp (WP) (arrow). Haematoxylin and Eosin X1000**
- 6d) Spleen of pig showing reticular fibers in the marginal zone (MZ) and around central artery (CA) (arrow). Gridley's X 400**



**Plate 7**

**Photomicrograph of**

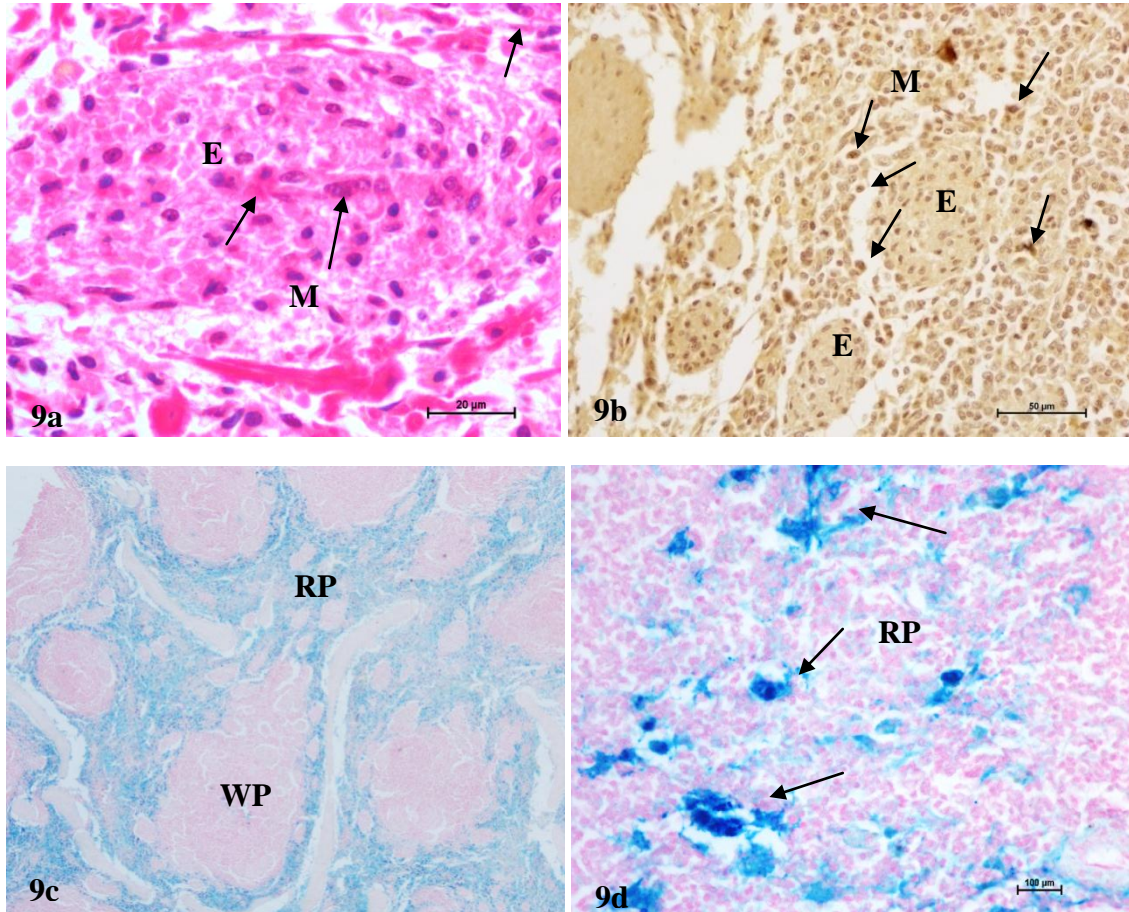
- 7a) Section of pig spleen showing macrophages (M), plasma cells (PC), reticular cells (RC) and lymphocytes (L) (arrow). Haematoxylin and Eosin X 1000**
- 7b) Section of spleen showing T-lymphocytes with cytoplasmic reaction in spleen present around ellipsoid (E) (arrow). HRP X 400**
- 7c) Section of spleen showing B-lymphocytes with cytoplasmic reaction in spleen (arrow). HRP X 400**
- 7d) Section of spleen showing B-lymphocytes with cytoplasmic reaction in spleen (arrow). HRP X 1000**



**Plate 8**

**Photomicrograph of**

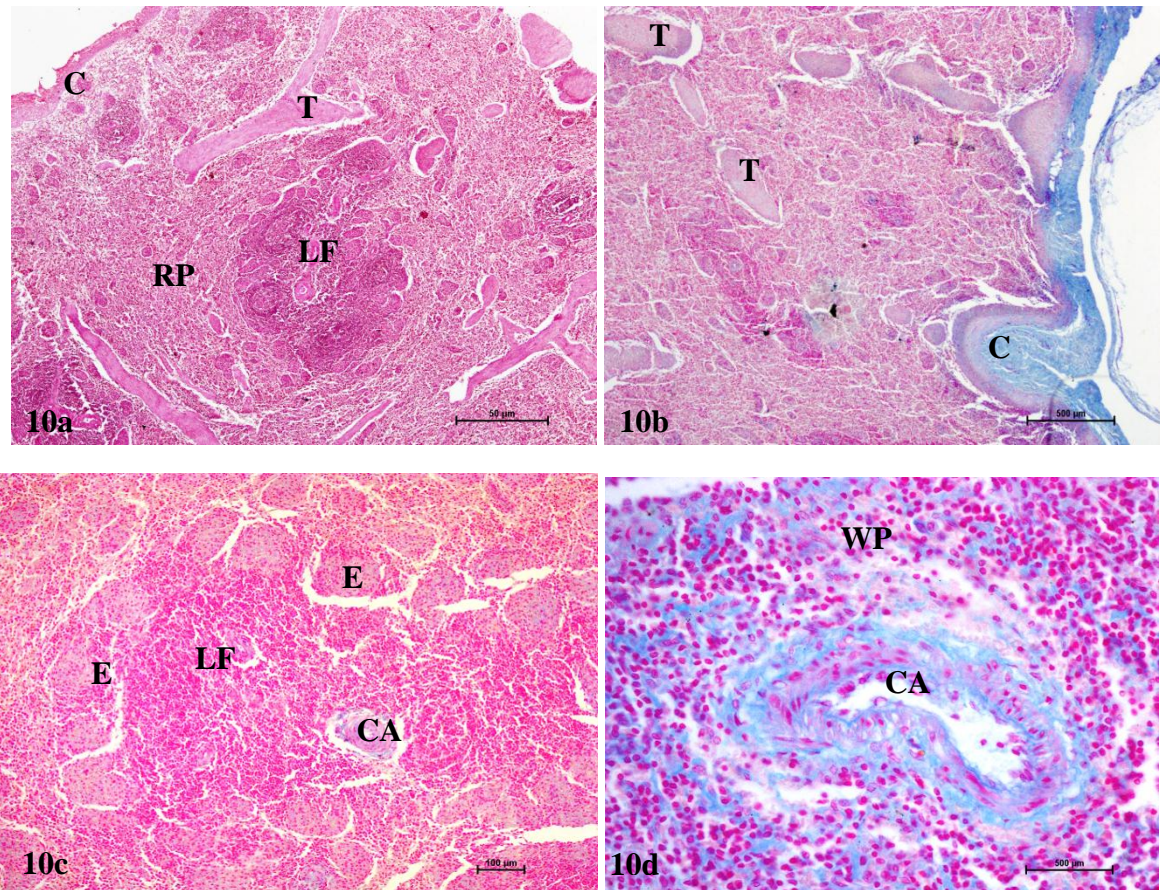
- 8a) Section of spleen of pig showing area of red pulp (RP) and white pulp (WP).  
Haematoxylin and Eosin X100**
- 8b) Section of pig spleen showing differential area of white pulp (WP) with nucleated cells and red pulp (RP) with splenic sinuses and erythrocytes. Haematoxylin and Eosin X1000**
- 8c) Section of spleen of pig showing ellipsoids (E) around the lymphoid follicle (LF).  
Haematoxylin and Eosin X100**
- 8d) Section of pig spleen showing central artery (CA) with erythrocytes and ellipsoids (E). Haematoxylin and Eosin X1000**



**Plate 9**

**Photomicrograph of**

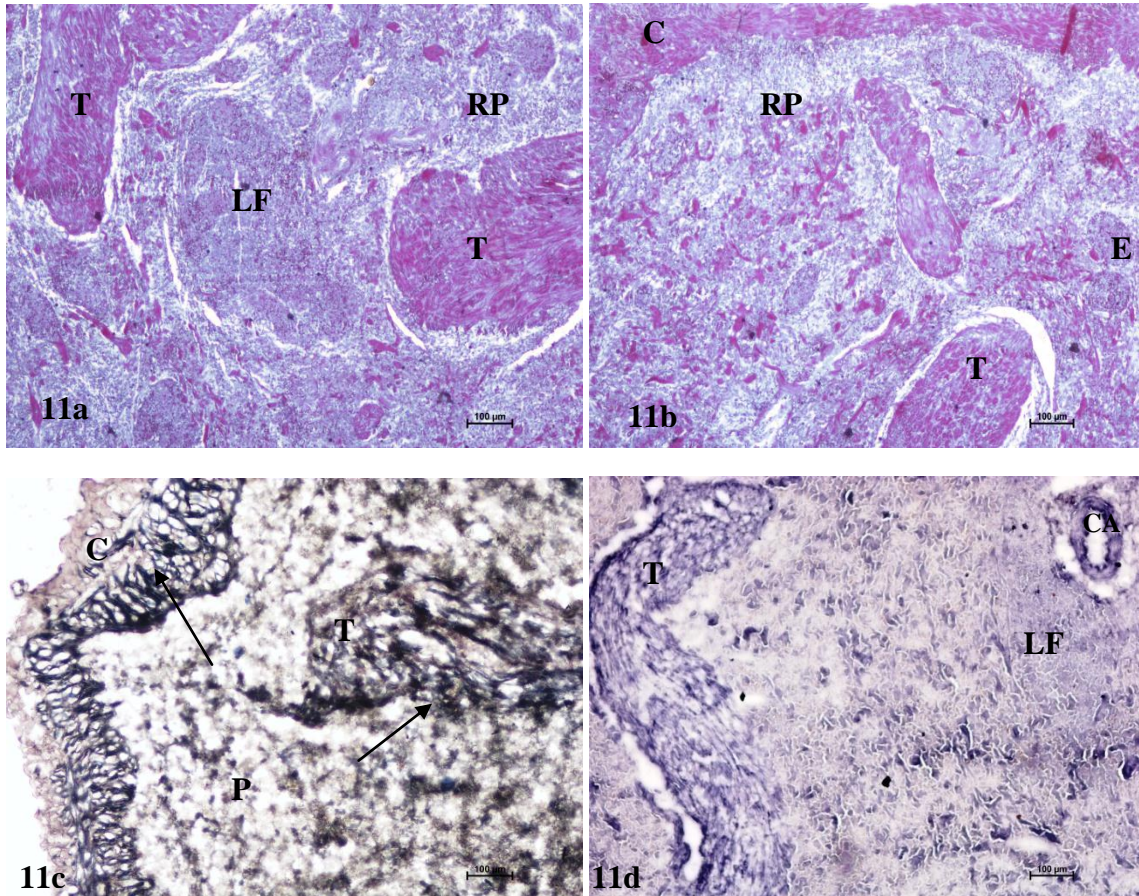
- 9a) Section of spleen of pig showing macrophages (M) present in ellipsoid (E) (arrow) in spleen. Hematoxylin and Eosin X 1000**
- 9b) Section of spleen showing macrophages (M) with cytoplasmic reaction around ellipsoids (E) in spleen (arrow). HRP X 1000**
- 9c) Section of pig spleen showing erythrophagocytosis in the red pulp (RP) with deposition of hemosiderin pigment and white pulp (WP) in pig spleen. Perl's Prussian blue X 40.**
- 9d) Section of pig spleen showing accumulation of bluish granules of hemosiderin pigment in red pulp macrophages (arrow). Perl's Prussian blue X 400.**



**Plate 10**

**Photomicrograph of**

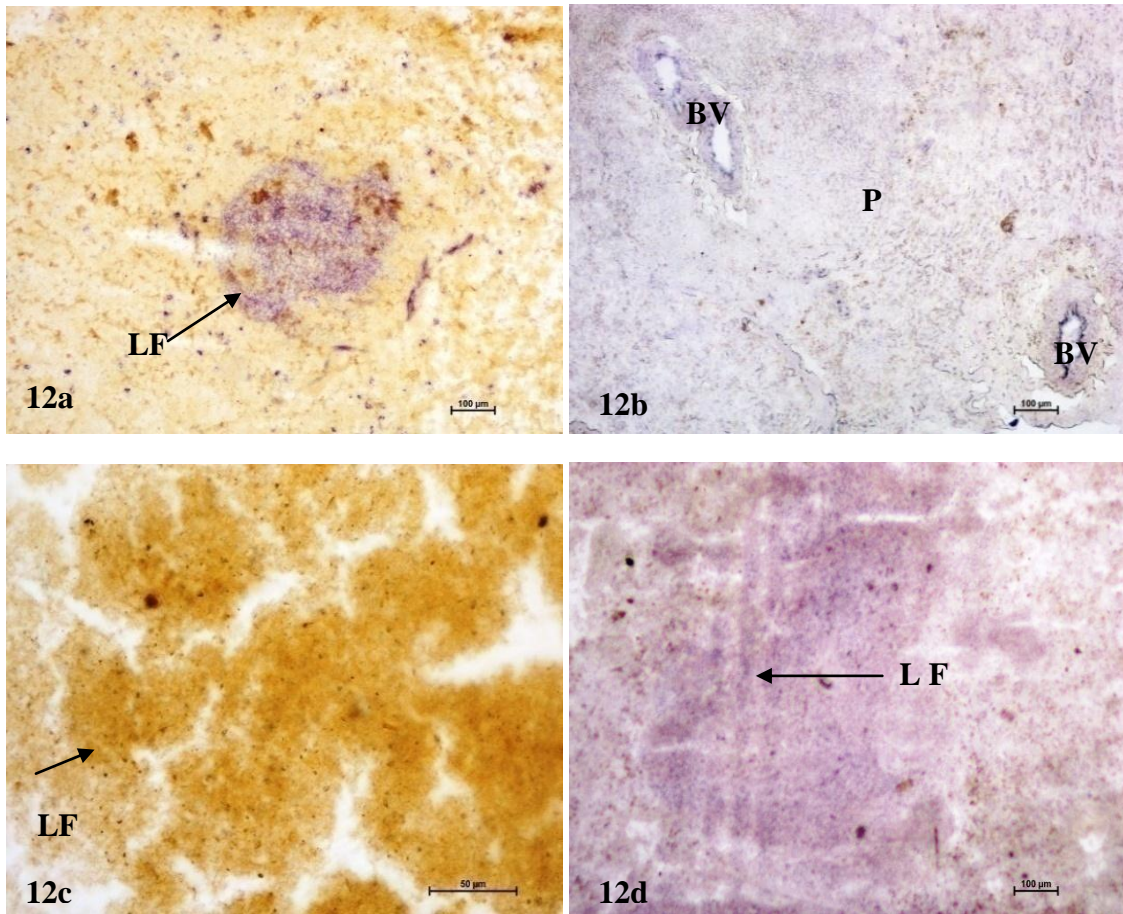
- 10a) Section of pig spleen showing moderate to strong activity for neutral mucopolysaccharides in capsule (C), trabeculae (T) and lymphoid follicles (LF). Moderate activity for neutral mucopolysaccharide is also present in red pulp (RP). PAS X 100.**
- 10b) Section of spleen of pig showing strong acidic mucopolysaccharides activity in outer fibrous layer of capsule (c). Trabeculae (T) showed mixed activity for acidic mucopolysaccharides. AB/PAS X 100.**
- 10c) Section of pig spleen showing activity for acidic mucopolysaccharides around central artery (CA), and ellipsoids (E). Lymphocytes in white pulp are strongly positive for neutral mucopolysaccharides. AB/PAS X 100.**
- 10d) Section of pig spleen showing activity for acid mucopolysaccharides around central artery (CA). AB/PAS X 1000**



**Plate 11**

**Photomicrograph of**

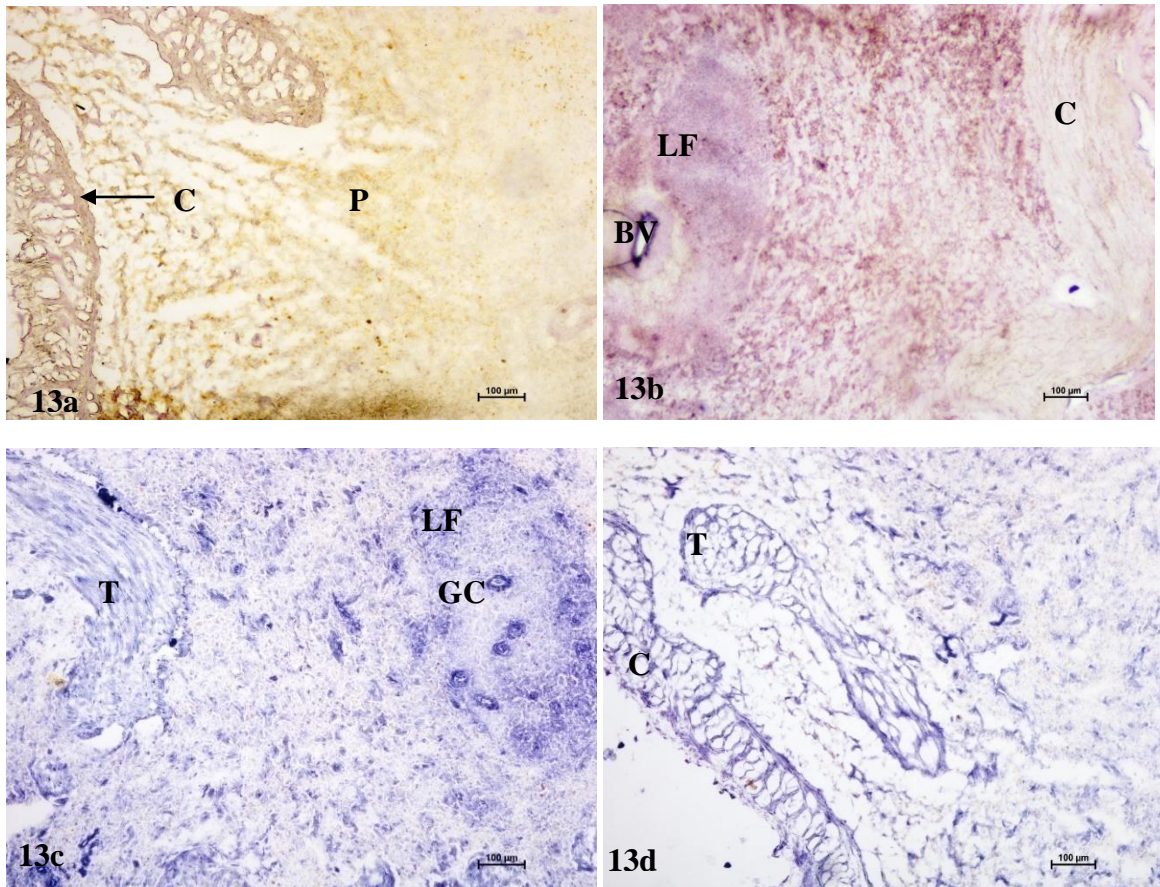
- 11a) Section of pig spleen showing moderate to strong protein activity in the trabeculae (T), lymphoid follicle (LF) and red pulp (RP). Bromphenol Blue X 100**
- 11b) Section showing moderate activity in red pulp (RP) and weak to moderate in trabeculae (T) and weak in ellipsoids (E). Bromphenol Blue X 100**
- 11c) Cryosection of pig spleen showing strong activity for phospholipids in capsule (C), trabeculae (T) and parenchyma (P). Acid hematin X 100**
- 11d) Cryosection of spleen showing activity for lipids in trabeculae (T), lymphoid follicle (LF) and central artery (CA). Sudan black X 100**



**Plate 12**

**Photomicrograph of**

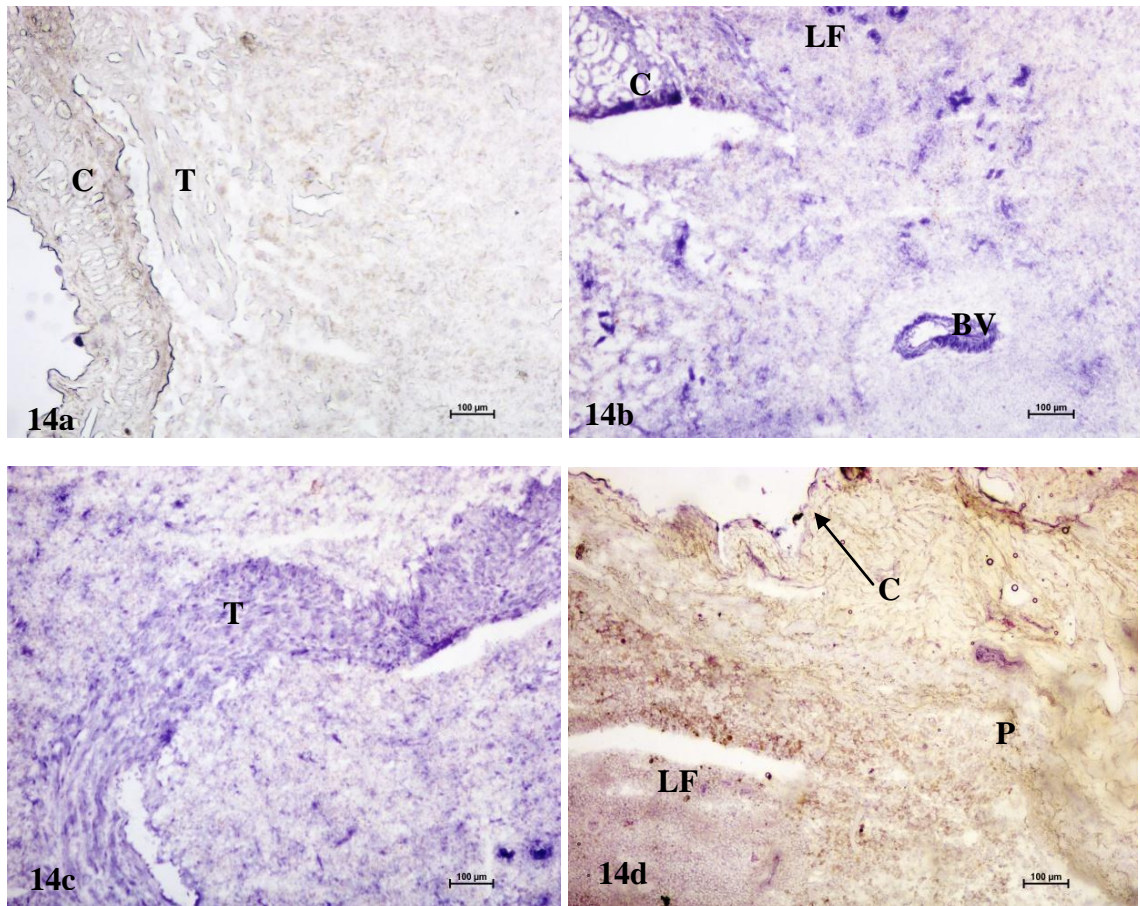
- 12a) Cryosection of pig spleen showing activity for AKPase in lymphoid follicle (arrow). AKPase X 100**
- 12b) Cryosection of spleen of pig showing weak to mild activity for G-6-phosphatase in parenchyma (P) and blood vessels (BV). Lead nitrate method X400**
- 12c) Cryosection of spleen of pig showing moderate to strong activity for ATPase in lymphoid follicle (arrow). ATPase X400**
- 12d) Cryosection of pig spleen showing moderate activity for succinate dehydrogenase (SDH) in capsule. Nitro BT method X400**



**Plate 13**

**Photomicrograph of**

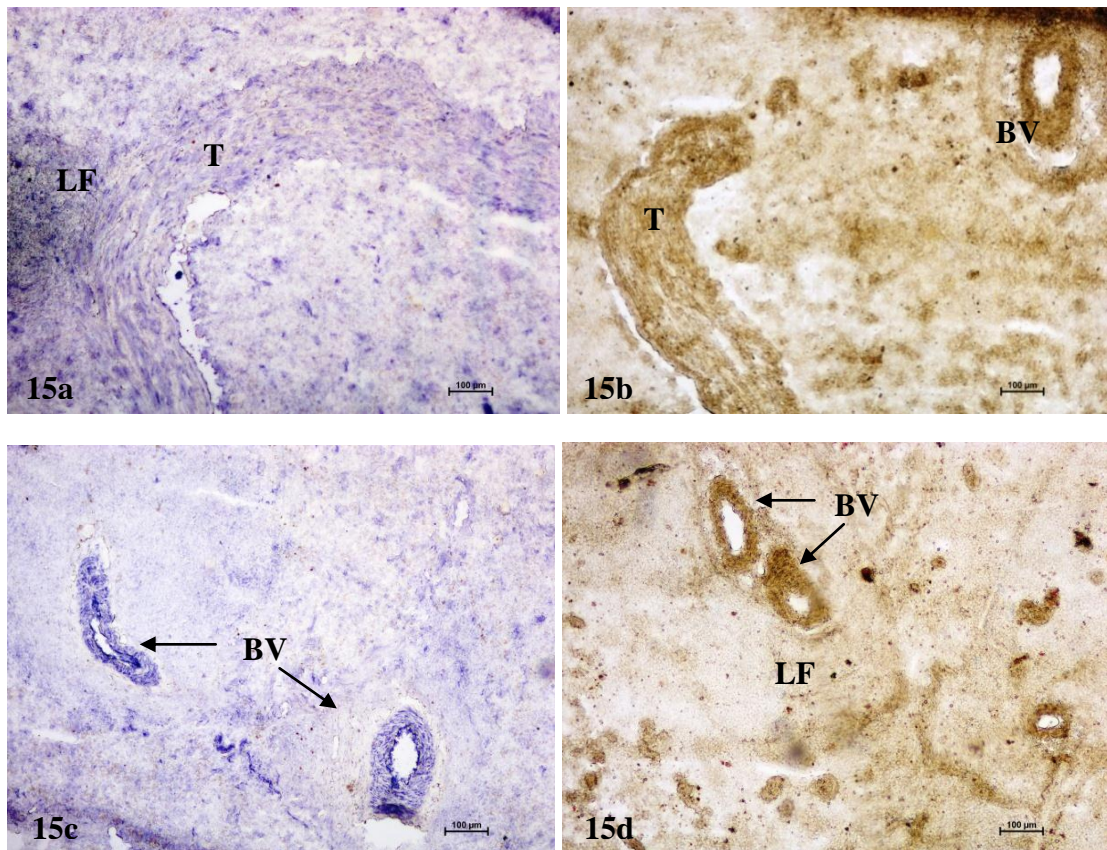
- 13a) Cryosection of spleen of pig showing weak to moderate MDH activity in the capsule (C) and parenchyma (P). Nitro BT method X 100**
- 13b) Cryosection of pig spleen showing weak activity for LDH in capsule (C), lymphoid follicle (LF) and blood vessel (BV). Nitro BT method X 100**
- 13c) Cryosection of pig spleen showing moderate activity of NADPH in trabeculae (T), germinal center (GC) and strong reaction at periphery in lymphoid follicle (LF). Nitro BT method X 100**
- 13d) Cryosection of spleen of pig showing moderate activity of NADPH in capsule (C) and trabeculae (T). Nitro BT method X 100**



**Plate 14**

**Photomicrograph of**

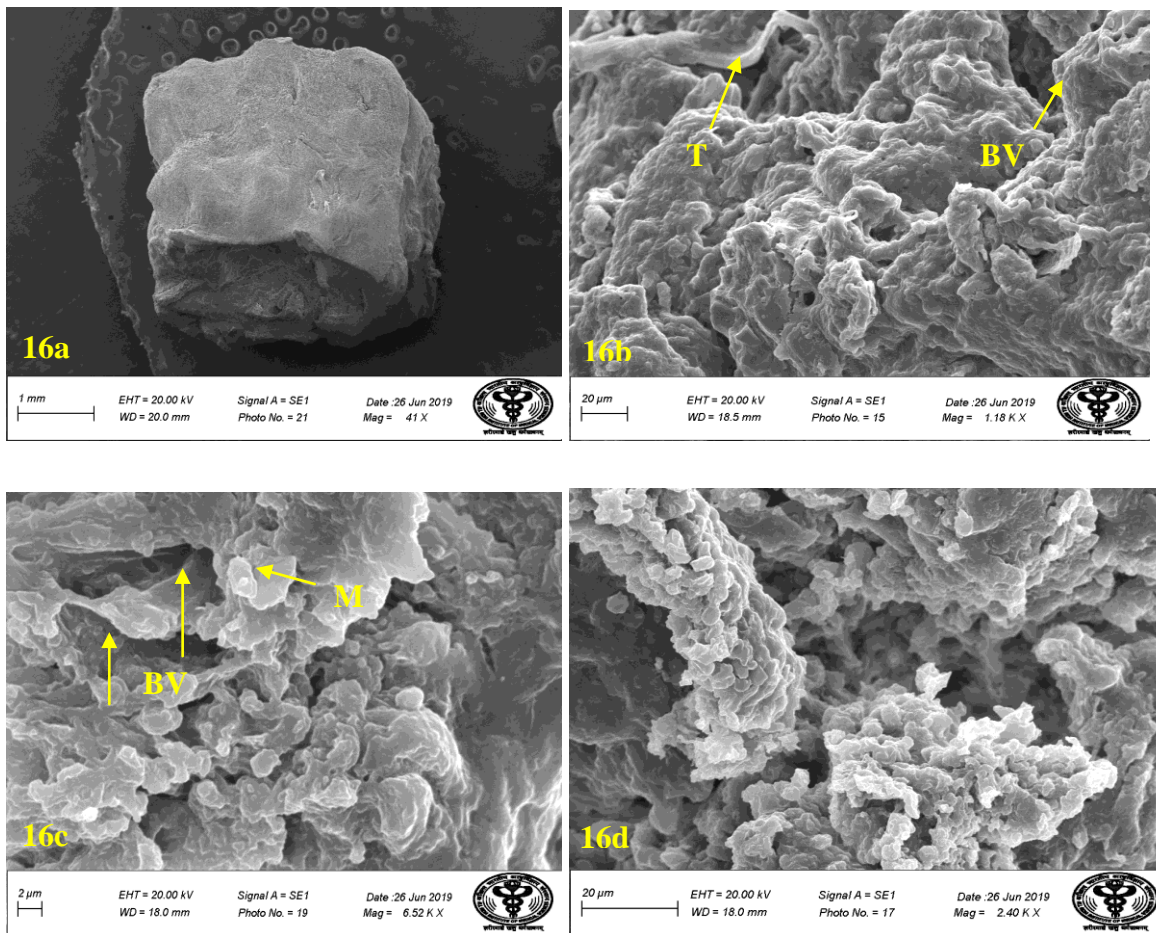
- 14a) Cryosection of spleen of pig showing weak to moderate ALC activity in capsule (C), trabeculae (T) and parenchyma (P). Nitro BT method X 100**
- 14b) Cryosection of pig spleen showing moderate activity for G-6-PD in the capsule (C), lymphoid follicle (LF) and strong activity in blood vessel (BV). Nitro BT method X 100**
- 14c) Cryosection of spleen of pig showing moderate to strong activity for G-6-PD in the trabeculae (T). Nitro BT method X 100**
- 14d) Cryosection of pig spleen showing moderate activity for GLD in capsule (C) and lymphoid follicle (LF). Weak activity in parenchyma (P) (arrow). Nitro BT method X 100**



**Plate 15**

**Photomicrograph of**

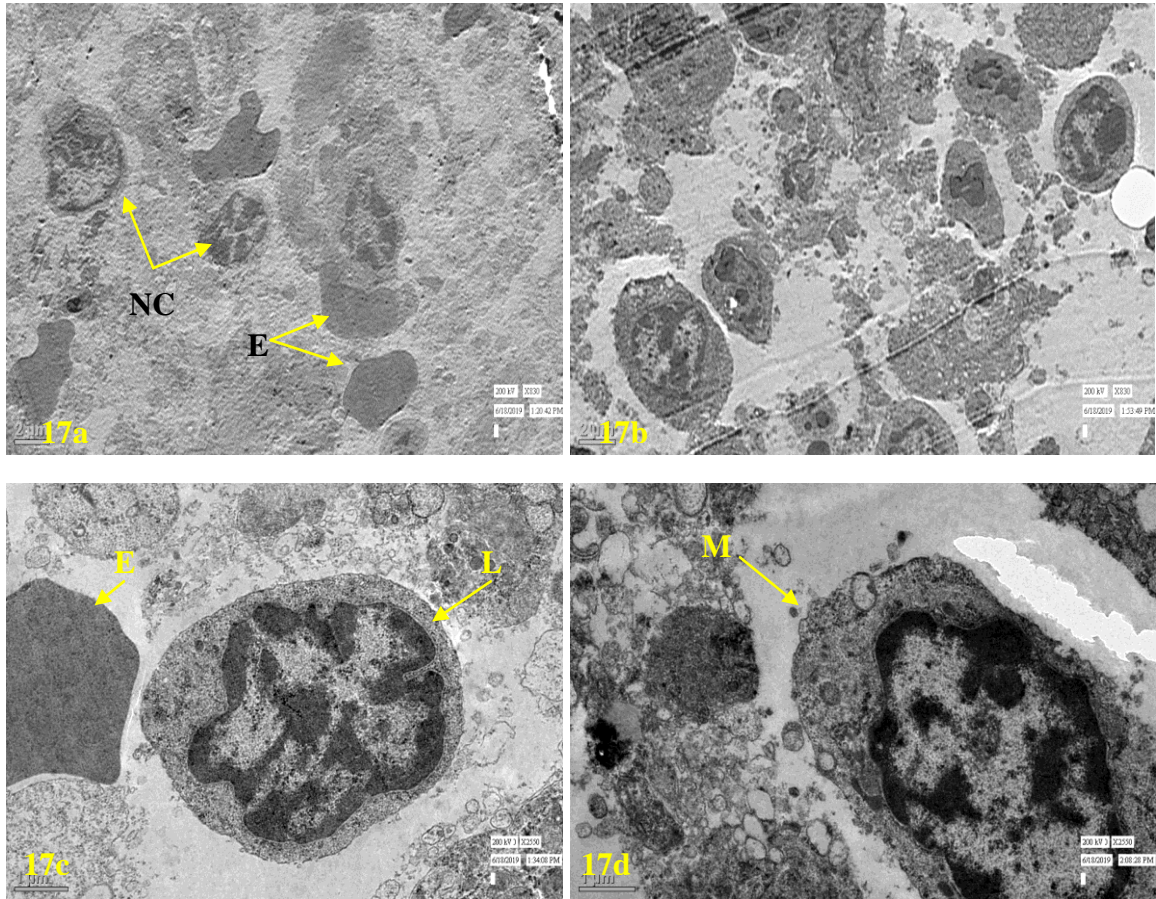
- 15a) Cryosection of spleen showing moderate to strong activity for NADH in trabeculae (T), lymphoid follicle (LF) and small blood vessels. Nitro BT method X 100**
- 15b) Cryosection of spleen showing positive NSE activity in trabeculae (T) and blood vessel (BV). Naphthol acetate method X100**
- 15c) Cryosection of spleen showing moderate activity for NADH in blood vessels (BV). Nitro BT method X 100**
- 15d) Cryosection of spleen showing moderate to strong activity in blood vessels (BV) and weak to moderate in lymphoid follicle (LF). Naphthol acetate method X100**



### Plate 16

#### Photomicrograph of

- 16a) Scanning electron micrograph showing uneven cut surface of splenic tissue. X 41
- 16b) Scanning electron micrograph showing trabeculae (T) extending into the parenchyma and opening of blood vessels (BV). X 1180
- 16c) Scanning electron micrograph showing opening of blood vessels (BV) and macrophages (M) around blood vessels. X 6520
- 16d) Scanning electron micrograph showing multiple nucleated cells of white pulp having processes. X 2400



**Plate 17**

**Photomicrograph of**

- 17a) Transmission electron micrograph (TEM) of spleen showing area of white pulp showing nucleated cells (NC) and few non-nucleated erythrocyte (E) (arrow). X 1180**
- 17b) Transmission electron micrograph (TEM) of spleen showing different nucleated cell with different morphology in white pulp.. X 2300**
- 17c) Transmission electron micrograph (TEM) of spleen showing nucleated lymphocyte (L) of white pulp and non-nucleated erythrocytes (E) in parenchyma (arrow).X 2550**
- 17d) Transmission electron micrograph (TEM) of spleen showing nucleated macrophage (M) cell in white pulp. X 2550**

## CHAPTER V

### SUMMARY AND CONCLUSIONS

The spleen of pig in the present study was elongated tongue like structure and reddish-brown in colour which exhibited two surfaces. The two surfaces were parietal surface and visceral surface. The parietal surface of the spleen was convex and the visceral surface was concave in pig. The present study on pig spleen showed that the hilus was present at the upper middle of visceral surface.

The biometrical observations on weight, length, width and volume were recorded. The average weight of the spleen was  $86.83 \pm 7.47$  gm. The average length of spleen in the present study was  $29.88 \pm 0.61$  cm, whereas average width of spleen was  $4.11 \pm 0.24$  cm. The volume of spleen in present study in pig was found to be  $72.66 \pm 5.59$  ml.

The average thickness of the splenic capsule in pig in the present study was  $151.14 \pm 8.82$   $\mu\text{m}$  and the average thickness of trabeculae was  $144.71 \pm 9.38$   $\mu\text{m}$ . The average diameter of lymphoid follicle was found to be  $395.68 \pm 15.97$   $\mu\text{m}$ . The central artery average diameter in present study in pig spleen was recorded as  $46.12 \pm 3.48$   $\mu\text{m}$ .

The spleen was enclosed by connective tissue capsule. The capsule was made up of two layers, outer fibrous connective tissue layer and inner muscular layer of smooth muscle fibers. The outer layer was composed of capsule mainly consisted of collagen, elastic and reticular fibers and inner muscular layer of smooth muscles. The capsule was of uneven thickness in pig spleen.

The connective tissue trabeculae extended from the inner muscular layer of capsule into splenic parenchyma and divided it into smaller compartments by forming a net like framework in the present study. The trabeculae were scattered throughout splenic tissue and were composed of smooth muscle fibers along with reticular and collagen fibers.

The red pulp and white pulp were the two distinct major components of splenic parenchyma in pig. The white pulp consists of lymphocytes, reticular cells plasma cells and macrophages which was distributed throughout splenic parenchyma.

The white pulp was composed of two components, which were splenic nodules or Malphigian corpuscles or lymphatic nodules and peri-arterial lymphatic sheath.

The peri-arteriolar lymphatic sheath was chiefly made up of lymphocytes with central artery placed eccentrically. The splenic nodules were composed of aggregation of the lymphoid tissue which occurred as aggregation of two or three nodules. The splenic nodules showed germinal center which appeared as lightly stained area which consisted of loosely arranged lymphocytes.

Ellipsoids (pericapillary macrophage sheath) were large and abundant in the marginal zone. The shape of pericapillary macrophage sheaths varied from oval to elongated and was composed of macrophages, reticular cells and lymphocytes around the arteries. The marginal zone was made up of two or more layers of reticular and collagen fibers in the present study.

Two or three central arteries were observed in the of spleen in the lymphatic nodule. The central artery extended out of splenic nodules and branched to form penicillar arteries in red pulp. These were further surrounded by macrophages, reticular cells and lymphocytes.

The red pulp occupied space between white pulp and trabeculae. Red pulp consisted of splenic sinuses which were observed as irregular anastomising channels between splenic cords and branching blood vessels.

The outer fibrous layer of splenic capsule was strongly positive for acidic mucopolysaccharides and inner muscular layer was weakly positive for acidic mucopolysaccharides. The trabeculae extending from the capsule also showed weak activity for presence of acidic mucopolysaccharides. The lymphocytes present in the lymphoid follicle of white pulp were devoid of acidic mucopolysaccharides activity. Ellipsoids were weakly positive for acidic mucopolysaccharides. The lymphocytes present in germinal centers were weakly positive for acid mucopolysachharides indicating presence of immature lymphocytes. Erythrocytes present in the sinuses were devoid of acid mucopolysaccharides but the periphery of the sinuses showed positive reaction for acid mucopolysaccharides.

The inner muscular layer of capsule covering the spleen was moderately positive and outer fibrous layer was weakly positive for neutral mucopolysachharides. The trabeculae extending from the capsule towards the parenchyma of spleen also

showed moderate reaction for neutral mucopolysaccharides. The lymphoid follicles present in the white pulp of spleen contained strongly positive cells especially lymphocytes for neutral mucopolysaccharides. The connective tissue framework was strongly positive for neutral mucopolysaccharides in the parenchyma of the spleen.

Fine sudanophilic lipid droplets were observed inside the capsule. The capsule was positive for phospholipids. The trabeculae extending from parenchyma showed positive activity for phospholipids.

The bromphenol blue positive activity for basic protein was observed throughout the spleen. The capsule showed moderate to strong activity for proteins. The trabeculae extending from the capsule showed moderate to strong activity for proteins. The lymphoid follicles present in pulp region showed moderate activity for proteins.

Alkaline phosphatase activity was constantly weak in the capsule of the spleen and was moderate to strong in the lymphoid follicles of pig. Weak to mild activity for glucose-6-phosphatase was observed in entire spleen. SDH activity was moderate in the lymphoid follicle. The capsule and trabeculae were weakly positive. A weak to moderate lactate dehydrogenase activity was observed in the capsule, trabeculae and lymphoid follicles of spleen.

The malic dehydrogenase and glutamic dehydrogenase activity was weak to moderate in the spleen. Weak to moderate alcoholic dehydrogenase activity was seen in capsule and trabeculae of pig spleen however, moderate activity was seen in blood vessels and lymphoid follicle of pig spleen. Glucose 6-phosphate dehydrogenase (G-6-PD) enzyme activity was moderate to strong in all components of spleen.

In the present study, moderate activity of nicotinamide adenine dinucleotide diaphorase was observed in the capsule, trabeculae and strong activity in lymphoid follicle of the spleen and moderate activity of nicotinamide adenine dinucleotide phosphate diaphorase was present in spleen. The non-specific esterases activity was seen in capsule, trabeculae and blood vessels of pig spleen. The activity was moderate in the capsule, trabeculae and blood vessels. Weak activity was observed in the periphery of lymphoid follicle.

The presence of B lymphocytes and T-lymphocytes in white pulp was observed using immunohistochemistry. T-lymphocytes were found more around

ellipsoids and possibly acted as phagocytic cells. Macrophages were abundant in the area of ellipsoids.

In the scanning electron microscopy, multiple opening of the blood capillaries were seen in the area of white pulp. There was variety of nucleated cells present in case of white pulp. The localization of macrophages were more in white pulp and ellipsoids so T-lymphocytes possibly acted as phagocytic cell in spleen.

Most of the capillaries were surrounded by macrophages. The white pulp consisted of variety of nucleated cells. In TEM, there was distinguishable area of non-nucleated red pulp and nucleated white pulp. The nucleated cells were lymphocytes, plasma cells and macrophages. The lymphocytes and the macrophages were found more in the area of ellipsoid and white pulp.

## **CONCLUSIONS**

- Spleen was enclosed by connective tissue capsule, made up of outer fibrous layer of collagen, elastic and reticular fibers and inner of smooth muscle fibers.
- Branching trabeculae arose from inner muscular layer of capsule and scattered throughout splenic tissue.
- White pulp consisted of aggregation of lymphoid follicle and PALS was well developed around central artery.
- Ellipsoids were found abundantly in marginal zone.
- Capsule and trabeculae showed moderate activity for neutral and acid mucopolysaccharides, proteins whereas white pulp showed weak activity for acidic mucopolysaccharides and moderate to strong for neutral mucopolysaccharides.
- Localisation of more T-lymphocytes and macrophages in white pulp and ellipsoid implied that T-lymphocytes possibly act as major phagocytic cell in spleen.
- Capsule, trabeculae, white pulp and red pulp exhibited a weak to moderate activity of AKPase, G-6-Pase, SDH, LDH, NADP, NADPH and G-6 PD.
- In SEM, variety of nucleated cells and capillaries were surrounded by macrophages.
- In TEM, distinguished area of grouped non-nucleated red pulp and nucleated white pulp was seen with different cell types.

## REFERENCES

- Abed Muslih M H and Mirhish S H. 2017. Histomorphological study of the spleen in indigenous gazelle (*Gazella subgutturosa*). *The Iraqi Journal of Veterinary Medicine* **41**(2): 100-05.
- Alam M S, Awal M A, Das S K and Islam M N. 2005. Morphometry of spleen with special emphasis on its arterial circulation of indigenous horse In Bangladesh. *Bangl Journal of Veterinary Medicine* **3**(2): 166-68.
- Alex L, Rajan M L, Xavier B, Jacob P, Rani K D, Lakshmi G V. 2015. Microscopic study of human spleen in different age groups. *International Journal of Research in Medical Sciences* **3**(7): 1701-06.
- Alim A, Nurunnabi A S M, Ara S, Mahbub S, Mohanta L C. 2012. Comparative histological study on the spleen of human (*Homo sapiens*), cow (*Bos indicus*), goat (*Capra hircus*). *Nepal Journal of Medical Sciences* **1**(2): 64-67.
- Alshamarry H A. 2010. Histological and histometric study on the spleen of Iraqi camel (*Camelus dromedarius*). *Emirates Journal of Food and Agriculture* **22**(1): 65-70.
- Anagnostou C K, Anagnostopoulou I D, Tiniakos D G, Karandrea D, Agapitos E, Karakitsos P, and Kittas C. 2007. Ontogeny of intrinsic innervation in the human thymus and spleen. *Journal of Histochemistry and Cytochemistry* **55**(8): 813-20.
- Aw D, Hilliard L, Nishikawa Y, Cadman E T, Lawrence R A and Palmer D B. 2016. Disorganization of the splenic microanatomy in ageing mice. *Journal of Cells, Molecule, System and Technologies* **148**: 92-101.
- Bacha W J and Bacha L M. 2000. *Color Atlas of Veterinary Histology*. 2<sup>nd</sup> Edn, Lippincott Williams and Wilkins, London.
- Barka T and Anderson P J. 1963. *Histochemistry: Theory, Practice and Bibliography*. pp 237-316. Harper and Row Publishers, Inc, New York.
- Balogh P, Horvath G, and Szakal A K. 2004. Immunoarchitecture of distinct reticular fibroblastic domains in the white pulp of mouse spleen. *Journal of Histochemistry and Cytochemistry* **52**: 1287-98.
- Barnhart M I and Lusher J M. 1976. The human spleen as revealed by scanning electron microscopy. *American Journal of Hematology* **1**: 243-264
- Basir Z. 2017. Histomorphology of spleen in non immature Iranian camel (*Camelus dromedaries*). *CIBTech Journal of Zoology* **6**(3): 9-13.
- Bhupender S, Ingole S P, Chaurasia D, Giri D and Kumar P. 2013. Gross and histometrical study of spleen in black bengal goats. *Indian Journal of Small Ruminants* **19**(2): 179-81.

- Birjandi S Z, Ippolito J A, Ramadorai A K and Witte P L. 2011. Alterations in Marginal zone macrophages and marginal zone b cells in old mice. *Journal of Immunology* **15**: 1-11.
- Blue J and Weiss L. 1981. Electron microscopy of the red pulp of the dog spleen including vascular arrangements, periarterial macrophage sheaths (ellipsoids), and the contractile, innervated reticular meshwork. *American Journal of Anatomy* **161**: 189-218.
- Cesta M F. 2006. Normal structure, function and histology of the spleen. *Toxicologic Pathology* **34**: 455-65.
- Chayen J, Butcher R G, Bitensky L and Poulter L W. 1969. *A Guide of Practical Histochemistry*. pp 83-174. Oliver and Boyd, Edinbury, England.
- DAHDF, GOI. 2018. Annual Report 2017-18. Department of Animal Husbandry, Dairying & Fisheries Ministry of Agriculture & Farmers Welfare Government of India.
- Das S K, Alam M S, Islam M N and Awal M A. 2005. Histology of the spleen of indigenous dog (*Canis familiaris*) of Bangladesh. *Journal of Veterinary Medicine* **3**(1): 59-61.
- Dellmann H D and Brown E M. 2006. *Textbook of Veterinary Histology*. 6<sup>th</sup> Edn. Iowa: Blackwell Publishing, p. 147-52.
- Devi H, Mathur R and Joshi S. 2016. Histological studies on the spleen of Marwari goat (*Capra hircus*). *Veterinary Practitioner* **17**(2): 190-91.
- Dyce K M, Sack W O and Wensing C G J. 2010. *Text Book of Veterinary Anatomy*. 4<sup>th</sup> Edn. Saunders Elsevier, Missouri, pp. 765-67.
- Ellenport C R. 2012. *The Anatomy of the Domestic Animals*. 5<sup>th</sup> Edn. vol 1, pp. 180. W.B. Saunders, Philadelphia
- Fennel R A and Pearse A G E. 1961. Some histochemical observations on the bursa of fabricius and thymus of the chicken. *Anatomical Record* **139**: 93-103.
- Gautam A K and Mishra U K. 2015. Histochemical and transmission electron microscopic studies of spleen in prenatal stages of goat. *Indian Journal of Animal Research* **49**(1): 59-62.
- Geetha R and Vijayaragava C. 1999. Microanatomical studies on the spleen of laboratory animals. *Indian Journal of Veterinary Anatomy* **11**(1): 62-63
- Geetha R, Kannan T A and Sivakumar M. 2016. Age related histochemical changes in thymus, spleen and mesenteric lymph nodes in mice, rat and guinea pig. *International Journal of Science and Research* **5**(5): 1088-91.

- Geetha R, Sivakumar M and Vijayragavan C. 2001. Age related changes in the structure of spleen in laboratory animals. *Indian Journal of Veterinary Anatomy* **13**(2): 137-41.
- Gnanadevi R, Senthilkumar S, Kannan T A and Ramesh G. 2019. Comparative histoarchitectural study of splenic components in sheep and goat. *International Journal of Current Microbiology and Applied Sciences* **8**(5): 1387-94.
- Gupta V, Farooqui M M, Prakash A, Archana and Kumar P. 2017. Morphological and cytological differentiation of goat spleen (*Capra hircus*). *Indian Journal of Animal Research* **51**(6): 1027-32.
- Hande S T, Pansambale S A, Pansare P A, Gaikwad S A and Dhande P L. 2002. Studies on macro and microscopic structures of spleen in rat. XVII IAVA Convention Technical Bulletin, pp. 21.
- Hataba Y, Kirino Y and Suzuki T. 1981. Scanning electron microscopic study of the red pulp of mouse spleen. *Journal of Electron Microscopy* **30**(1): 46-56.
- Ibe C S, Onyeanusi I, Salami S O, Ajayi I E and Nzalak J O. 2010. On the structure of the spleen in the African gaint pouched rat (*Cricetomys gambianus*). *Veterinary Research* **3**(4): 70-74.
- Ikpegbu E, Nlebedum U C, Nnadozie O and Agbakwuru I O. 2014. The spleen of the African palm squirrel (*Epixerusebii*): A Micromorpholglcal Observation. *Journal of Veterinary Advances* **4**(6): 564-69.
- Kannan T A and Ramesh G. 2013. Light and electron microscopic details of blood spleen barrier in Nandanam chicken (*Gallus domesticus*). *International Journal of Science and Research* **4**(6): 2203-06.
- Karad N M, Bhosle N S, Mamde G S and Nimse R G. 2002. Microanatomy of spleen in goat (*Capra chircus*). xvll IAVA Convention Technical Bulletin. pp 34.
- Kaur J, Singh O and Pathak D. 2018. Enzyme histochemistry of hemal nodes of Indian buffalo (*Bubalus bubalis*). *International Journal of Livestock Research* **9**(3): 249-55.
- Khalel E M. 2010. Anatomical and histological study of the spleen in Iraqi sheep (Awasi sheep). *Basrah Journal of Veterinary Research* **10**(2): 163-71.
- Khan H, Miyandad P, Rahamtullah P and Muhammad M R. 2003. Biometrical study on normal spleen of cattle. *Journal of Animal and Veterinary Advances* **2**(2): 92-94.
- Khan M, Baba M A, Choudhury A R, Mansoor M. 2006. A comparative macroscopic study on the spleen of Musk deer (*Moschus moschiferus*) and goat (*Capra hircus*). *Zoos Print* **21**(8): 11-12.

- Kimura K, Fusano T and Tanaka Y. 2001. A scanning and transmission electron microscopic study of contractile in the rat spleen. *Okajimas Folia Anatomica Japonica* **78**(5): 187-200.
- Kumaravel A, Sivagnanam S and Paramasivan S. 2019. Scanning electron microscopy of paraffin embedded white pulp of spleen of goats. *International Journal of Chemical Studies* **7**(1): 26-28
- Kuper D F, de Heer E, VanLoveren H and Vos J G. 2002. Immune System. In Handbook of Toxicologic Pathology (W. M. Haschek, C. G. Rousseaux and M. A. Wallig, eds.), Vol. 2, pp. 585–646. Academic Press, San Diego.
- Luna L G. 1968. *Manual of Histological Staining Methods of Armed Forces Institute of Pathology*. 3<sup>rd</sup> Edn. pp 38-196. McGraw Hill Book Company, New York, USA.
- Maina M M, Usende I L, Igwenagu E, Onyiche T E, Yusuf Z M and Ntung N O. 2014. Gross, histological and histomorphometric studies on the spleen of one-humped camel (*Camelus dromedarius*) found in the semi-arid region of north eastern Nigeria. *Journal of Veterinary Advances* **4**(10): 703-11.
- Mebius R E and Kraal G. 2005. Structure and function of the spleen. *Nature Reviews Immunology* **5**: 606-16.
- Mishra U K, Singh G K and Chauhan R S. 2001. Gross biometrical attributes of the spleen of the developing chicks. *Indian Journal of Veterinary Anatomy* **13**(2): 159-62.
- Mishra U K, Singh G K and Chauhan R S. 2003. Histological and immunocytochemical development of spleen in chicken. XVIII IAVA Convention Souvenir and Abstr., pp. 32-33.
- Mukhia R, Mukherjee A and Sabnis A. 2016. Histogenesis of human fetal spleen. *International Journal of Anatomy Research* **4**(1):2119-24.
- Nolte M A, Hamann A, Kraal, G and Mebius R E. 2002. The strict regulation of lymphocyte migration to splenic white pulp does not involve common homing receptors. *Immunology* **106**: 299-307.
- Nolte M A, Arens R, Kraus M, van Oers M H J, Kraal G, van Lier R A W, and Mebius R E. 2018. B cells are crucial for both development and maintenance of the splenic marginal zone. *Journal of Immunology* **172**: 3620-27.
- Onkar D P and Govardhan S A. 2013. Comparative histology of human and dog spleen. *Journal of Morphology Science* **30**(1): 16-20.
- Pantic I, Paunovic J, Jovanovic G B, Perovic M, Pantic S and Milosevic N T. 2013. Age-related reduction of structural complexity in spleen hematopoietic tissue architecture in mice. *Experimental Gerontology* **48**: 926-32.

- Pathak D and Bansal N. 2011. A transmission electron microscopic study on the buffalo uterus during follicular and luteal phases of estrous cycle. *Indian Journal of Animal Sciences* **81**(7): 704-07.
- Pathak D, Bansal N, Singh O, Gupta K and Ghuman S P S. 2019. Immunohistochemical localization of estrogen receptor alpha (ER $\alpha$ ) in the oviduct of Indian buffalo during follicular and luteal phases of estrous cycle. *Tropical Animal Health and Production* **51**(6): 1601-09.
- Pathak D, Nagpal S K and Singh G. 2008. Scanning electron microscopic studies on uterus of sheep in follicular and luteal phases of estrous cycle. *Indian Journal of Animal Sciences* **78**(12): 1361-64.
- Pearse A G E. 1972. *Histochemistry: Theoretical and Applied*. 3<sup>rd</sup> Edn. Churchill Livingstone, London.
- Pereira-Sampaio M A and Marques-Sampaio B P de S. 2006. Anatomical study and proportional analysis of the pig spleen arterial segments. *Cells Tissues Organs* **182**: 32-34.
- Piao Z, Min H, Wei Y L J, He M, He Y and Xu D. 2018. Age-related histomorphological studies on the spleen of Chinese yellow quail. *INDIAN Journal of Animal Research* **15**: 1-4.
- Polak S, Galfiova P and Varga I. 2009. Ultrastructure of human spleen in transmission and scanning electron microscope. *Biologia* **64**: 402-08.
- Press C, Mc L and Landsverk T. 2006. Immune System. In: Eurell J A and Frappier B L (Eds.) *Textbook of Veterinary Histology*. 6<sup>th</sup> Edn. Blackwell Publishing, Iowa, 151.
- Radhika D, Saila R N, Kanchanlatha G, Murli M K V, Kumar L A and Hemiliamma N M. 2012. Prenatal histogenesis of human spleen. *Indian Journal of Public Health and Research* **3**(1): 129-31.
- Rahman N, Tandon R, Ghaus F, Moinuddin A, Akram W and Faruqi N A. 2016. Comparative anatomy of spleen: Histomorphometric study in human, goat, buffalo, rabbit and rat. *Academia Anatomica International* **2**: 28-32.
- Raju N K B, Lyyengar M P and Gopinath S. 2003. Histological study on the spleen of adult Indian goat (*Capra hircus*). *Indian Journal of Veterinary Anatomy* **15**(12): 44-47.
- Ramesh G, Kannan T A and Sivakumar M. 2016. Age related histochemical changes in thymus, spleen and mesenteric lymph nodes in mice, rat and guinea pig. *International Journal of Science and Research* **5**(9): 1088-91.
- Reshag A F and Hamza R A. 2017. Anatomical and histological changes in the spleen of post hatching indigenous chicken in Iraq. *The Iraqi Journal of Veterinary Medicine* **41**(2): 174-78.

- Sheehan D C and Hrapchak B B. 1973. *Theory and Practice of Histotechnology*. pp 80-172. The C V Masby Company, Saint Louis.
- Shringi N, Mathur R, Rohlan K, Kumar V and Ganguly S. 2017. Morphometry of Spleen in White Yorkshire Pig (*Sus scrofa*). *International Journal of Pure and Applied Bioscience* **5**(4): 755-57.
- Shringi N, Mathur R, Rohlan K, Kumar V and Ganguly S. 2018. Histological studies on the spleen of large White Yorkshire Pig (*Sus scrofa*). *Journal of Entomology and Zoology Studies* **6**(1): 1142-44.
- Sinha B, Ingole S P, Chaurasia D, Giri D and Kumar P. 2013. Gross and Histometrical study of spleen in Black Bengal goats. *Indian Journal of small Ruminants* **19**(2): 179-81.
- Sivagnanam S, Muthukrishnan S, Paramasivan S, Selvaraj J and Ronald B S M. 2017. Postnatal chronological morphometric study on the spleen of non-descript goats in Cauvery delta districts. *International Journal of Science, Environment and Technology* **6**(3): 2011-17.
- Souza A D, Bangera H, Ankolekar V H, Das A, Padmashali S, Souza A S D and Hosapatna M. 2015. Microscopic appearance of human spleen at different gestational age group: A fetal histological study. *Cukurova Medical Journal* **40**(1): 36-41.
- Smith C. 1969. Studies on the thymus of mammals XVI. Lipopigment cells in the cortex of the thymus of the mouse. *American Journal of Anatomy* **124**: 389-410.
- Song H, Peng K, Shenghe L I, Wang Y, Lan W E I and Tang L. 2012. Morphological characterization of the immune organs in ostrich chicks. *Turkish Journal of Veterinary and Animal Sciences* **36**(2): 89-100.
- Suri S, Sasan J S, Sarma K, Chakraborty D. 2017. Comparative gross and histomorphological studies in the spleen of sheep and goat of Jammu region of India. *Exploratory Animal and Medical Research* **7**(2): 179-83.
- Turner V M and Mabbott N A. 2017. Influence of ageing on the microarchitecture of the spleen and lymph nodes. *Biogerontology* **18**: 723-38.
- Usende I L, Okafor C L, Aina O O, Onyiche T E, Durotoye T I, Omonuwa A O, Jarikre T A, Maina M M and Falohun O O. 2014. Comparative studies and clinical significance of the spleens of Nigerian indigenous pig (*Sus scrofa*) and goat (*Capra hircus*). *Journal of Veterinary Advances* **4**(7): 604-12.
- Venkatesan S and Vijayaragavan C. 1999. Microanatomical studies on the spleen of Japanese quail (*Coturnix coturnix japonica*). *Indian Journal of Veterinary Anatomy* **11**(1): 61.

- Venkatesan S, Ramesh G and Vijayaragavan C. 2004. Histochemical studies on the spleen of the Japanese quail (*Coturnix coturnix japonica*). *Indian Journal of Veterinary* **16** (1-2): 42-44.
- Waghaye J Y, Banubakode S B, Charjan R, Rana J, Nandeshwar N C and Mainde U P. (2017). Age related histomorphological changes of splenic connective tissue in goat (*Capra hircus*). *Indian journal of Veterinary Science and Biotechnology* **13**(2): 1-6.
- Wilson D A, Sharma N N and Brown E R. 1972. Alkaline and Acid Phosphatase in Murine Leukemia. *Infection and Immunity* **5**(4): 461-66.
- Xue M, Baba T, Terada N, Kato Y, Fujii Y and Ohno S. 2001. Morphological study of erythrocyte shapes in red pulp of mouse spleens revealed by an in vivo cryotechnique. *Histology and Histopathology* **16**: 123-29.
- Zidan M, Kassem A, Dougbag A, Ghazzawi E E, Aziz M A E and Pabst R. 2000. The spleen of the one humped camel (*Camelus dromedarius*) has a unique histological structure. *Journal of Anatomy* **196**: 425-32.

## **VITA**

Name of the student : Harmanpreet Kaur  
Father's name : Mohan Singh  
Mother's name : Manjeet Kaur  
Nationality : Indian  
Date of birth : 16-10-1994  
Permanent home address : H/N 428A, New Sant Nagar, Gurdaspur  
E-mail : harmanpreetwahla@gmail.com

## **EDUCATIONAL QUALIFICATION**

Bachelor degree : B.V.Sc. & A.H.  
University : Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana  
Year of Award : 2017  
OCPA : 7.51/10.00  
Master's degree : M.V.Sc.  
OCPA : 8.65/10.00  
Awards/ Distinction : University Merit Fellowship for M.V.Sc.  
Fellowships/Scholarship : programme