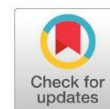


Research Article

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Anatomical and histological study of the spleen of Barqi sheep in Libya

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Abstract

The anatomical and histological characteristics of the spleen were investigated in ten apparently and clinically healthy adult rams Barqi sheep in Libya. Anatomical examination revealed that the spleen in this breed exhibited a triangular shape with rounded corners, obtuse margins and a distinct reddish-brown color. Also, biometric analysis revealed that the mean values of length, width, and weight were 13.3 ± 1.0 cm, 8.2 ± 0.9 cm, and 113.2 ± 30.9 gm respectively. Histological examination demonstrated that the spleen of the Barqi sheep is enveloped by a delicate fibrous capsule composed of collagen fibers and smooth muscle fibers. Trabeculae of varying thickness extend from the capsule into the splenic parenchyma which composed of red and white pulp, these trabeculae were composed of smooth muscle fibers with sparse distribution of collagen and each trabecula was surrounded by peritrabecular sinus. The white pulp composed of splenic follicles most of them had light germinal center. The splenic follicle was surrounded by marginal zone and there was no distinct demarcation between red and white pulp. The red pulp consisted of splenic cords and sinusoids.

Keywords: Gross anatomy, morphometric measurements, Histological, Spleen, rams Barqi sheep

قسم التشريح والأنسجة وعلم الأجنة،
كلية الطب البيطري، جامعة عمر
المختار، البيضاء، ليبيا.

دراسة تشريحية ونسجية لطحال الأغنام البرقية في ليبيا المستخلص:

تم دراسة الخصائص المورفومترية والنسجية للطحال في عشرة كباش بالغة سليمة ظاهرياً وطبياً من الخراف البرقية في ليبيا، وقد أظهر الفحص التشريحي أن الطحال في هذه السلالة مثلث الشكل ذو زوايا مستديرة وحواف غير حادة، ولون بني محمر مميز، كما كشف التحليل البيومتري أن متوسط قيم الطول والعرض والوزن كانت 13.3 ± 1.0 سم و 8.2 ± 0.9 سم و 113.2 ± 30.9 سم جم على التوالي، وقد أظهر الفحص النسيجي أن طحال الخروف البرقي محاط بمحفظة ليفية رقيقة تتكون من ألياف كولاجينية وألياف عضلية ملساء، ويمتد من المحفظة تريباق (حويجزات) ذات سماكة متفاوتة إلى لحمة الطحال التي تتكون من لب أحمر وأبيض، وقد تكونت هذه الحويجزات من ألياف عضلية ملساء مع توزيع متناثر للألياف الكولاجينية، وكل حويجز كان محاطاً بجيب حول حويجز، وقد لم تكون اللب الأبيض من جريبات طحالية معظمها ذات مركز جرثومي فاتح، وكانت محاطة بمنطقة هامشية يكن هناك حد فاصل واضح بين اللب الأحمر واللبن الأبيض، وقد تكون اللب الأحمر من حبال وجيوب طحالية.

الكلمات المفتاحية: التشريح العياني، القياسات البيومترية، علم الأنسجة، الطحال، كباش أغنام برقية

Introduction:

Sheep (*Ovis aries*) represent a cornerstone of livestock production, serving as a primary source of meat, milk, and wool (Holocomb, 1994). It is well-established that lymphoid tissues play a pivotal role in systemic defense mechanisms against pathogenic microorganisms (Khalel, 2010). While the immunohistochemical properties of splenic compartments have been characterized in bovine (Keresztes et al., 1996) and certain ovine species (Gupta et al., 1998), precise data regarding cellular composition remains essential for understanding the organ's immunological function and diagnosing various animal pathologies (Ngeranwa et al., 1993).

As the largest secondary lymphatic organ, the spleen is specialized to mount immune responses against blood-borne antigens (Elhussieny et al., 2022; Madhu et al., 2023). Its structural organization, particularly the spatial relationship between the red and white pulp, is governed by vascular distribution and varies significantly across species (Bhagyalakshmi, 2025). Although lymphoid organs persist throughout a mammal's lifespan, they undergo age-related transitions; for instance, the spleen transitions from an embryonic hematopoietic site to a lifelong producer of lymphoid cells (Krause and Cutts, 1994).

Despite previous studies on caprine splenic morphology (Waghaye et al., 2017; Gupta et al., 2017). Historically, the splenic surface is described as being covered by a mesothelium of simple squamous cells, although some reports suggest a discontinuous layer overlying the parenchyma (Gupta et al., 2017). Histologically, the red pulp serves as a reservoir for erythrocytes, reticular cells, and macrophages (Yogesh, 2007). The scarcity of information and published research on the anatomical and histological structure of the spleen in Barki sheep in Libya encouraged us to conduct this research to provide information on the morphological and histological structure of the spleen and to encourage scientific research on these animals in Libya.

Material and methods:

This study was conducted on ten spleen samples (5 for morphological and morphometric study and 5 for histological study) obtained from apparently and clinically healthy adult rams Barqi sheep (*Ovis aries*) weighted about 15 Kg and aged 6-8 month freshly slaughtered for human consumption at the municipal slaughterhouse at El Bayda city, Eastern Libya for anatomical and histological studies. Immediately after animals slaughter the splenic specimens were excised and transported to the laboratory in a icebox to maintain tissue integrity for subsequent anatomical and histological evaluation.

Gross anatomy evaluation and morphometric measurements including splenic length, width, thickness, and absolute weight were performed on 5 samples. Additionally, the total body weight of each animal was recorded to establish the relative organ-to-body weight ratio. This index was calculated according with the formula established by Federova (1987):

$$\text{Relative spleen weight} = \frac{\text{weight of spleen}}{\text{body weight}} \times 100$$

Data analysis:

The excel programs were used to analysis and draw the figure, and the data were presented as mean $\pm$ standard error values.

Histological study was conducted on 5 samples of spleen. After animals slaughter the splenic specimens were harvested and small pieces (3mm) from each sample were fixed in fresh prepared Bouin's fixative for 24hr, then the samples were washed with 70% alcohol to remove excess of picric acid from the tissues and dehydrated in graded series of ethanol cleared in xylol, embedding in paraffin wax, sectioned by rotary microtome (Leica RM 2125) at 5 μ m thick then the histological sections stained with Harri's Hematoxylin and Eosin (H&E) and Crossman's trichrome stain according to Bancroft and Gamble (2008). Stained sections were examined under light microscope with digital camera (Nikon Eclipse E400, Japan).

Results:

Gross anatomical observation revealed that the spleen of Barqi sheep had triangular shape with rounded corners, obtuse margins, reddish-brown color in fresh state and granular appearance which appeared as white spots in the splenic parenchyma. The spleen was found to consist of two surfaces include parietal convex surface and visceral concave surface containing the hilus. There were also two ends in the spleen include broad, thick basal dorsal extremity (edge) and narrow, thin ventral extremity. It was also found that the spleen covered by thick transparent fibrous capsule. The average length width and the weight of the spleen were 13.3 ± 1.0 cm, 8.2 ± 0.9 and 113.2 ± 30.9 gm respectively (Figs.1-3).

Histological examination of the spleen sections stained with H&E and Crossman's trichrome stains revealed that the spleen was surrounded with a thick fibrous capsule formed of outer collagen fibers interspersed with fibroblasts and inner smooth muscle fibers from which extended trabeculae with different length and thickness to the splenic parenchyma. The trabeculae consisted mainly of smooth muscle fibers with very few sporadic collagen fibers. Each trabecula was surrounded by peritrabecular sinus which appeared with subcapsular sinus engorged with blood cells. The splenic parenchyma was clearly found to consist of splenic white pulp and splenic red pulp. The white pulp consisted of splenic follicles that showed varying size and round to oval shape had prominent eccentrically placed arteriole branched from the central artery and surrounded by periarterial lymphatic sheath. These follicles consisted of densely packed small and medium sized lymphocytes with different type of cells as few plasma cells, macrophages and granulocytes also, most of the splenic follicles had light germinal centers. The splenic white pulp was surrounded by the slightly darker marginal zone and there was less clear distinct demarcation between red and white pulp. Splenic red pulp occupied the interstitial spaces between the white pulp and consisted of splenic cords which consisted of diffusely distributed cells mainly of lymphocytes, erythrocytes, granulocytes, macrophages and occasional scattered large megakaryocytes which characterized by acidophilic cytoplasm and lobated nuclei and irregular splenic sinusoids which lined by endothelial cells with large nuclei protruding into the sinusoidal lumen (Figs.4A-L).

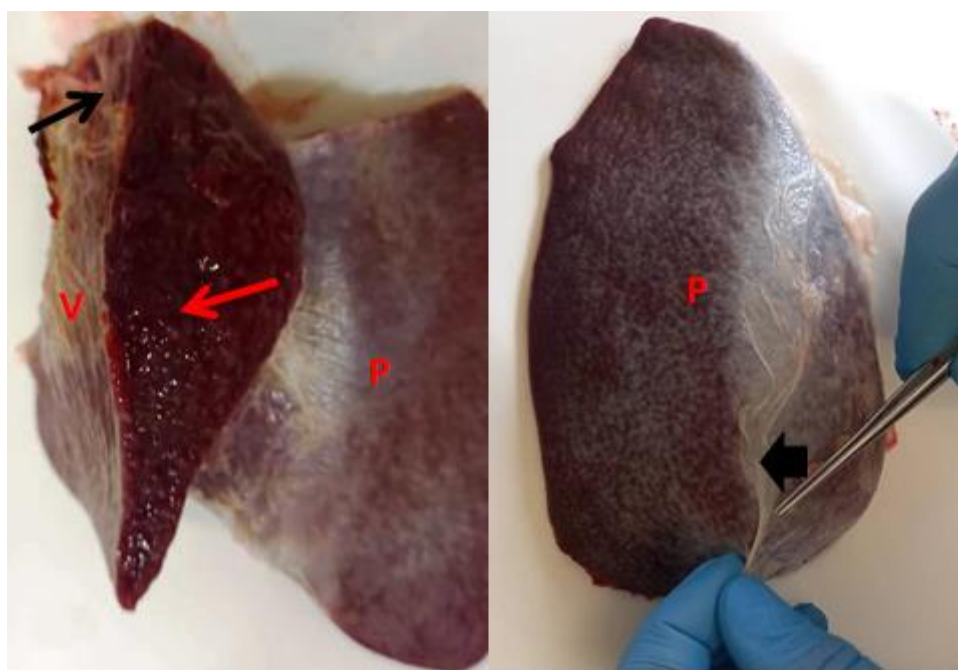


Fig. (1): A photograph of the spleen of Barqi sheep showing triangular shape of spleen with rounded corners and reddish-brown color, parietal surface (P), visceral surface (V) containing the hilus (Arrow), thick transparent fibrous capsule (Arrowhead), granular appearance which appeared as white spots in the splenic parenchyma represent the splenic follicles in the cut surface of spleen (Red arrow).

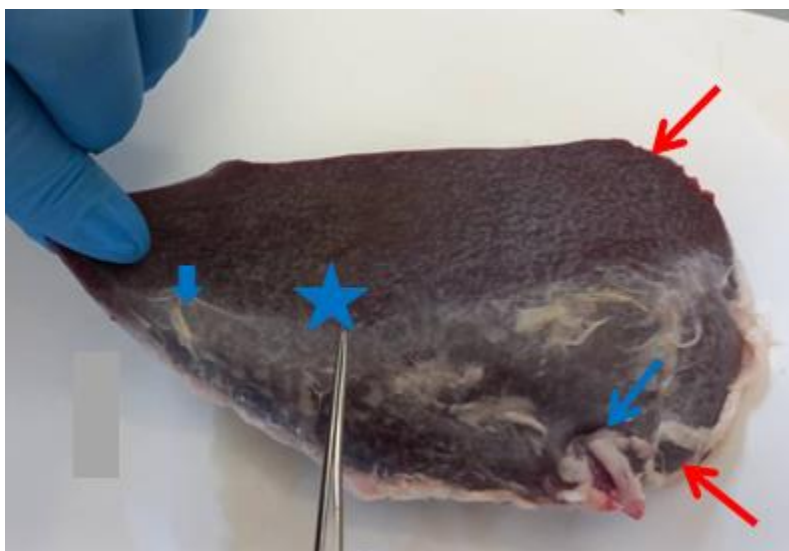


Fig. (2): A photograph of the spleen of Barqi sheep showing reddish-brown color, triangular shape of spleen with rounded corners (Red Arrows) and granular appearance of visceral surface (Star) containing the hilus (Blue Arrow), thick transparent fibrous capsule (Arrowhead).

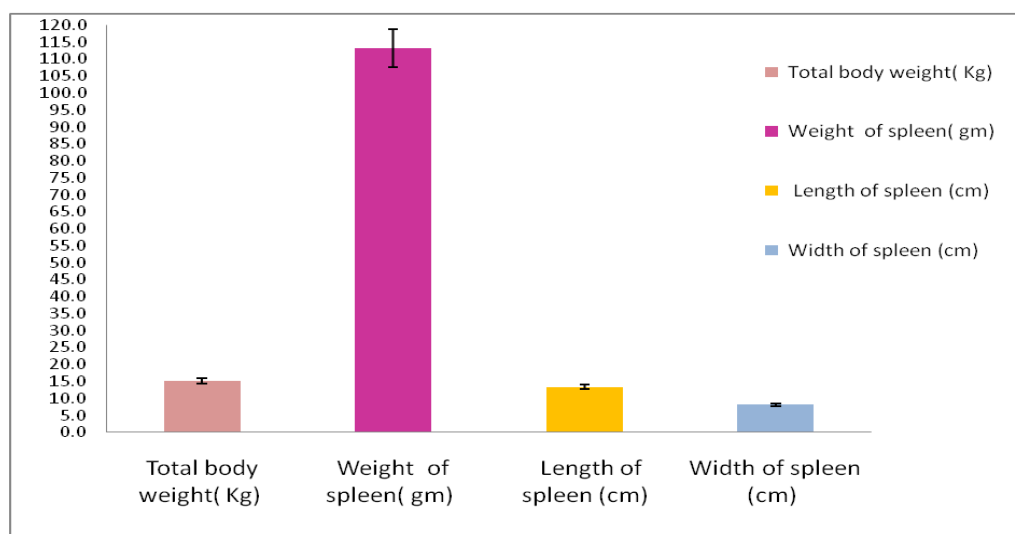


Fig. (3): Diagram showing the average length width and the weight of the spleen

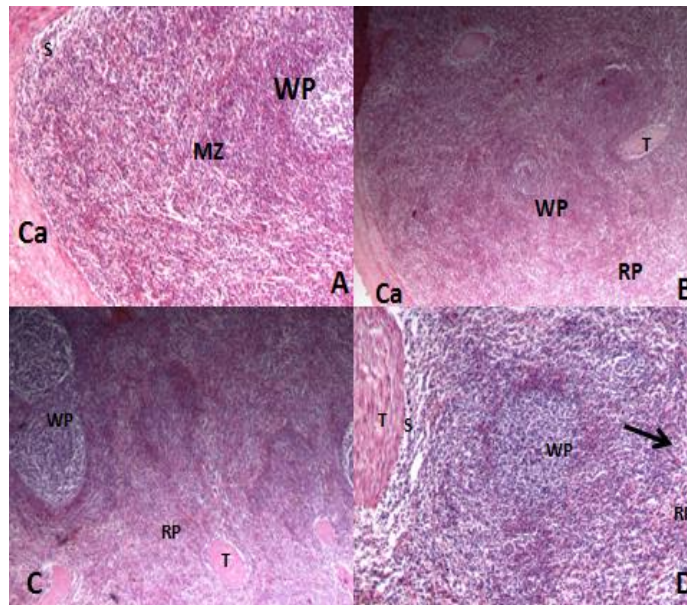


Fig. (4A-D): Photomicrographs of a transverse histological sections of spleen of Barqi sheep ram showing capsule (Ca), trabeculae with different thickness (T), peritrabecular sinus (S), white pulp splenic follicles (WP), red pulp (RP), marginal zone (MZ), megakaryocyte (Arrow) (H and E stain , X 200).

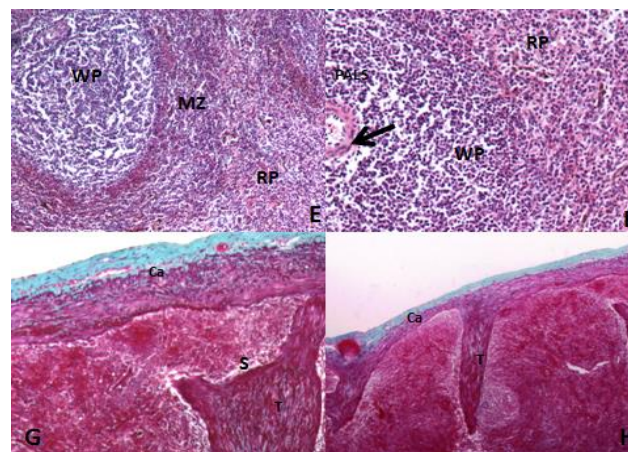


Fig. 4 (E-F): Photomicrographs of a transverse histological sections of spleen of Barqi sheep ram illustrating the microanatomical architecture. Panels E-F (H&E stain, X 200) showing organization of the white pulp (WP) with central artery (Arrow) surrounded by periarterial lymphatic sheath (PALS), marginal zone (MZ) and red pulp (RP) consist of splenic cords and sinusoids. Panels G-H (Crossman's Trichrome, X 200) showing capsule (Ca), trabeculae with different thickness (T) consists mainly of smooth muscle fibers with red color and a few sporadic collagen fibers with green color, peritrabecular sinus (S) engorged with blood cells.

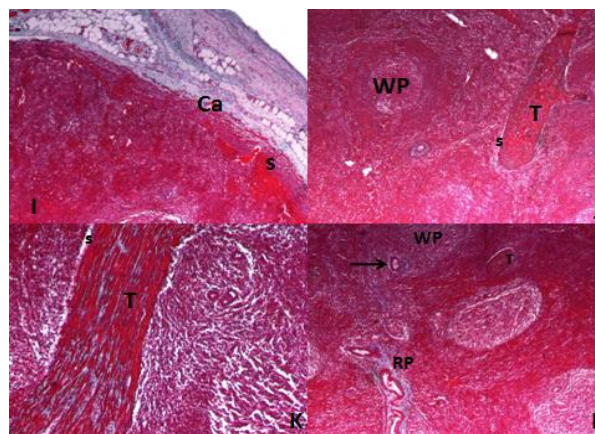


Fig. (4I-L): Photomicrographs of a transverse histological sections of spleen of Barqi sheep ram showing capsule (Ca), trabeculae with different thickness (T) consists mainly of smooth muscle fibers with red color and a few sporadic collagen fibers with green color, pericapsular and peritrabecular sinuses (S), white pulp splenic follicle (WP), central artery (Arrow), red pulp(RP) (Crossman's Trichrome, X200).

Discussion:

Our findings regarding the triangular shape, color, borders, parietal convex surface and visceral concave surface with hilus of the spleen in Barqi sheep were consistent with Suri *et al.*, (2017) in sheep of Jammu region in India, Khalel (2010) in adult Awasi sheep and Elhussieny *et al.*, (2022) in Barki Sheep. Thanvi *et al.*, (2021) reported that the shape of the spleen in sheep (*Ovis aries*) was somewhat triangular with round corners, two surfaces, three borders and had bluish red to dark purple color. Spleen of sheep had three borders was in accordance with the findings of Thanvi *et al.*(2021) . On the contrary the different shape and color of the spleen in other animals were documented Elhussieny *et al.*, (2022) reported that the color of the bovine spleen varies according to the age and sex of the animal. Our findings regarding the length width and the weight (13.3 ± 1.0 cm, 8.2 ± 0.9 and 113.2 ± 30.9 gm respectively) of the Barqi sheep ram spleen were slightly larger than those documented by Khalel (2010) who reported that the average measurements of length, width and weight of 10 spleen of adult Awasi sheep were 9.93 ± 0.342 , 6.48 ± 0.233 and 69 ± 6.663 gm respectively and relatively similar observation was documented by Thanvi *et al.*, (2021) who reported that the average length, width and weight of the spleen in 30 apparently healthy adult sheep (*Ovis aries*) of either sex was 10.03 ± 0.10 cm, 4.29 ± 0.35 and 39.576 ± 2.034 gm respectively. The dimensions of the spleen vary by sex and age and the splenic dimensions were higher in males than females (Demissie *et al.*,2021). Lee *et al.*(2021) add that the spleen length is influenced by age, sex, and body mass .

Our results revealed that the Barqi sheep spleen was surrounded by a thick fibrous capsule of collagen and smooth muscle fibers send trabeculae consist mainly of smooth muscle fibers with very few sporadic collagen fibers. Our findings were in agreement with Eurell and Frappier (2013) who reported that the spleen surrounded by a thick capsule of connective tissue composed of two layers of outer dense irregular connective tissue and inner smooth muscle fibers and the thickness and amount of smooth muscle vary with the species and the same author also confirmed extend trabeculae from the capsule and the hilus into the parenchyma of the spleen. Similar finding was also noticed in the spleen of adult Awasi sheep by Khalel (2010), and also (Suri *et al.*, 2017) showed that the sheep had a thick fibro-muscular capsule and from the innermost zone of capsule, connective tissue trabeculae extended into the splenic parenchyma in form of bands. Similar findings in domestic animals were reported Dellmann and Brown (1987). (Zidan *et al.*,2002) also reported that the camel spleen was composed of a thick capsule surrounding the splenic parenchyma. The cap-

sule is divided into outer layer consists mainly of connective tissue including collagen and inner layers is composed predominantly of smooth muscle cells. The presence collagen and muscle fibers in parallel orientation in the capsule and trabeculae help in changing the volume of spleen and pumping out the excess blood in circulation (Shringi *et al.*, 2018). Also, (Gnanadevi *et al.*, 2019) reported that the spleen in both sheep and goat was surrounded by a thick fibro-elastic capsule predominantly composed of collagen fibres with elastic and smooth muscle fibres. Cesta (2006) and Suri *et al.*, (2017) reported that the presence of smooth muscle fibers in the capsule and trabeculae indicated the capacity of spleen to expand and store large number of erythrocytes and also the ability for rapid contraction.

Our findings regarding splenic parenchyma are consistent with other mammalians in previous study by Khalel (2010) and (Gnanadevi *et al.*, 2019) in sheep. Also, similar architecture of the splenic parenchyma was documented by (Waghaye *et al.*, 2017) and Devi *et al.* (2016) in goats and by Mirhish (2018) in gazelle. Similar histological architecture of the spleen of sheep and goat was also reported by (Suri *et al.*, 2017) but the same author add that there was clear demarcation between white and red pulp. The demarcation was more distinct in goat as compared to sheep. (Nicander *et al.*, 1993) reported that lymphocytes, macrophages, other accessory cells and all cellular elements of blood were present in white pulp in domestic animals. (Copenhaver *et al.*, 1975) stated that the plasma cells and macrophages were present on the germinal centers, which were developed due to antigenic stimulation necessary for increasing phagocytic activity. (Zidan *et al.*, 2002) reported that the red pulp in one humped camel (*Camelus dromedarius*) is characteristically divided into cords by secondary trabeculae and contains venous sinusoids of different sizes.

Conclusion: Barqi sheep spleen characterized by fibrous capsule composed of collagenous and smooth muscle fibers and the splenic parenchyma composed of white pulp and red pulp as another mammalian with slight different in morphological and histological architecture specialized for Barqi sheep. Future research utilizing immunohistochemical markers, electron microscopy study and Polymerase chain reaction (PCR) technology are recommended.

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