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Development and Maturation of the Dromedary Spleen: Anatomical and Histological Analysis During the First Three Years of Life

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Development of the Dromedary's spleen

Abstract

BACKGROUND: The dromedary spleen plays a crucial role in the immune system and maintaining homeostasis. However, there is limited research on the developmental changes that occur in the spleen during the first few years of a dromedary's life

OBJECTIVES: The objective of this study was to comprehensively investigate the developmental changes that transpire in the dromedary spleen during the crucial first three years of life. This was achieved through a multi-faceted approach, involving both macroscopic examination, which entailed measuring the absolute and relative mass of the spleen, as well as morphometric analysis. Additionally, histological and histomorphometric analysis were employed to study the cellular composition of the spleen at different ages.

METHODS: Five groups of dromedaries were examined in Southeastern Algeria. The spleens were analyzed using both gross anatomy and histological examination. The cellular composition of the spleen was studied on histological slides of spleens of different ages, which were stained with hematoxylin and eosin.

RESULTS: The results showed that the size, weight, and volume of the spleen significantly increased as the animals aged. The splenic mass of the animal of the first group showed an average absolute mass of 251±14.19 g, whereas in the fourth group they showed an average mass of 318±23.91 g, while the length, the width and the thickness showed average values of 43.78±1.95 cm, 19.44±1.52 cm, and 1.88±0.16 cm respectively at the age of eight months, while they showed average values of 49.6±1.86 cm, 24.32±1.69 cm and 3.18±0.34 cm respectively at the age of three years. The cellular composition of the spleen changed over time, with a higher percentage of lymphoid tissue at eight months, more red pulp at one year, and a higher

percentage of white pulp at two years. By three years, the spleen had a mature mix of red and white pulp and its immune function was fully developed.
CONCLUSION: This study provides new insights into the development and maturation of the dromedary spleen. The findings have important implications for understanding the health and well-being of these animals, and could potentially contribute to the development of better strategies for the management and care of dromedaries.

Keywords*: Cytology*, *Dromedary*, *Histology*, *Spleen*, *Maturation.*

Introduction

The dromedary, or one-humped camel (*Camelus dromedarius*), is an important livestock animal in dry, arid regions of the world (Nagy *et al*., 2020), dromedaries are adapted to survive in harsh environments with limited access to water, and are used for a variety of purposes including transportation, milk production, and meat (Ho *et al*., 2022). They have a unique physiology that allows them to survive prolonged periods without water, and they are able to tolerate high body temperatures and maintain their body water balance, in addition to their physiological adaptations, dromedaries also have a complex immune system that helps them combat disease and maintain health (Gossner *et al*., 2016; Hajinejad-Bamroud *et al*., 2020; Mohamed Amine *et al*., 2023; Fares *et al*., 2023c).

The spleen is a vital organ in the immune system of all mammals, including dromedaries. It functions as a blood filter, storing red blood cells and releasing them, when necessary, as well as storing and activating immune cells. The spleen also plays a role in the removal of senescent erythrocytes, or red blood cells that are no longer functional. In addition, the spleen is involved in the immune response to infections, and it contains a variety of immune cells such as T cells, B cells, and macrophages (Mebius and Kraal, 2005).

The first three years of life for dromedaries are critical for their overall development and productivity. During this period, they undergo significant physiological changes that affect their

growth and immune system. Understanding the changes that occur during this period is important for animal production and management, as it can help identify potential health problems and implement appropriate interventions.

One important aspect of dromedary development during the first three years of life is the maturation of the spleen, which is a vital organ in the immune system of all mammals. The spleen plays a crucial role in filtering the blood and activating immune cells to combat infections. However, little is known about the development and maturation of the dromedary spleen during this critical period (Fares *et al*., 2023a; Fares et al., 2023b).

The development and maturation of the dromedary spleen is not well understood, with the current state of knowledge, and more research is needed to fully understand the immune function of these animals (Hussen and Schuberth, 2021).The findings of this study contribute to our understanding of the development of the dromedary spleen and may have practical implications for the management and care of these animals, also, understanding the changes that occur in the spleen during the first three years of life may help veterinarians to identify potential health problems and implement appropriate interventions. Additionally, this information may be useful for researchers studying the immune function of dromedaries and developing vaccines and other preventive measures to protect these animals from diseases.

Despite the importance of the spleen in the immune system of dromedaries, the development and maturation of this organ is not well understood (Lewis *et al*., 2021), few researches were carried out. In particular, there is a lack of information on the changes that occur in the gross and microscopic anatomy, size, and cellular composition of the dromedary spleen during the first three years of life. This knowledge gap limits our understanding of the immune function of these animals and hinders the development of effective management strategies for dromedary health. However, there have been some studies conducted on the postnatal development of the spleen in other domestic animals, such as rabbits (Rahmoun *et al*., 2019) and lambs (Rahmoun *et al*., 2020), which may provide some insights into the developmental changes that occur in the dromedary spleen.

Materials and methods

In this study, 25 healthy dromedary camels were selected and divided into five age groups (eight months, one year, 18 months, two years, and three years of age), consisting of five animals per group. The animals were sourced from a single herd to control for any extraneous variables that might affect the outcome of the study (Cero *et al*., 2021). The animals were humanely slaughtered in adherence to the Algerian Islamic customs and in accordance with stringent sanitary protocols, under the supervision of licensed veterinarians. The ages of the dromedaries were obtained through a survey of their owners. The bodyweight of the dromedaries was determined by using a specific equation, which takes into account various physical measurements of the dromedaries to estimate their bodyweight, according to (Kamili *et al*., 2006; Gherissi *et al*., 2022). The spleens were meticulously collected and subjected to both morphometric and mass analysis. The gross anatomy of the spleen was carefully documented through detailed description and photographs taken with a digital camera. The size and weight of the spleen were also measured using established techniques to ensure accurate and precise data collection.

For the microscopic study, the spleen fragments were fixed in formalin and processed for paraffin embedding. Five-micrometer sections were cut and stained with both Hematoxylin and Eosin (H&E) and Masson Trichrome staining techniques to highlight the various cell types and tissues in the spleen. The stained sections were examined under a B382PLi-ALC light microscope t x400 magnification (Iezzoni, 2018).

The statistical results are expressed as mean ± standard deviation of the mean, also, a One Way ANOVA was performed to compare the differences between the different age groups. Additionally, Pearson correlation analysis was conducted to evaluate the relationship between the different physical factors of the spleen. The statistical analysis was conducted using R

software (Posit, PBC, USA, 2011-2023). This allowed us to determine if there were any significant differences between the groups and to identify any potential correlations between the various physical characteristics of the spleen.

The equation of estimation of the dromedary's bodyweight according to (Kamili *et al*., 2006):

Bodyweight (kg) = 4.06 x Age (years) + 3.05 x Circumference of the neck (cm) + 3.38 x Circumference of the thigh (cm) + 1.38 x Hump length (cm) - 191.

Results

Our investigation into the characteristics of the one-humped camel's spleen has revealed a number of intriguing details about this organ. Notably, we have discovered that it is characterized by a soft texture and is colored in a distinctive shade of red-brown, as indicated by our observations and depicted in (Figure 1). However, one of the most remarkable revelations to emerge from our study is that the spleen of camels possesses a concave surface, which represents a unique and previously unknown feature of this anatomical structure.

Our results also revealed that the spleen of the one-humped camel is located under the transverse processes of the lumbar vertebrae and extends from the upper caudal end of the dorsal sac of the rumen to the caudal end of the left kidney, it has two surfaces: a convex parietal surface in contact with the internal obliques muscle and the subcutaneous region, and a concave visceral surface in contact with the upper-caudal surface and the left face of the dorsal sacrum for the rumen. The dorsal end of the spleen attaches to the upper surface of the dorsal sac of the rumen, while the caudal edge wraps around.

The results of this study revealed a range of variation in the physical characteristics of dromedaries within different age groups, the dromedaries in the first group, which were 8 months old, presented splenic masses of 251±14.19 g and body weights of 339.4±9.24 kg, whereas the relative mass of the in the animals of this group was 0.0738±0.0036 %, with a

splenic length of 43.78±1.95 cm. The width of their spleen was 19.44±1.52 cm, with a splenic thickness of 1.88±0.16 cm (Figure 2).

On the other hand, the dromedaries of the second group (1 year of age), presented splenic masse was 267±12.59 g and body weight of 364.2±9.36 kg. While the relative mass of their spleen was 0.0732±0.0043 %, with a splenic length of 45.7±1.42 cm, while the width of their spleen was 19.8±1.69 cm, with a thickness ranging from 2.24±.29 cm (Figure 2). Similarly, the dromedaries of the third group (18 months old), had splenic masse of 280±21.02 g with a mean body weight of 368.5±5.31 kg, while the relative mass of their spleen was 0.0774±0.0083 %, with a splenic length of 46.2±1.62 cm, whereas the splenic width was 21.7±1.78 cm, with a splenic thickness of 2.58±0.24 cm (Figure 2).

The dromedaries in the fourth group, which were two years old, presented splenic masse of 297±12.98 g with body weight of 404.6±10.41 kg, whereas the relative mass of their spleen was comprised between from 0.0736±0.0021 %. The splenic length in the animals of the fourth group was 47.42±1.46 cm. with a splenic width of 22.1±1.99 cm, and splenic thickness 2.89±0.32 cm. Finally, the dromedaries in the fifth group (three years old), had splenic masse of 318±23.91 g and body weight of 426.2±5.4 kg. whereas the relative mass of their spleen was comprised between 0.0748±0.0058 %. The splenic length was 49.6±1.86 cm, with a splenic width of 24.32±1.69 cm, and a splenic thickness of 3.18±0.34 cm (Table 1) (Figure 2).

To further analyze the differences between the groups, a One Way ANOVA was performed. The results of the ANOVA showed that there was a significant difference between the groups in terms of splenic mass ($p < 0.001$), relative mass of the spleen ($p < 0.001$), splenic length ($p < 0.001$), splenic width ($p = 0.017$), and thickness ($p < 0.001$), (Table 2;3;4;5). In all tables, the p-value is less than 0.05, indicating that there is a significant difference in the mass, length, width, and thickness of the dromedary spleen across different age groups.

Regarding the histological analysis of the spleen of the dromedary (*Camelus dromedarius*), our results revealed a unique structure that is characterized by the presence of white pulp and red pulp. The white pulp was composed of lymphoid follicles, which appear

densely packed with lymphocytes, macrophages, and dendritic cells, and they were surrounded by a dense network of reticular fibers, which form the framework for the follicles and provide structural support (Figure 3).

On the other hand, the red pulp of the spleen was composed of sinusoids, which are large, thin-walled blood vessels that are lined with macrophages and lined by reticular cells. It was also rich in blood vessels and is responsible for maintaining the blood volume and blood pressure.

Additionally, we found that the splenic capsule is a thin layer of fibrous tissue that surrounds the spleen and separates it from the surrounding organs and sending trabecular tissue towards the inner layers of the organ (Figure 4),

The cellular composition of the spleen changes significantly over time, with significant differences in term of cellular populations and density, at eight months of age, the spleen contains mostly lymphocytes and a small number of erythrocytes and macrophages, whereas at one year of age, the number of erythrocytes increases while the number of lymphocytes decreases. At two years of age, the spleen presented a higher rate of macrophages and a lower number of erythrocytes, while at 3 years, the spleen presented a highest rate of lymphocytes, which suggests that the immune function of the spleen is fully developed by three years of age and the organ is fully functional in carrying out its various roles in the immune system.

Our results revealed that the spleen undergoes developmental changes over time in both its structure and immune function, at eight months of age, the spleen was composed mostly of lymphoid tissue and a sparse population of splenic cords, whereas at one year of age, the number of splenic cords increases and the spleen contains more red pulp, which is important for the removal of senescent red blood cells and storage of iron, at two years of age, the spleen contains a higher ratio of white pulp, and the splenic cords become more prominent. By three years of age, the spleen presented a mature mix of red and white pulp and has fully developed its immune function,

Our statistical results revealed a moderate to strong positive correlation between age and various physical characteristics of the spleen in one-humped camels. As the age of the animals increased, there was a corresponding increase in the thickness, length, and mass of the spleen. These findings suggest that there may be a physiological relationship between the age of the dromedaries and the development of their spleen

Additionally, there was a moderate positive correlation between the width of the spleen and age, indicating that as the dromedary ages, there may also be a slight increase in the width of their spleen. Furthermore, our study found a strong correlation between splenic mass and its thickness, width, and length. This suggests that there is a strong relationship between the size and dimensions of the spleen and its overall mass, also, we were able to observe a strong positive correlation between the body weight of the camels and the mass of the splenic mass, indicating that as the body weight of the camels increases, so too does the mass of their spleen (Figure 5).

Our findings indicate that there are clear correlations between the age of dromedaries and the physical characteristics of their spleen. Also, the correlations between splenic mass and its thickness, width, and length, as well as the correlation between the body weight of the dromedaries and the mass of their spleen, suggest that there may be strong relationships between these factors.

Discussion

The one-humped camel's spleen is an important organ that has been the subject of numerous studies. Despite this, our recent research has revealed a surprising discrepancy in earlier reports regarding the anatomy of the spleen.

Specifically, we found that the groove between the dorsal end of the spleen and the body, which had been described in previous studies by (Zidan *et al.,* 2000a; Nawal and Maher, 2018), was not present in our observations. This unexpected finding raises important questions

about the structure and function of the one-humped camel's spleen and highlights the need for further investigation.

Our results on the anatomy of the dromedary's spleen have yielded some intriguing findings. Perhaps the most fascinating among them is the discovery that the spleen of camels has a concave surface, which differs from the convex surface typically found in other mammals such as cows, sheep, and horses.

This unique characteristic of the camel's spleen sets it apart from other animals and underscores the importance of comparative anatomy in understanding the evolutionary history and functional significance of this organ. Previous studies by (Chadburn, 2000; Khalel, 2010; Maina *et al.,* 2014; Nawal and Maher, 2018) have also highlighted the distinctive features of the camel's spleen, including its coloration and texture.

Moreover, our study revealed that the shape of the camel's spleen is also distinct from other animals. Cows have an elongated and elliptical spleen, while sheep have an almost round shape. Dogs have a sickle-shaped spleen, and cats have a broad, curved, flattened, and elongated one, according to (Hassankhani *et al*., 2017; Wang *et al*., 2021).

Also, the researcher (Jaji *et al*., 2019) conducted a study on the anatomical and histological characteristics of the spleen in one-humped camels and reported similar observations regarding the location and surfaces of the camel's spleen. The concave surface of the spleen is particularly noteworthy, as it differs from the convex surface typically found in other mammals. The researcher (Sty and Conway, 1985) studied the histology of the camel's spleen and reported that the spleen undergoes a significant increase in size and mass as the animal grows. Similarly, (Kamath *et al*., 2000) investigated the gross anatomy of the camel's spleen and found that the organ's size and weight increased with age.

Our results about the morphometric variables provide further evidence of the changes in the one-humped camel's spleen as the animal grows. The significant differences in the mass, length, width, and thickness of the spleen across different age groups suggest that the organ undergoes structural adaptations to meet the needs of the growing animal.

Regarding the histological structure of the dromedary's spleen, (Zidan *et al*., 2000b) reported that the red pulp of the camel's spleen is composed of sinusoids lined with macrophages and reticular cells. Our study supports this finding, indicating that the red pulp of the spleen in one-humped camels is also composed of sinusoids and rich in blood vessels, suggesting that the spleen plays an important role in regulating blood volume and pressure.

Furthermore, our study found that the splenic capsule is a thin layer of fibrous tissue that separates the spleen from surrounding organs and sends trabecular tissue toward the inner layers of the organ. These results are consistent with previous research by (Zidan *et al*., 2000a; Zidan *et al*., 2000b), indicating that the structure of the splenic capsule in one-humped camels is similar to that of other mammalian species.

The results of our study also showed that the red pulp of the spleen is composed of sinusoids that are rich in blood vessels and responsible for maintaining blood volume and pressure, which is consistent with the function of the red pulp reported by (Fares *et al*., 2023a). Furthermore, our study found that the splenic capsule is a thin layer of fibrous tissue that surrounds the spleen and separates it from the surrounding organs, which is in agreement with the results reported by (Fares *et al*., 2023a).

Finally, the correlations observed in our study between the physical characteristics of the spleen and the age and body weight of the dromedaries are consistent with the results reported by (Brendolan *et al*., 2007; Burn *et al*., 2008). This suggests that the physical changes in the spleen of dromedaries over time are not unique to our study, but rather are widely observed in the scientific literature. These correlations can provide important insights into the development of the spleen and its functions in the dromedary, as well as potential implications for the health and well-being of the animal.

Furthermore, the relationships between splenic mass, thickness, width, and length, and body weight of the dromedaries are significant, indicating that as the dromedary grows, its spleen also grows proportionally. This is consistent with the results of previous studies that have investigated the correlation between body weight and splenic mass in other animal

species (Brendolan *et al*., 2007; Wang *et al*., 2021). These findings may have implications for veterinary medicine and animal welfare, particularly in relation to the diagnosis and treatment of diseases that affect the spleen, as well as the potential effects of physiological changes associated with growth and development.

Conclusion

The dromedary spleen undergoes significant changes in size, weight, and cellular composition during the first three years of life. These changes are important for understanding the immune function of the dromedary and may have practical implications for the management and care of these animals. Understanding the changes in the size and weight of the spleen may help to inform the development of body weight standards for dromedaries, which are important for the evaluation of animal health and well-being. In this study, the size and weight of the spleen increased significantly between eight months and three years of age, as determined by statistical analysis. Specifically, the mean size of the spleen and the splenic mass increased by age, which concludes that the dromedary spleen undergoes significant growth and development during the first three years of life.

Similarly, understanding the changes in the cellular composition of the spleen may help to identify potential health problems or changes in immune function that could impact the health of the dromedary. In this study, the cellular composition of the spleen changed significantly over time. At eight months of age, the spleen contained mostly lymphocytes and a small number of erythrocytes and macrophages. At one year of age, the number of erythrocytes increased and the percentage of lymphocytes decreased. By two years of age, the spleen contained a higher percentage of macrophages and the number of erythrocytes decreased. At three years of age, the spleen contained a mix of all three cell types, with the highest percentage of lymphocytes. These changes in cell composition may be due to the different functions of the spleen at different ages. For example, the increase in erythrocytes at 1 year of age may be related to the increased storage of red blood cells in the spleen at this

age, while the increase in macrophages at three years of age may be related to the increased immune function of the spleen at this age.

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Conflict of interest

The authors declare no conflict of interest.

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Tables

Table 1: Means and standard deviations for the morphometric characteristics of the dromedary spleen across different age groups

Age	Groups (n)	Mean (SD) of the mass	Mean (SD) of the length	Mean (SD) of the width	Mean (SD) of the thickness
8 months	Groupe 1 (5)	251 (14.19)	43.78 (1.95)	19.44 (1.52)	1.88 (0.16)
One year	Groupe 2 (5)	267 (12.59)	45.7 (1.42)	19.8 (1.69)	2.24 (0.29)
18 months	Groupe 3 (5)	280 (21.02)	46.2 (1.62)	21.7 (1.78)	2.58 (0.24)
Two years	Groupe 4 (5)	297 (12.98)	47.42 (1.46)	22.1 (1.99)	2.89 (0.32)
Three years	Groupe 5 (5)	319.8 (21.91)	49.6 (1.86)	24.32 (1.69)	3.18 (0.34)

443 SD: Standard Deviation of the mean
444
445
446 Table 2: ANOVA results for the mass of the dromedary spleen across different age groups

Source of Variaion	Sum of squares SS	Degrees of freedom ν	Mean square MS	F statistic	p-value
Age groups	14,196.1600	4	3,549.0400	11.5064	5.1841e-05
Error	6,168.8000	20	308.4400		
Total	20,364.9600	24			

447 A significant F statistic and small p-value indicate a significant difference in the splenic mass
448 between the age groups, $P < 0.001$.
449
450 Table 3: ANOVA results for the length of the dromedary spleen across different age groups

Source of Variaion	Sum of squares SS	Degrees of freedom v	Mean square MS	F statistic	p-value
Age groups	92.8840	4	23.2210	8.2438	0.0004
Error	56.3360	20	2.8168		
Total	149.2200	24			

A significant F statistic and small p-value indicate a significant difference in the splenic length between the age groups, $P < 0.001$.

Table 4: ANOVA results for the width of the dromedary spleen across different age groups

Source of Variaion	Sum of squares SS	Degrees of freedom v	Mean square MS	F statistic	p-value
Age groups	77.4104	4	19.3526	6.3976	0.0017
Error	60.5000	20	3.0250		
Total	137.9104	24			

A significant F statistic and small p-value indicate a significant difference in the splenic width between the age groups, $P < 0.01$.

Table 5: ANOVA results for the thickness of the dromedary spleen across different age groups

Source of Variaion	Sum of squares SS	Degrees of freedom v	Mean square MS	F statistic	p-value
Age groups	5.2916	4	1.3229	16.8587	3.4167e-06
Error	1.5694	20	0.0785		
Total	6.8610	24			

A significant F statistic and small p-value indicate a significant difference in the splenic thickness between the age groups, $P < 0.001$.

480

481 **Figure 1:** Spleen morphology according to age groups

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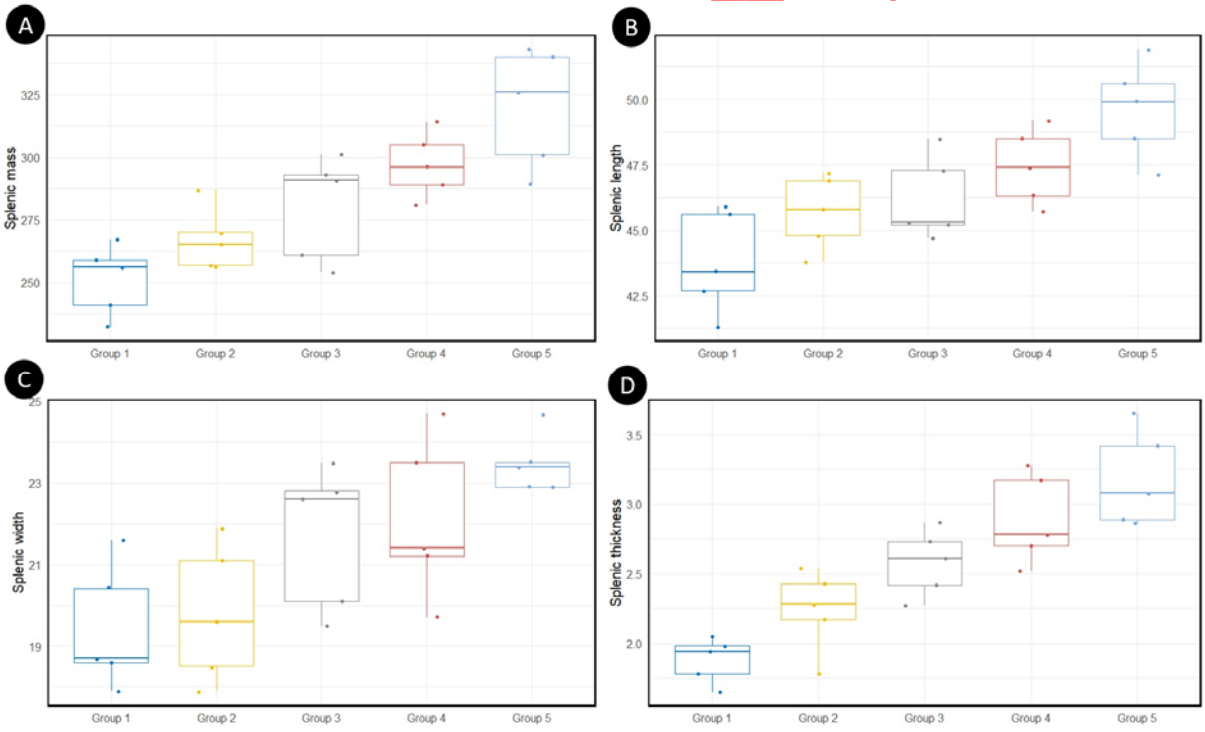

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Figure 2: Dynamic of the splenic mass (A), length (B), width (C) and thickness (D) according to age groups

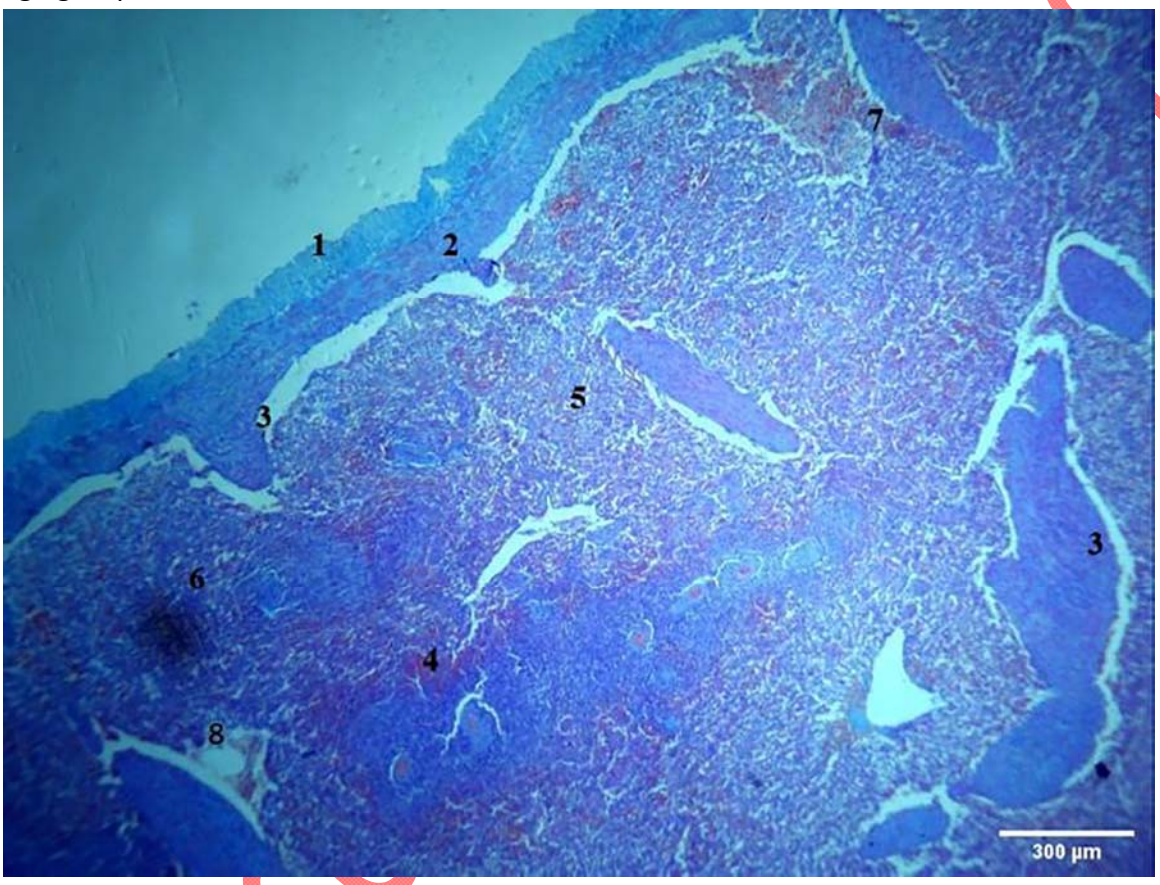


Figure 3: Histological section of the spleen in Dromedary during the first three years of life (x40 Masson Trichrome Staining), 1-Capsule, 2-Mesothelium (Smooth Muscle Bundles), 3-Trabeculae, 4-Red Pulp, 5-White Pulp, 6-Lymphatic Follicle, 7-Peri-arterial Zone, 8-Splenic Ellipsoids

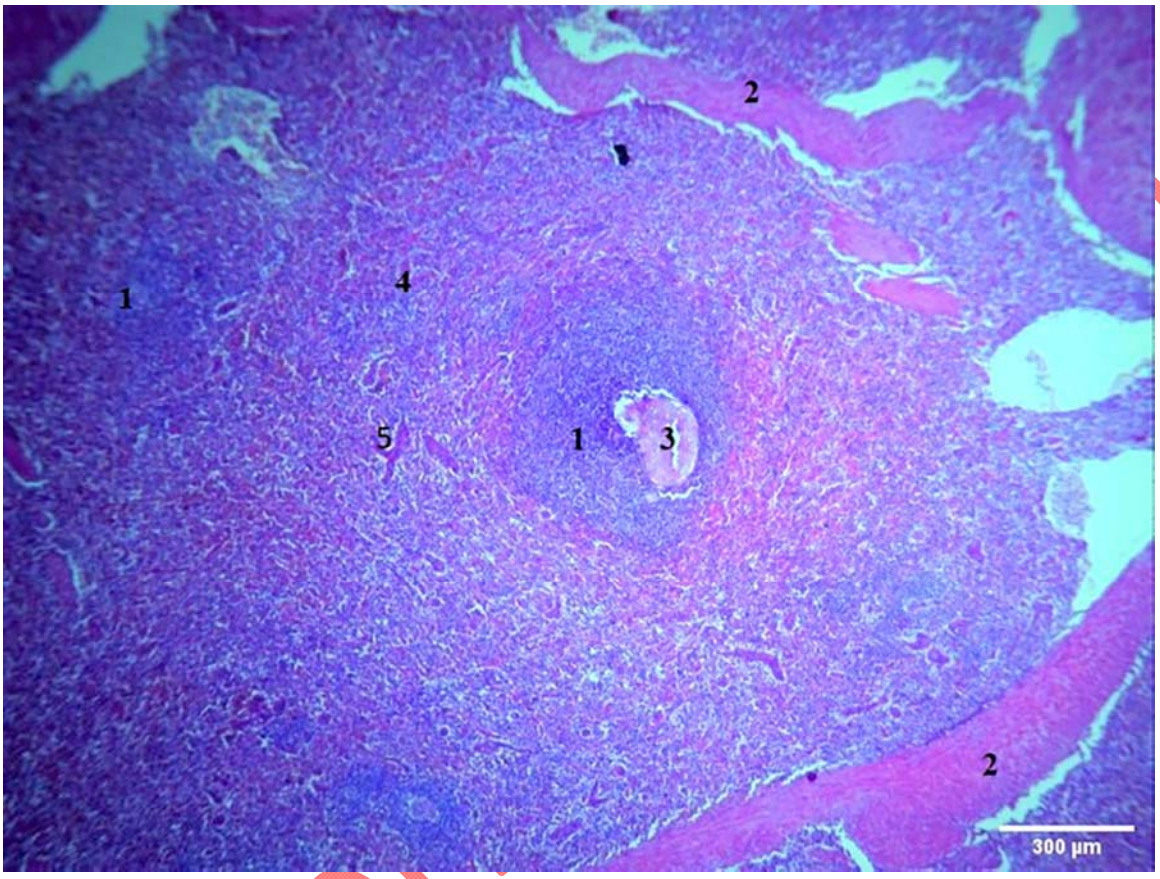
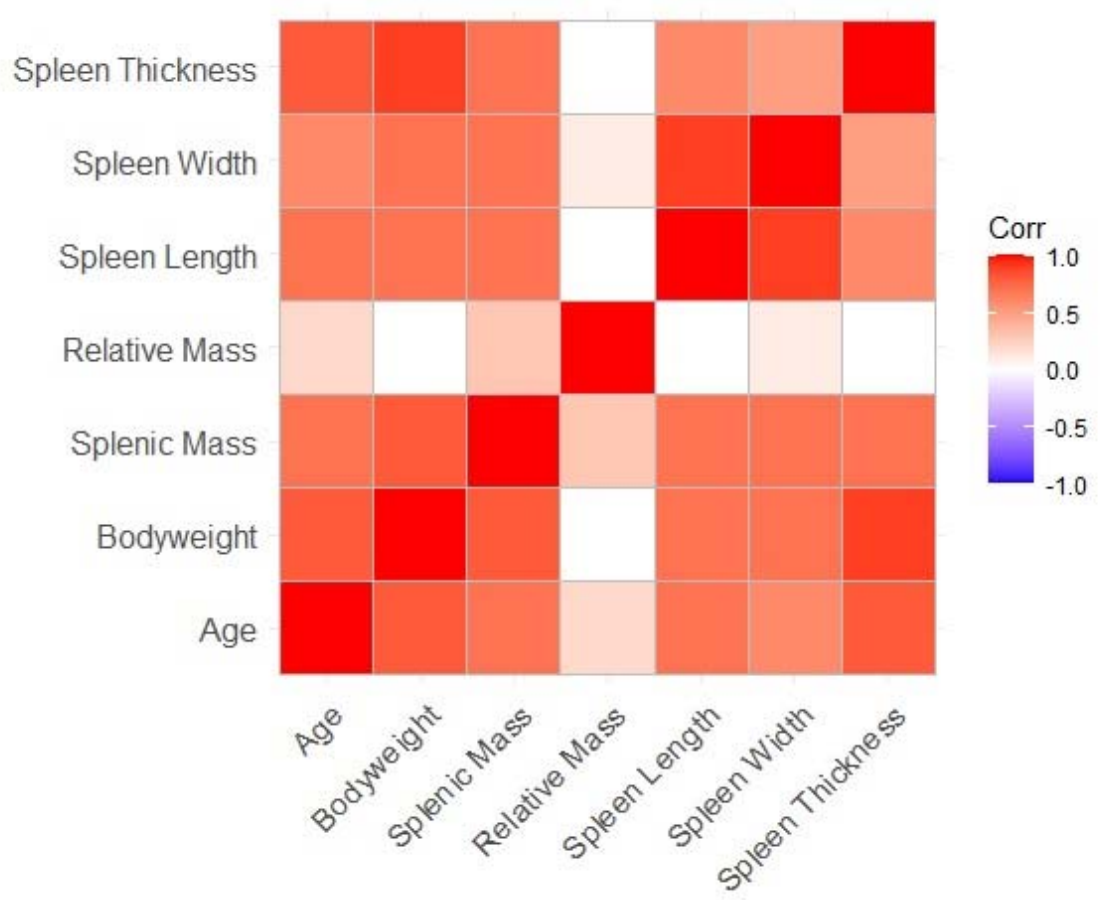


Figure 4: Histological section of the parenchyma of the spleen in Dromedary during the first three years of life (x40 Hematoxylin and Eosin Staining), 1- White Pulp (Mantle Zone), 2- Trabeculae, 3-Pulp Artery, 4-Red Pulp, 5-Sinusoidal Frames



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500 **Figure 5:** Correlogram of Age and Morphometric Parameters in Dr

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