Original Article

Gross Anatomical, Histological, and Cytological Study of the One-humped Camel (*Camelus dromedarius*) Lymph Nodes in Southeastern Algeria

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ABSTRACT

Background: The immune system of the dromedary has remained a subject that has not been extensively researched in immunology. Researchers in morphology and immunology have long sought to delve into the structure and function of the dromedary's immune system to gain a deeper understanding of its mechanisms and potential applications in human and animal health.

Objectives: This study aims to elucidate the histological architecture and cellular composition of the lymph nodes in the indigenous dromedary breed of the El Oued region in Algeria and to compare the results with those of prior investigations of lymph node structures in other mammalian species.

Methods: Hematoxylin, eosin stain, and Masson's trichrome stain techniques were used for histological analysis. In contrast, methylene blue, eosin, and May-Grünwald Giemsa staining techniques were used for cytological analysis. The study data were collected and analyzed using qualitative and quantitative methods to identify the histological and cellular features of the lymph nodes.

Results: Our study revealed that the lymphatic follicles in the dromedary's lymph nodes have a higher concentration of lymphocytes within the follicles' germinal center than other species. The lymph nodes were observed to be divided into conglomerates. The cytological study showed that the major cellular population consisted of lymphocytes, followed by macrophages and reticulocytes according to the localization and the functional zone.

Conclusion: The study provided novel insights into the architecture and cellular composition of the lymph nodes of dromedaries, distinct from those of other species. These findings may have implications for the understanding and treatment of immune-related conditions in dromedaries.

Keywords: Cytology, Dromedary, Histology, Lymph nodes, Morphology

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1. Introduction

The immune system of the dromedary consists of primary and secondary lymphoid organs. The secondary lymphoid organs, which include the spleen and lymph nodes, are responsible for filtering fluids, recognizing and removing foreign agents, and promoting the proliferation and differentiation of lymphocytes (Rahmoun et al., 2020b). Therefore, anatomy, histology, and morphology specialists have focused on the structure of these organs in various species to better understand immunological processes and functional organization. Numerous articles have been published on the organization of lymphatic compartments in different lymph nodes from various species. Gavrilin et al. (2017) demonstrated that the absence of morphological polarity is a key feature of the dromedary's lymph nodes, resulting in a fragmented tissue structure with incomplete compartments separated by spreading trabecular tissue that diffuses throughout the functional parenchyma. According to Elmore (2006), this structure of the dromedary's lymph nodes may play a role in the mechanisms of defense.

The dromedary's lymph nodes are characterized by a unique structure, with large layers of sinuses and connective tissue surrounding the parenchyma. The connective tissue is organized in two layers (Gavrilin et al., 2017; Al-Ramadan, 2022), which partially aligns with the established understanding of mammalian lymph nodes presented by Lobov and Pan'kova (2011). However, there are notable differences in the medullary zone of the dromedary's lymph nodes, which consist of irregular-shaped lymphatic cords isolated by several units, with the sinus formed by endothelial cells being the most important unit (Zidan & Pabst, 2004). In contrast, other species occasionally have lymphatic extensions connected with the paracortical zone, composed of a mixed cellular population consisting of B lymphocytes, plasma cells, and T lymphocytes (Von Andrian & Mempel, 2003).

It is essential to highlight the morphological variations between different mammalian species and even within different breeds of dromedaries. Scientific hypotheses that account for the unique structure of the dromedary's lymph nodes should be proposed to improve our understanding of the immunological process and to develop better strategies for treating immune-related conditions in dromedaries.

2. Materials and Methods

The study was conducted in the Laboratory of Sciences and Techniques of the Livings in Taoura's Institute of Agriculture and Veterinary Sciences, University of Souk.

Sampling

The study utilized lymph nodes collected from 10 fully healthy and sexually mature male dromedaries at the El Oued regional slaughterhouse in South East of Algeria. The authors confirm that the animals were slaughtered in adherence to the Algerian Islamic traditions and strict hygienic conditions under the supervision of qualified veterinarians. From each dromedary, 6 lymph nodes were collected, of which 3 were somatic; the axillary lymph node (nodi lymphoidei axillares), the popliteal lymph node (nodi lymphoidei poplitei), the prescapular lymph node (nodi lymphatiei cervicales), and 3 were visceral; tracheobronchial lymph node (nodi lymphoidei tracheobronchiales), retropharyngeal lymph node (nodi lymphoideiretro pharyngei) and mesenteric lymph nodes (nodi lymphoidei mesenterici) yielding a total of 60 lymph nodes sampled for this study.

Histological study

The samples were fixed for 10 days in a 5% neutral formalin solution, followed by 15 days in a 10% formalin solution. Using a sharp knife, the samples underwent a perpendicular cut; 5 segments were chosen from each lymph node. Lately, all selected fragments were merged in paraffin; dehydration was accomplished using ethanol with an increasing concentration, and the casting process was finished according to the method given by Fischer et al. (2008). Histological sections (10-15 μm thick) have undergone the process of the hematoxylin and eosin staining technique. However, the thinnest sections (2-5 μm) have undergone the process cited by Hu et al. (2015) to create sections stained with methylene blue and eosin to visualize and quantify the cellular population of different compartments of the lymph nodes. Also, May-Grünwald Giemsa staining technique was applied for other sections (4-5 μm of thickness), according to the protocol of Gupta et al. (2021). In addition, Masson's trichrome staining technique was applied to determine the histoarchitectural structure of the lymph nodes, according to Foot (1933). Finally, the histological slides have undergone the microscopic examination process using B-382PLi-ALC light microscope.

Statistical analysis

We took 5 photos from each section to analyze each slide's structural components. The "spot count" method, as described by Avtandilov and Zukakova (1975), was used for quantitative (morphometric) analysis. Additionally, the length of the follicles' crown and the thickness of the capsule were measured using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA. 1997-2023). The statistical results were presented as Mean±SD and visualized with boxplots using Meshroom software version: 2023.2.0, based on Python version: 3.7.4, Rstudio version: RStudio 2022.12.0 (Posit, PBC, USA, 2011-2023).

3D modeling

The 3D modeling of lymph nodes in this study was done to obtain a more detailed and accurate understanding of the structure and morphology of the lymph nodes being analyzed. The 3D models can provide a better visualization of the lymph node components' internal structures and spatial relationships, which can aid in identifying and localizing specific features within the lymph nodes.

The 3D models were created using Meshroom software (Stark et al., 2022), a photogrammetry software that uses 2D images to construct a 3D model. This study captured a set of high-resolution images of each lymph node from multiple angles and then processed them using the Meshroom software to generate the 3D models.

The resulting 3D models of the lymph nodes will be used for various purposes, including the measurement of various features of the lymph nodes, such as volume, surface area, and the dimensions of specific structures within the nodes. Additionally, the 3D models will aid in the visualization and analysis of the lymph nodes, ultimately contributing to a more detailed understanding of the lymphatic system and its function (Stark et al., 2022).

3. Results

Following the dissection of the animals, the location of the axillary lymph node was identified on the right thoracic muscle at the lower end of the first intercostal space, surrounded by a thick layer of adipose tissue. Similarly, the popliteal lymph node was identified as a pair of lymph nodes located on the caudal surface of the gastrocnemius muscle in the caudal part of the stifle joint, between the biceps femoris muscle and the semitendinosus muscle and surrounded by a layer of adipose tissue. The prescapular lymph node was also identified as a pair of lymph nodes located in the interstitial space of the neck's omo-transverse, biceps, and serratus muscles.

Also, the location of the tracheobronchial lymph node was identified at the tracheal bronchus bifurcation. The retropharyngeal lymph node was cranially related to the mandibular gland. It was bulky and had a gutter where the common carotid artery lodges. Furthermore, it was discovered that there are approximately 20 cranial mesenteric lymph nodes in the great mesentery. They have a median position with irregular shapes (Figure 1).

Examining the lymph node morphology in dromedaries in the El Oued region has revealed several noteworthy macroscopic and histological characteristics. The lymph nodes possess a unique structure consisting of multiple conglomerates comprised of varying numbers of small nodules dispersed within adipose tissue and separated by thick connective tissue of varying colors and consistency, dependent on the location of the lymph node.

Microscopic examination of histological slides of the dromedary's lymph nodes in the El Oued region revealed several distinct features. The capsule, which envelops the parenchyma, varied in thickness, with the thickest portions surrounding the subcapsular sinuses and blood vessels originating from the trabeculae in the hilum. The Mean±SD capsule thickness was 47.44±19.63 µm for the axillary lymph node, 44.504±17.79 µm for the prescapular lymph nodes, and 59.3±19.19 µm for the popliteal lymph node. Regarding the visceral lymph nodes, the Mean±SD capsule thickness was 71.28±20.27 µm for the tracheobronchial lymph node, 67.37±21.82 µm for the retropharyngeal lymph node, and 75.78±18.32 µm for the mesenteric lymph node. The number of sinuses and blood vessels was greater in the visceral lymph nodes than in the somatic lymph nodes.

Notably, there was an absence of an extracellular zone within the capsular myocytes. Microvilli infiltrated the interior zones of the parenchyma. The capsule structure was rich in elastic muscle fiber. Lacerations of the capsular tissue corresponded to the areas where the afferent vessels penetrated the organ for lymph admission. Myocytes were found to be fused in the hilar zone. The capsules of visceral lymph nodes were thicker than the somatic lymph node capsules. Trabecular extensions were observed to spread towards the cortico-medullary zone, forming lobules, which are the morpho-functional zones. Cortical and medullary sinuses separated the lobules by sine lines of variable number on both sides. The axillary lymph node trabecula was rich in blood vessels. In most slides, the trabecula of lymph nodes did not reach the medullary zone. In contrast, the trabecular trajectory was prominent towards the paracortical zone, dividing the parenchyma into lobules rich in active follicles (Figure 2).

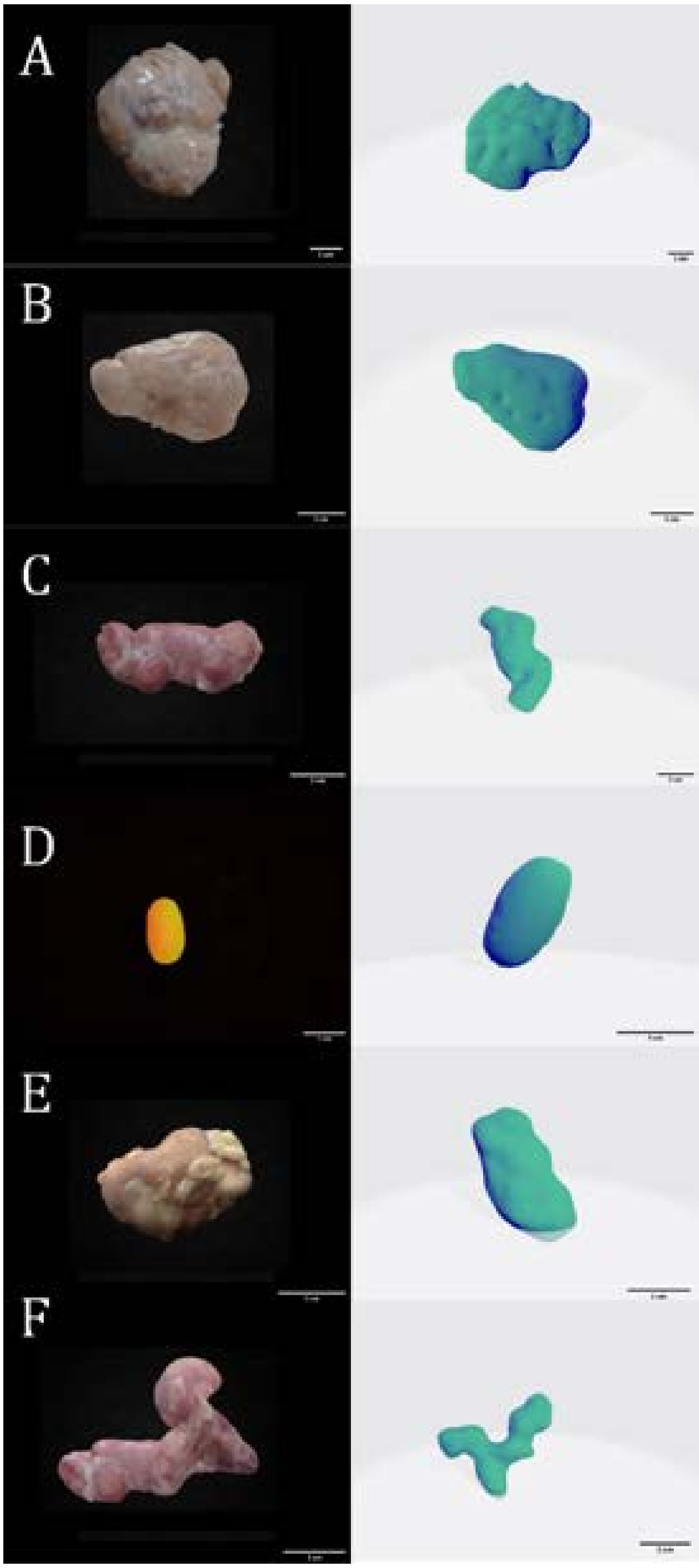

Figure 1. Macroscopic aspect and three-dimensional models of lymph

A) Axillary lymph, B) Popliteal lymph, C) Prescapular lymph, D) Tracheobronchial lymph, E) Retropharyngeal lymph, F) Mesenteric lymph

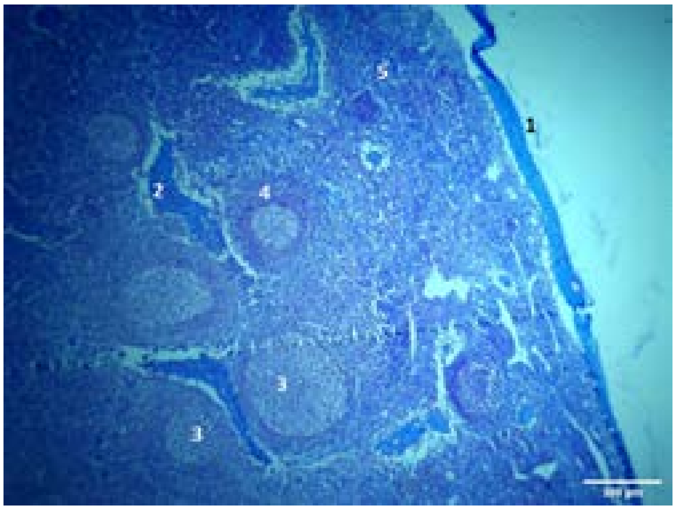

Figure 2. Histological section of the retropharyngeal lymph node

1) Capsule, 2) Trabecula, 3) Follicle with large germinal center, 4) Active follicle surrounded with large crown, 5) Cortical zone (stained with Masson's trichrome X40, scale bar 300 µm)

The microscopic examination of the lymph node cortex revealed two layers: The cortex and the deep cortex. More paracortical sinuses were observed in the visceral lymph nodes compared to the somatic lymph nodes. The morpho-functional units of the axillary lymph nodes' lymphatic follicles have a rounded sigmoid shape and were present throughout the parenchyma with varying diameters. A large germinal center surrounded by a variable-thickness crown was visible in most follicles. The most voluminous follicles were on the periphery of the parenchyma in the visceral lymph nodes, surrounded by a larger crown.

A smaller crown surrounded active follicles in the somatic lymph nodes. The Mean±SD thickness of the crown was found to be 34.69±7.638 µm in active follicles and 21.487±4.687 µm in inactive follicles. Many cortical sinuses of varying shapes and sizes occupied the interfollicular space. In the marginal zone of the visceral lymph nodes, we noticed the presence of high endothelial venules (Figure 3).

Upon further microscopic examination, we observed that the medullary zone in both somatic and visceral lymph nodes occupied a larger surface area than the cortex. Trabecular spaces were observed on both sides of the medulla, surrounded by some blood vessels of small caliber. In addition, we noticed the presence of diffuse lymphoid tissue surrounded by a network of small medullary sinuses in both somatic and visceral lymph nodes.

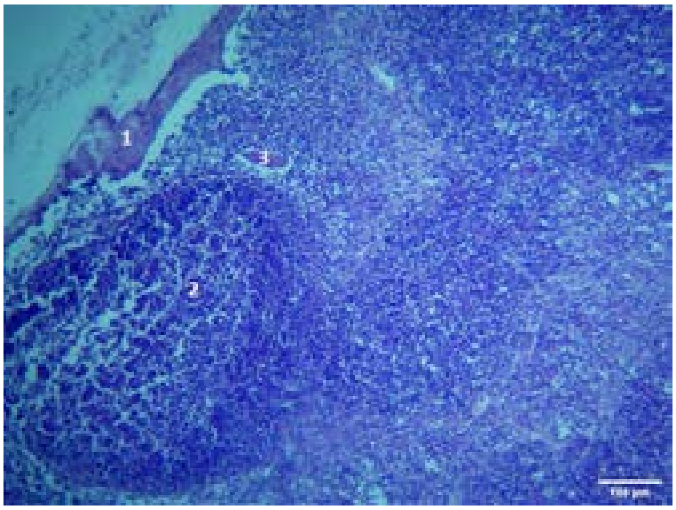

Figure 3. Histological section of the prescapular lymph node

1) Capsule, 2) Follicle occupying cortical sinuses, 3) Blood vessel (stained with hematoxylin and eosin X100, scale bar=100 µm)

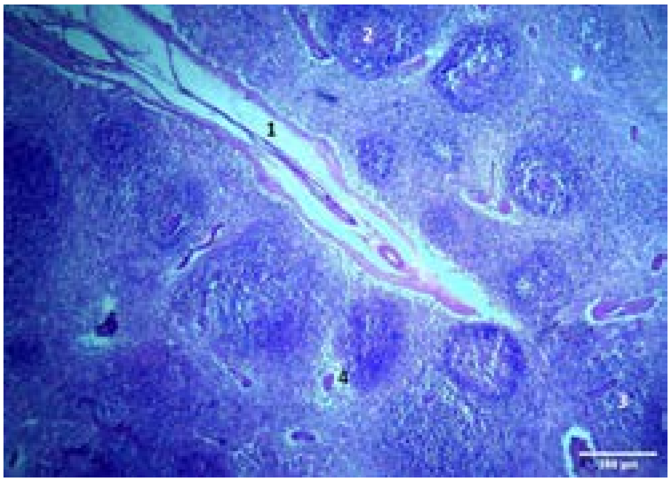

Figure 4. Histological section of the axillary lymph node

1) Trabecula, 2) Active follicle, 3) Medullary sinuses, 4) Inactive follicle (stained with hematoxylin and eosin X40, scale bar=300 µm)

Furthermore, we found that the areas occupied by reticular tissue were more extensive in the medullary zone of both somatic and visceral lymph nodes (Figure 4).

The quantitative analysis revealed that active lymphatic follicles occupied an Mean±SD surface area of 7.4%±0.86% in the axillary lymph node, 7.16%±1.01% in the popliteal lymph node, and 7.65%±0.61% in the prescapular lymph node. In contrast, active follicles occupied a significantly Mean±SD larger area in the visceral lymph nodes with values of 14.81%±1.76%, 13.038%±0.8%, and 15.528%±1.07% in the tracheobronchial, retropharyngeal, and mesenteric lymph nodes, respectively. Inactive follicles, on the other hand, occupied an Mean±SD area of 13.87%±1.025%, 12.46%±0.81%, and 13.31%±0.91% in the axillary, popliteal, and prescapular lymph nodes, respectively. In contrast, they occupied 5.99%±1.22%, 6.61%±1.34%, and 5.27%±1.24% in the tracheobronchial, retropharyngeal, and mesenteric lymph nodes. In terms of the paracortical zone, they occupied an Mean±SD areas of 18.47%±1.29%, 19.12%±0.74%, and 18.32%±1.69% in the axillary, popliteal, and prescapular lymph nodes, respectively. Furthermore, they occupied Mean±SD areas of 15.72%±1.03%, 16.09%±1.28%, and 16.46%±0.83% in the tracheobronchial, retropharyngeal, and mesenteric lymph nodes, respectively (Figure 5).

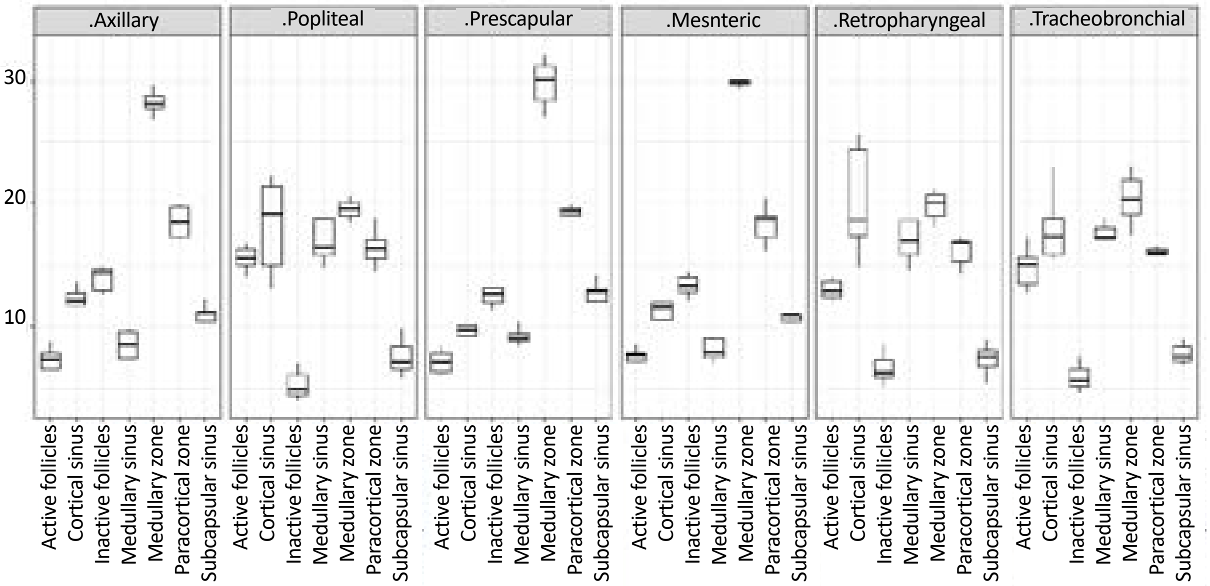

Figure 5. Histomorphometry of dromedary's lymph nodes

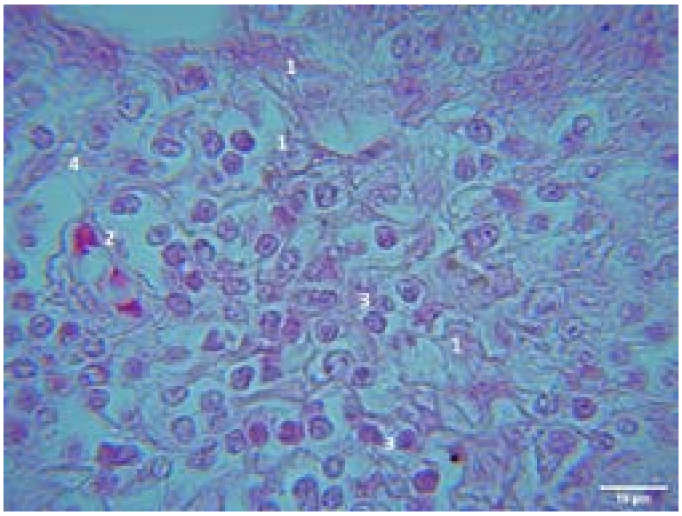

Figure 6. Histological section of the mesenteric lymph node

1) Reticulocytes, 2) Macrophage, 3) Lymphocytes, 4) Plasmocytes (stained with methylene blue and eosin X1000, scale bar=10 µm).

On the other hand, the medullary zone occupied an Mean±SD surface areas of 28.17%±1.05%, 29.74%±2.07%, and 30.21%±1.16% in the axillary, popliteal, and prescapular lymph nodes, respectively. In the visceral lymph nodes, they occupied Mean±SD areas of 20.37%±2.2%, 19.79%±1.29%, and 19.49±0.83% in the tracheobronchial, retropharyngeal, and mesenteric lymph nodes, respectively. Regarding the subscapular, cortical, and medullary sinuses, they occupied an Mean±SD surface areas of 31.11%±2.13%, 31.36%±3.24%, and 30.49%±2.57% in the axillary, popliteal, and prescapular lymph nodes, respectively. In contrast, they occupied an Mean±SD surface areas of 43.12%±3.89%, 44.45%±3.19%, and 42.63%±3.47% in the tracheobronchial, retropharyngeal, and mesenteric lymph nodes, respectively (Figure 5).

The cytological analysis of the parenchyma in the somatic lymph nodes revealed a rich para-cortical zone containing lymphocytes of varying diameters, mostly large lymphocytes scattered throughout the para-cortical zone. Additionally, a small number of reticulocytes with a starry shape were observed. Macrophages were triangular, occupying a smaller surface area, and were much fewer in number compared to lymphocytes and reticulocytes. Plasmacytes were restricted to a small surface area in the para-cortical zone, with a large size and rounded shape. In the active follicles, lymphocytes were domi-

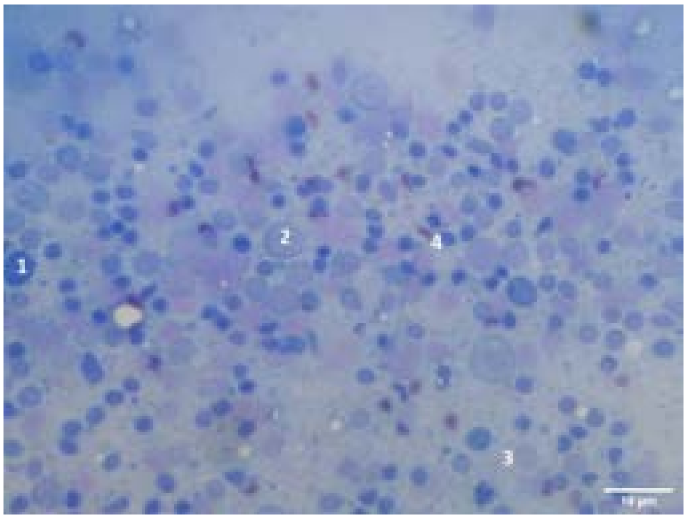

Figure 7. Histological section of the axillary lymph node

1) Plasma cell, 2) Macrophage, 3) Lymphocyte, 4) Reticulocyte (stained with May-Grünwald Giemsa technique, X1000, scale bar=10 µm)

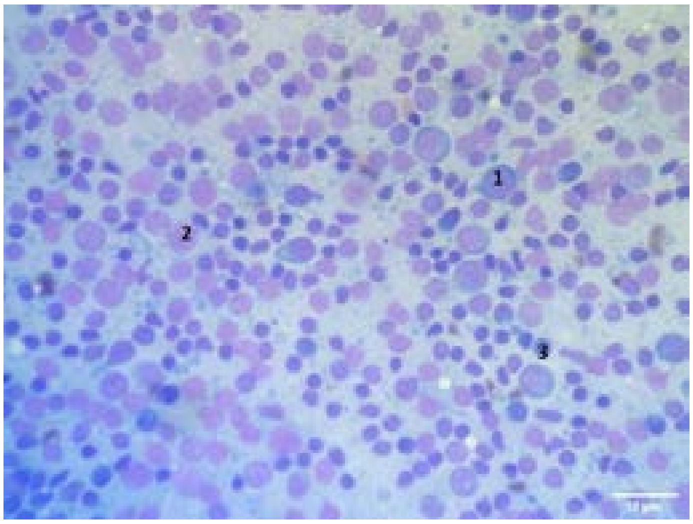

Figure 8. Histological section of the popliteal lymph node

1) Plasma cell, 2) Lymphocyte, 3) Reticulocyte (stained with May-Grünwald Giemsa technique, X1000, scale bar=10 µm)

nant, with diameters ranging from medium to large, followed by triangular-shaped reticulocytes, macrophages, and a limited number of plasma cells (Figure 6).

Regarding the inactive follicles, it has also been noticed that the cellular distribution is similar to that of the active follicles but with a lower rate of reticulocytes and macrophage; on the other hand, the medullary zone showed a larger number of reticulocytes with a much more developed volume, we also observed that lymphocytes were distributed throughout both sides of the medullary zone, likely due to the presence of active follicles in this region (Figure 7).

Also, the cytological analysis of the paracortical zone of the visceral lymph nodes demonstrated a high concentration of lymphocytes with varying diameters. Reticulocytes were observed in very low numbers, while macrophages were few and had a small diameter. The plasma cells were present in a relatively small area and had a rounded shape. Moreover, the cytological analysis of the active follicles revealed that lymphocytes dominated this area, with fewer macrophages and reticulocytes concentrated in the center of the follicles. Inactive follicles showed a similar cellular distribution to active follicles, with a lower proportion of macrophages.

Regarding the medullary zone, we observed the presence of certain active follicles that allowed us to notice a predominance of lymphocytes in this area, with a high number of larger reticulocytes.

The quantitative research revealed that in the active lymph node follicles, lymphocytes occupied a maximum value in the tracheobronchial lymph node, with a Mean±SD value of 77.93%±1.28%. In contrast, they occupied a minimum Mean±SD value of 61.7%±0.04% in the popliteal lymph node. Regarding plasma cells, it was found that the maximum Mean±SD value found was 5.93%±1.63% in the tracheobronchial lymph node. In contrast, the minimum Mean±SD value was 0.65%±0.47% in the popliteal lymph node, while reticulocytes occupied a Mean±SD area of 25.93%±1.16% in the pre-scapular lymph node. In contrast, they occupied a minimum Mean±SD area of 6.87±0.68 in the tracheobronchial lymph node. Thus, the maximum Mean±SD area occupied by the granulocytes and the erythrocytes was 13.36%±1.55% in the popliteal lymph node, while the minimum value was, on the other hand, it was found that the maximum surface occupied by macrophages was 11.36%±1.47% in the retropharyngeal lymph node. In contrast, the minimum Mean±SD value was 1.98%±0.22% in the pre-scapular lymph node, while the minimum was 1.25%±0.37% in the retropharyngeal lymph node (Figure 8).

Regarding the inactive follicles, the maximum Mean±SD area occupied by lymphocytes was 75.32%±1.58% in the tracheobronchial lymph node, while the minimum Mean±SD value was 59.75%±2.12% in the popliteal lymph node. On the other hand, the maximum Mean±SD area occupied by plasma cells was 8.36%±1.96% in the tracheobronchial lymph node, while the minimum Mean±SD value was 1.65%±0.12% in the popliteal lymph node. In contrast, the maximum Mean±SD area occupied by reticulocytes was 27.89%±2.06% in the pre-scapular lymph node. Thus,

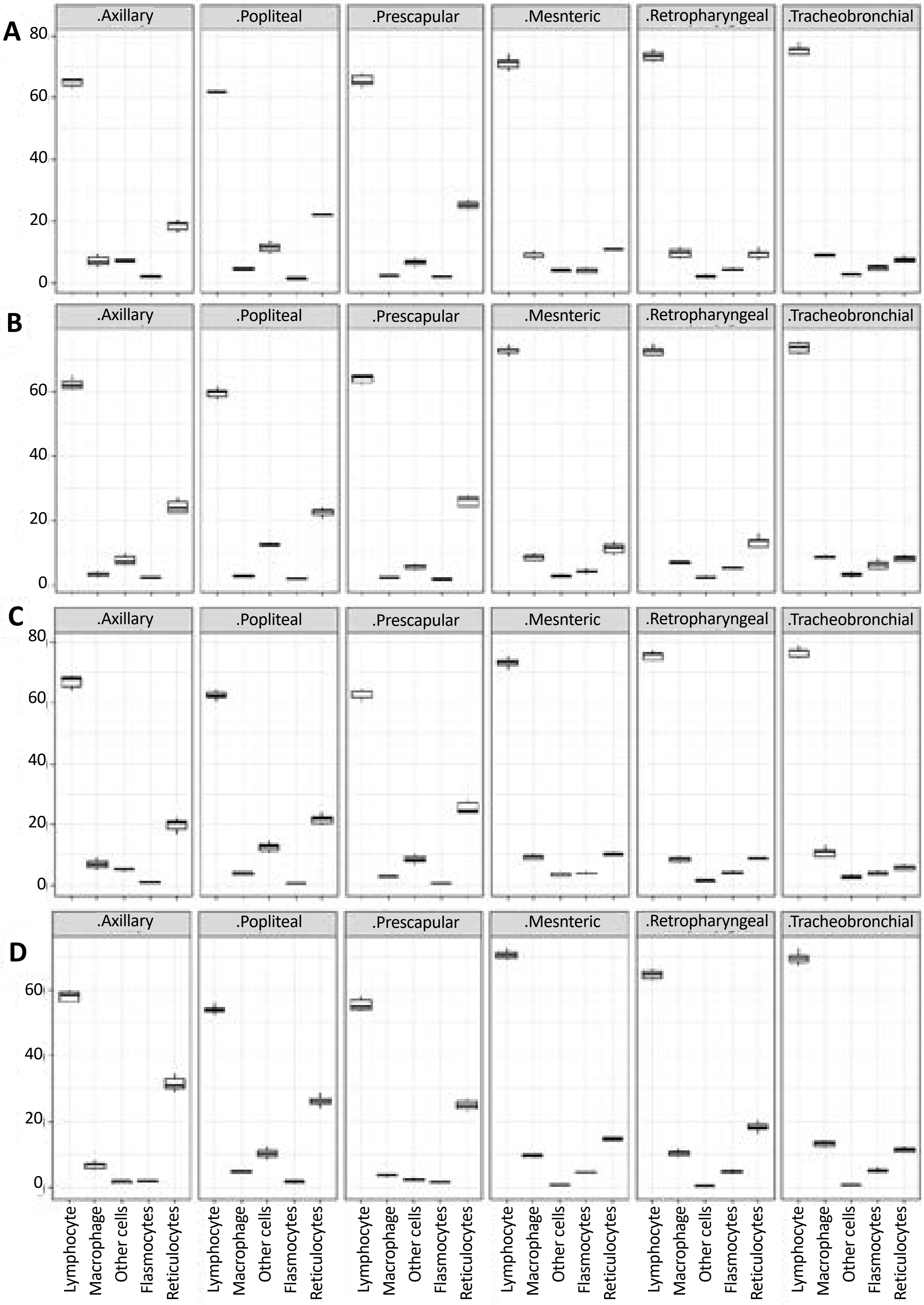

Figure 9. Cytomorphometry of dromedary's lymph nodes in each compartment

A) Active follicles, B) Inactive follicles, C) Paracortical zone, D) Medullary zone the maximum Mean±SD area occupied by macrophages was 9.82%±0.65% in the mesenteric lymph node, while the minimum Mean±SD value was 2.12%±0.21% in the pre-scapular lymph node (Figure 9).

In the paracortical zone, the maximum Mean±SD area occupied by lymphocytes was 78.93%±0.59% in the tracheobronchial lymph node. At the same time, the minimum Mean±SD value was 60.32%±1.23% in the pre-scapular lymph node, while plasma cells occupied a maximum Mean±SD area of 4.79±1.02 in the tracheobronchial lymph node. In contrast, the minimum Mean±SD value was 0.54%±0.13% in the popliteal lymph node. While the maximum Mean±SD area occupied by reticulocytes was 27.82%±2.12% in the pre-scapular lymph node, while the minimum Mean±SD value was 5.02%±0.79% in the tracheobronchial lymph node. Also, it was found that the maximum Mean±SD surface occupied by macrophages was 12.93%±1.77% in the tracheobronchial lymph node. In contrast, the minimum Mean±SD value was 2.49%±0.01% in the pre-scapular lymph node, while the maximum Mean±SD areas occupied by other cells were 13.35%±1.58% in the popliteal lymph node and 1.18%±0.56% in the retropharyngeal lymph node.

In the medullary zone, the maximum Mean±SD area occupied by lymphocytes was 72.36%±1.8% in the mesenteric lymph node, while the minimum Mean±SD value was 51.96%±1.98% in the popliteal lymph node. On the other hand, the maximum Mean±SD area occupied by plasma cells was 6.63%±0.65% in the tracheobronchial lymph node,

Regarding the reticulocytes, they occupied a maximum Mean±SD surface of 32.82%±2.98% in the axillary lymph node. At the same time, the minimum Mean±SD value was 10.43%±1.12% in the tracheobronchial lymph node. In contrast, macrophages occupied a maximum Mean±SD area of 14.35%±0.79% in the tracheobronchial lymph node. At the same time, the minimum Mean±SD value was 3.15%±0.14% in the pre-scapular lymph node. The granulocytes and the erythrocytes occupied a maximum Mean±SD area of 12.32%±1.12% in the popliteal lymph node. In contrast, the minimum Mean±SD value was 0.13±0.09 % in the retropharyngeal lymph node (Figure 9).

4. Discussion

Our research findings demonstrate that the organization of lymph nodes in dromedaries follows the general principles of structural and functional organization observed in other mammalian species (Gavrilin & Kolesnyk, 2019; Rahmoun et al., 2020a). However, certain morphological features observed in dromedaries may be related to adaptations necessary for survival in extreme environmental conditions, which place a high demand on immune system efficiency to sustain life, as previously reported by Kamoun et al. (1992), Faraz et al. (2021), and Moradi et al. (2021).

Anatomically some aspects of the topography of the popliteal lymph node were noted. It was revealed that it was displaced slightly above the gastrocnemius tendon and somehow a little bit higher than the hock joint, and also, the conglomerates' organization of these organs was very complicated. Their number was variable from one lymph node to another, so far from the simple organization described by (Lawson et al., 1985).

The variability in the surface of body regions occupied by certain lymph nodes may contribute to differences in the morphometric properties of the lymph nodes. Additionally, the degree to which different barrier systems (skin, mucous membranes) interact with antigenic elements of the external environment affects the size of the lymph node and its growth index in mammals. As a result, the groups of the most numerous and developed lymph nodes of the dromedary were typically found in the folds of the skin (the fold of the knee), the hila of the lungs, the dorsal surface of the lungs, and the mesentery of the small intestine which aligns with the findings of Hajinejad-Bamroud et al. (2020).

Thus, the results of our research revealed that, like all other mammalian species, the lymph nodes' parenchyma in dromedaries present an orderly lobular structure (compartmentalized). In contrast to other mammals, the compartments located in the lymph nodes of the dromedary are much more developed and present a localization character at several levels. According to previous research (Giuliano et al., 2010; Al-Ramadan et al., 2021), the characteristics of dromedary's lymph node structure may be due to an increased function under harsh environmental conditions, which leads to this species of mammal's higher viability and high immunological reactivity.

The histomorphometry analysis of the dromedary's lymph nodes revealed that the parenchyma is organized into several compartments, with the medulla occupying the largest area, then the cortical and paracortical zones, and finally, the sinuses. These findings are consistent with the radiological studies by Rahmoun et alb. (2020) and Hussen and Al-Sukruwah (2022) and similar to results observed in lymph nodes of other species, including small laboratory animals (Fares et al., 2019).

The statistical analysis showed that lymphocytes occupy the largest areas in the different compartments, followed by macrophages and reticulocytes, with fewer plasmacytes, granulocytes, and erythrocytes. These results agree with previous research by Fares et al. (2023).

These findings suggest that the dromedary's lymph nodes have a similar structural organization to those of other mammals, with certain differences related to their adaptation to extreme living conditions. Further studies are needed to fully understand the functional implications of these structural differences in the dromedary's lymph nodes.

5. Conclusion

In conclusion, our study revealed important anatomical and histological features of the dromedary's lymph nodes. Anatomically, we observed that the lymph nodes comprise conglomerates of small nodules dispersed throughout adipose tissue and separated by thick layers of connective tissue. Additionally, the histological examination revealed the presence of a capsule surrounding the lymph nodes, which was thicker in somatic lymph nodes due to their direct approach to the skin. Visceral lymph nodes, on the other hand, showed a higher number of follicles, indicating a direct immunogenic reaction in these lymph nodes. Moreover, we observed that the reticular tissue was highly developed in the mesenteric lymph nodes, likely due to antigens at the visceral level. Our findings contribute to a better understanding of the structural and functional organization of the lymph nodes in dromedaries. They also suggest that these animals have adapted to harsh environmental conditions by developing a complex lymph node structure that enhances their immune system's functioning. Further studies should investigate the specific mechanisms underlying this adaptation and explore potential implications for human health.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization and supervision: Fares Mohamed Amine, Khenenou Tarek and Rahmoun Djallal Eddine; Methodology: Fares Mohamed Amine and Rahmoun Djallal Eddine; Investigation, Writing review & editing: Fares Mohamed Amine; Data collection: Fares Mohamed Amine and Rahmoun Djallal Eddine; Data analysis: Fares Mohamed Amine.

Conflict of interest

The authors declared no conflict of interest.

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