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Morphological Variation of the Mesenteric Lymph Node in Dromedary Camels: The Impact of Rearing Systems

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Abstract

The study intends to evaluate the morphological changes in the mesenteric lymph nodes of dromedaries in different rearing systems. we aimed to evaluate the adaptative behavior of the animal's immune system with environmental variations, and to conduct a comparative analysis on the morphological features of the mesenteric lymph node of the one-humped camel (*Camelus dromedarius*) in the region of El Oued, with two different rearing systems, with different practices and different purposes. The study was conducted using histo-morphometric techniques to analyze the morphological features of the mesenteric lymph node of the one-humped camel (*Camelus dromedarius*) in the region of El Oued. Two groups of dromedaries were used in the study, one group raised in a free-roaming housing system and another group raised in a restricted-roaming housing system. The results revealed that there were significant differences between the two groups in terms of active follicle ratio and size and also the cellular population of functional zones. Animals living and roaming outside the farm barriers were more exposed to pathogens, which leads to the installation of an adaptative process, whereas the animals living under restricted-roaming housing system were not exposed to pathogens. This study indicated that the adaptative behavior of the animal's immune system with environmental variations is the functional translation of morphological changes. The obtained findings revealed that the morphological features of the mesenteric lymph node of the one-humped camel (*Camelus dromedarius*) in the region of El Oued are directly linked to the rearing system practices.

KEYWORDS
Adaptative behavior, Dromedary, Lymph node, Morphology, Rearing systems.

INTRODUCTION

In arid and semi-arid regions, the characterization and identification of livestock systems relies on the capacity of animals and plants to adapt to biotic and abiotic climatic fluctuations and constraints. The understanding of physiological and genetic mechanisms of adaptation is a major challenge for the scientific community, especially in the context of climate change. Animal populations in Saharan areas, to address this challenge, researchers have turned to animal populations in Saharan areas, particularly the dromedary, as models for studying adaptive processes and anatomo-histological features. Through rigorous research, scientists aim to unravel the complex biological mechanisms that allow these animals to survive and thrive in extreme environments. The insights gained from such researches have the potential to revolutionize livestock management in temperate regions by applying the knowledge gained from Saharan animal populations to other regions, according to Theusme *et al.* (2021), Depending on the geographical area, their ability to withstand extreme climatic conditions, including high temperatures, variable availability of food and water in quantity and quality, as well as a wide variety of diseases. Adaptation in animal husbandry is a very general term that describes the ability of an animal to cope with the constraints of the environment, by mobilizing its major physiological functions. The objective of the adaptive process for the animal is to maintain its well-being and to guarantee its survival and that of its descendants. In difficult areas, the sustainability of livestock systems depends on the ability of animals to survive and reproduce under severe food constraints (pastoral livestock in arid regions). The resilience of livestock systems is partly based on the adaptive potential of animals to undernourishment and on the efficiency of behavioral and physiological regulations involved in the adaptive response (Bocquier and González-García, 2010), Among the domestic animal species likely to exploit the semi-arid and desert zones in Algeria, the dromedary occupies a place of choice thanks to its anatomical-physiological particularities that allow it to valorize the scarce plant resources of the Sahelian environment. Thus, the dromedary breeding knows a not negligible craze since these last decades. This is precisely because of the exceptional potential of this species for populations living in areas where the breeding of other domestic animals and agriculture are often marginalized by an overly arid environment.

The development of dromedary breeding is however hindered by constraints such as neonatal pathologies among which diarrhea of the young dromedary figures prominently. Apart from parasitic diseases, dromedaries are also exposed to infections which cause inflammatory reactions. The latter are mainly characterized by changes in the leucocyte formula

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This aspect of adaptation has most often been addressed by understanding the genetic control of resistance in the hosts, while understanding the mechanisms results secondarily from knowledge of the observed variability. Anato-histological approaches have also been carried out to highlight factors of variation in the risks of exposure of the hosts to diseases. Morphological research on the dromedary's immune system has helped to understand the structure and function of various immune cells and tissues and how they work together to defend the body. The spleen and lymph nodes, for instance, are important structures for the dromedary's immune system as they produce and activate immune cells. B cells and T cells are specific immune cells that also play a role in the dromedary's immune defense (Zidan and Pabst, 2004). Lymph nodes, which are found throughout the body and are a vital part of the immune system, filter lymph, a clear fluid rich in immune cells, and produce and activate immune cells in response to infections or other threats to the body. The structure of lymph nodes is similar in many mammals, including the dromedary, which has an outer cortex and an inner medulla surrounded by a capsule made of dense connective tissue. The cortex contains lymphoid follicles, clusters of B cells, while the medulla contains T cells and other immune cells and tissues, such as macrophages and plasma cells (Gavrilin *et al*., 2017). The dromedary's lymph nodes are made up of several conglomerates, each containing a varying number of small nodules and covered by a thick capsule of connective tissue. The immune system interacts with environmental conditions, which can influence the morphological features of its functional compartments, such as the diameter of follicles and the thickness of the mantle (Kunisawa and Kiyono, 2010; Hilton *et al*., 2006), as well as the number of immune cells of different populations, which adapt to ensure an adequate immune response. These adaptive processes are impacted by factors such as housing systems and practices, medical procedures and vaccinations, and feeding systems (Ruddle and Akirav, 2009; Palacios *et al*., 2020). The aim of this study was to investigate the relationship between the diameter of active follicles and the ratio of lymphocytes and plasma cells in the lymph nodes of dromedaries living in different livestock systems.

In the current state of knowledge on this species, the topography of the lymph nodes has received little attention from researchers working in the field of animal health. The present study allowed us to fill this gap, by bringing our contribution to a better knowledge of the anatomy and the effect of environmental conditions on the histology of the lymph nodes of the dromedary in Algeria.

MATERIALS AND METHODS

Sampling and histological study

In this study, ten mesenteric lymph nodes (*Nodi lymphoidei mesenterici)* were collected from healthy, sexually mature dromedaries during 2022's summer season, from July to August at the regional slaughterhouse in El Oued, Algeria. The authors declare that all animal subjects were humanely and ethically treated in accordance with the guidelines of Algerian Islamic slaughter practices. Half of the lymph nodes were collected from dromedaries living in a free-roaming system, while the other half were collected from dromedaries living in a restricted-roaming system. The samples were fixed in formalin, embedded in paraffin, dehydrated with ethanol, and thinly sectioned for histological analysis. The sections were then stained using the hematoxylin and eosin technique in the Laboratory of Sciences and Techniques of the Livings, in the Institute of Agriculture and Veterinary Sciences, Taoura, University of Souk Ahras, and also in the Department of Biomedicine, Veterinary Sciences Institute, University of Tiaret in Algeria.

Quantitative study

The histological sections were photographed and analyzed using a combination of morphometric and image analysis techniques. The "spot count" method described by Avtandilov and Zukakova (1975) was used to perform a quantitative analysis of the structural components of the tissue, while the length of the follicles crown and the thickness of the capsule were measured using ImageJ software.

T-test

The T-test was used to compare the morphological parameters of lymph nodes in dromedary camels living in free-roaming and restricted-roaming breeding systems. The T-test table shows four variables: diameter of active follicles, ratio of active follicles, ratio of lymphocytes in the active follicles, and ratio of plasma cells in the medullary zone (Table 1). The results for each variable are presented for the two groups, free-roaming breeding system and restricted-roaming breeding system. The T test table also includes the p-value and significance degree for each variable. Knowing that the measure of the probability p-value less than 0.05 considered to be statistically significant, In the current study, *** indicates a p-value of less than 0.001, ** indicates a p-value of less than 0.01, and * indicates a p-value of less than 0.05, Our hypothesis was that the mean values of the dependent variables would be equal across the different levels of the independent variable (the livestock system).

Principal component analysis (PCA)

Multivariate analysis was utilized in this study to examine the relationships between multiple variables simultaneously. The use of multivariate techniques allowed for the identification of patterns and relationships within the data and an understanding of its underlying structure. The specific methods employed included principal component analysis (PCA), hierarchical cluster analysis (HCA), (Petersen, 2000). These techniques were useful for exploring the relationships between livestock systems and the structure of lymph nodes.

Hierarchical Ascendant Classification (HAC)

The Hierarchical Ascendant Classification (HAC) technique, a form of multivariate analysis, was carried out to identify patterns and relationships between variables and create a simplified structure summarizing the relationships between individuals. This technique is based on a correspondence matrix, which displays the relationships between the units under analysis (Behaeghel *et al*., 2015).

Statistical analysis

The statistical analysis was carried out using R-studio software (Posit, PBC, USA, 2011-2023).

RESULTS

The histological study revealed that the lymph nodes were entirely surrounded by capsule rich in elastic and muscle fiber, it was also reported that the regions of shredding in the capsular tissue matched the regions where the afferent vessel penetrated the organ, it was also crucial to draw attention to the presence of trabecular extensions that spread towards the cortico-medullary zone, creating the lobules that are the morpho-functional zones and being divided by sine lines of cortical and medullary sinuses with a highly variable number.

The study revealed that the lymph node structure includes two layers, the cortex and the deep cortex, and that the para-cortical sinuses are found within these layers. The shape of the lymphatic follicles in the mesenteric lymph nodes was described as rounded and sigmoid, while the medullary zone was found to have a diffuse lymphoid tissue encircled by small medullary sinuses. It was also reported that the medullary zone covers a larger surface area than the cortex (Fig. 1 and Fig. 2).

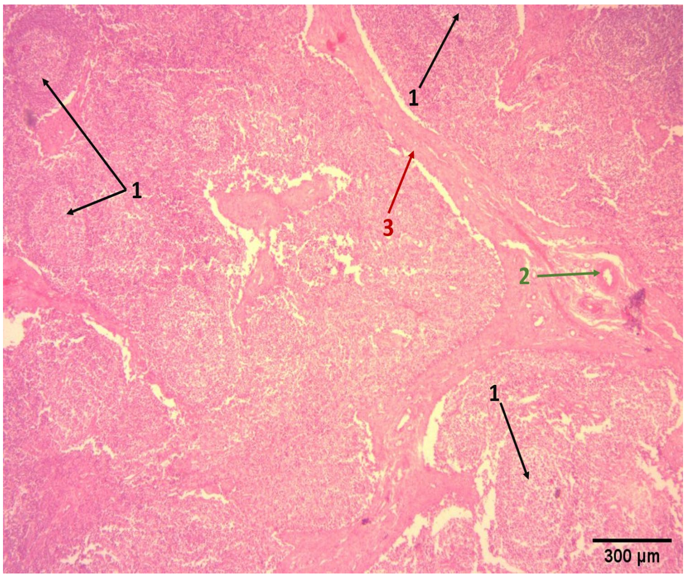

Fig 1. Histological section of the mesenteric lymph node, stained with hematoxylin and eosin. X40, scale bar = 300 µm: 1- Active follicles, 2- Trabecula ,3- Blood vessel. In dromedaries are raised under extensive rearing systems, these active follicles tend to congregate in the medullary regions of the parenchyma of the lymph nodes.

The histological examination conducted in the current study revealed that the ratio of active follicles in dromedaries living in a free-roaming breeding system was significantly higher than that in the group of animals raised and living under restricted-roaming housing conditions. Specifically, the ratio of active follicles in the free-roaming group was found to be 29.12±3.39 %, whereas the ratio of active follicles in the restricted-roaming group was 15.53±1.07. This result indicates a clear difference in the histological components of lymph nodes between the two groups of dromedaries, with the free-roaming animals displaying a higher ratio of active follicles (Table 1).

In the other hand, it was found that the diameter of the active follicles including both the germinal center and the surrounding mantle was larger in dromedaries living in free-roaming housing system, in which it was found that the mean diameter of active follicles was 178.07±23.9 µm, whereas the animals living under restricted-roaming housing system presented an average diameter of 355.9±66.97 µm (Table 1).

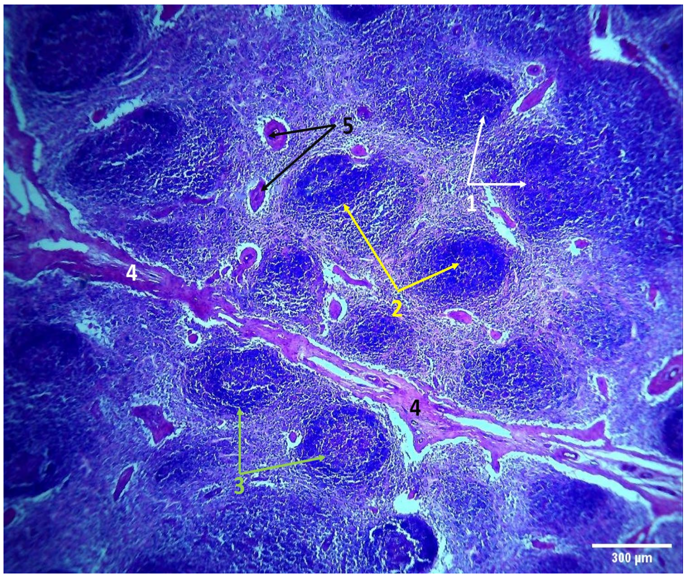

Fig 2. Histological section of the mesenteric lymph node, stained with hematoxylin and eosin. X40, scale bar = 300 µm: 1- Inactive follicles, 2- Follicles during the activation process,3- Active follicles,4- Trabecula, 5- Sinus. Conversely to the Fig 1, The active follicles in dromedaries were found to be dispersed throughout the peripheral regions of the parenchyma of the lymph nodes of dromedaries living under intensive rearing systems.

Regarding the cytological population, our results revealed that the general architecture of each functional compartment is almost the same, the paracortical zone including both active and inactive follicles was found to be invaded by lymphocytes, with the presence of some macrophages and collagen fibers, the high endothelial venule were found to be surrounded by lymphocytes, and plasma cells, (Fig. 3) and (Fig. 4).

In addition to the differences in the ratio of active follicles. It was observed that the diameter of the active follicles, including both the germinal center and the surrounding mantle, was larger in dromedaries living in free-roaming housing system than those living under restricted-roaming housing system. Specifically, the mean diameter of active follicles in the group of animals living under restricted-roaming rearing system was found to be 178.07±23.9 µm, whereas the animals living in a free-roaming housing system presented an average diameter of 355.9±66.97 µm. These results indicate that there is a significant difference in the size of the active follicles between the two groups of dromedaries (Fig. 5).

The cyto-morphometric results, showed a variation of the amount of plasma cells in the medullary zone, which presented a ratio of 1.69±0.65 % of the medullary zone of the lymph nodes of dromedaries living under restricted-roaming housing system, whereas they presented a ratio of 5.32±6.65 % in the medullary zone of the dromedaries living in free-roaming housing system (Table 1), it was also found that the ratio of the lymphocytes in the active follicles in the lymph nodes of dromedaries living under restricted-roaming rearing system presented a ratio of 63.96

Table 1. Statistical analysis of T-test with the p-value and the significance degree

Variables	Free-roaming Breeding system	Restricted-roaming Breeding system	p-value	Significance degree
Diameter of active follicles (µm)	355.9±66.98 µm	178.07±23.94 µm	0.003	**
Ratio of active follicles (%)	29.12±3.31 µm	15.53±1.07 µm	0.000	***
Ratio of lymphocytes in the active follicles (%)	74.9±2.52 %	63.96±1.87 %	0.005	**
Ratio of plasma cells in the medullary zone (%)	5.32±0.65 %	1.69±0.4 %	0.002	**

Significance degree p =< 0.001 ***; p =< 0.01 **; p=< 0.05 *; p> 0.05 NS

±1.87 %, whereas this value was higher in the active follicles of animals living in a free-roaming rearing system in which the ratio was 74.9±2.52 %, (Table 1).

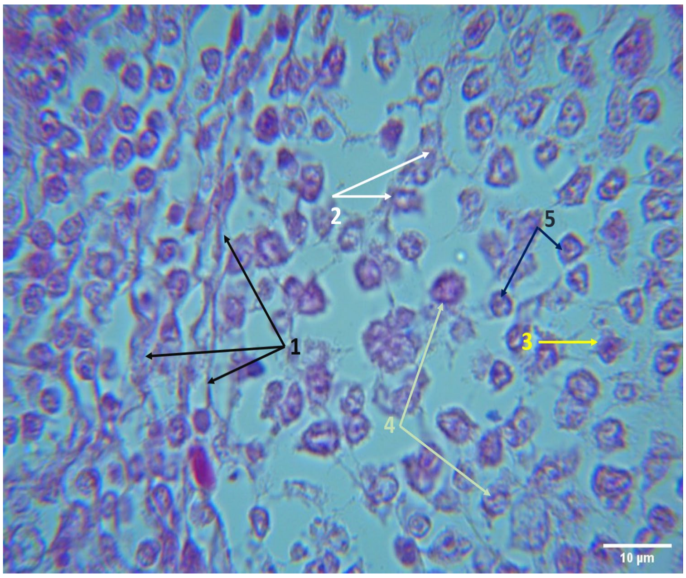

Fig 3. Histological section of the mesenteric lymph node, stained with methylene blue and eosin, X1000, scale bar = 10 µm: 1- Fibrin, 2- Reticulocyte, 3- Macrophages, 4- Polynuclear cells, 5- Small lymphocyte. The interstitial space appears wide in the lymph nodes of animals living under restricted-roaming rearing system.

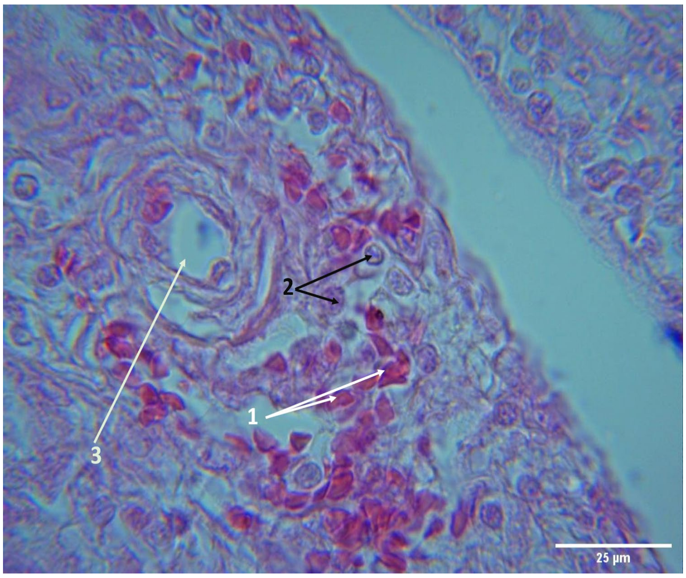

Fig 4. Histological section of the mesenteric lymph node, stained with methylene blue and eosin, X1000, scale bar = 25 µm: 1- Granulocyte, 2- Medium size lymphocyte, 3- Lymphatic vessel. The interstitial space appears narrow in the lymph nodes of animals living in free-roaming rearing system.

In the other hand, the Principal Component Analysis (PCA) was conducted on the following variables: the ratio of active follicles, the diameter of active follicles, the ratio of plasma cells in the medullary zone, and the ratio of lymphocytes in active follicles, and it was found that these variables explained 97.78% of the total variation in the data, (Table 2).

The graphic representation of the variables on the axes 1 and 2 in the circle of correlations represents the projection of the initial variables on a two-dimensional plane consisting of the two factors (PC1 and PC2). The distance of the points from the center of the circle demonstrates the effect of the contribution of the variables. The variables 'ratio of lymphocytes in the active follicles', 'ratio of plasma cells', and 'diameter of active follicles' are strongly positively correlated and are related to each other. On the other hand, the variable 'ratio of lymphocytes in the active follicles' and the variable 'ratio of active follicles' are negatively correlated (Fig. 6).

Table 2. Principal Component Analysis (PCA) table of the analyzed variables defining the coefficient of variance and the explained variation by PC1 and PC2.

Axes	Total	% of variance	Cumulative %
PC1	3.75	93.84	93.84
PC2	0.16	3.94	97.78
Cumulative proportion		97.78	191.62

Regarding the projection of individuals, the 1-2 factorial plane showed the formation of two main group; the first one included individuals 6,7,8,9, and 10 on the positive ordinates of axis 1 and 2 and which included the individuals represented by the variables Ratio of lymphocytes in the active, ratio of plasma cells and diameter of active follicles, so there is a connection with this livestock system (Free roaming livestock system) which indicates higher values in particular, for the diameters of the active follicles compared to the restricted-roaming livestock system. And the second one located on the negative ordinates of axis 1 and 2 and which includes 1,2,3,4, and 5 represented by ratio of active follicles, which is complimentary to the previous findings (Fig. 7).

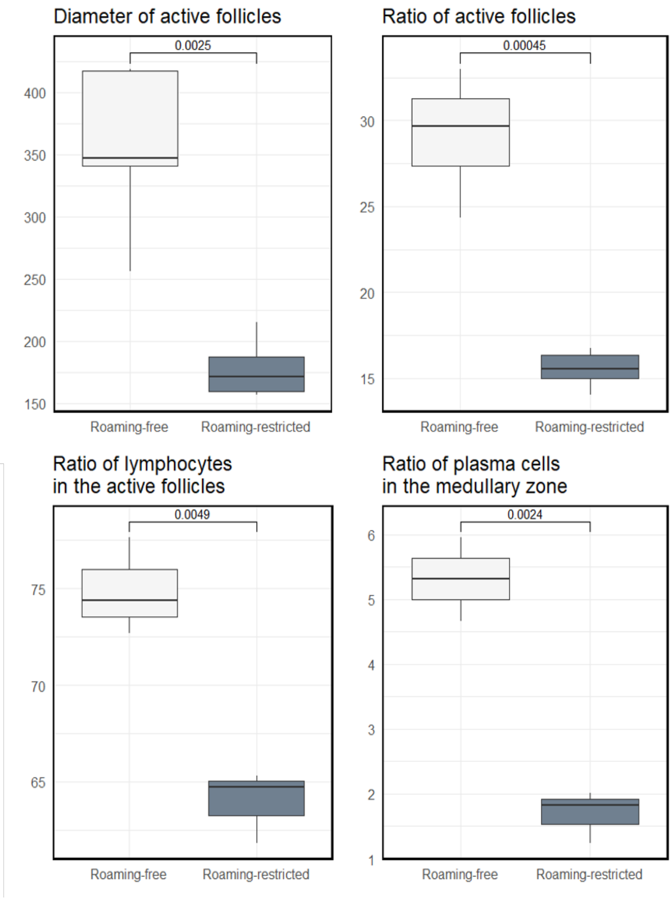

Fig 5. Boxplot presentation of the statistical analysis of T-test results with the P-value.

The resulting presentation of the individual allowed to determine two class groups as mentioned in (Fig. 7). The first group (shown inside the magenta ellipse) is showing the individual presentation of animals living under restricted-roaming housing system, while the second band (shown inside the red ellipse) which present the animals living in a free-roaming rearing system, regarding the presentation of the variables, it was found the factor that affects the most of the animals living under a restricted roaming housing system was 'the ratio of active follicles', and to know the similarities and dissimilarities between the individuals of the dromedary population studied, an Ascending Hierarchical Classification (HCA) was used and made by dissimilarity using Euclidean distances.

The dendrogram clearly represents how the algorithm proceeds to group the individuals into groups and then the subgroups (individuals) the selection of the truncation resulted in obtaining two classes, the algorithm gradually regroups all the individuals. The truncation makes it possible to visualize that the homogeneous groups have been identified according the histo-morphometric and cyto-morphometric results. This structuring composed of classes with a profile of two classes (Fig. 8).

DISCUSSION

The variation in the structure of lymph node parenchyma in dromedaries living in different rearing systems is due to the exposure to pathogens. Studies have shown that animals living in a free-roaming breeding system are exposed to a higher number of pathogens, especially during their early life stage. This exposure plays a significant role in the development of lymphatic follicles in dromedaries. The adaptation to exposure to a high number of pathogens in the early stages of life leads to the production of more antibodies and an increase in the number of active follicles. This is supported by previous studies, (Gaashan *et al*., 2020; Nera

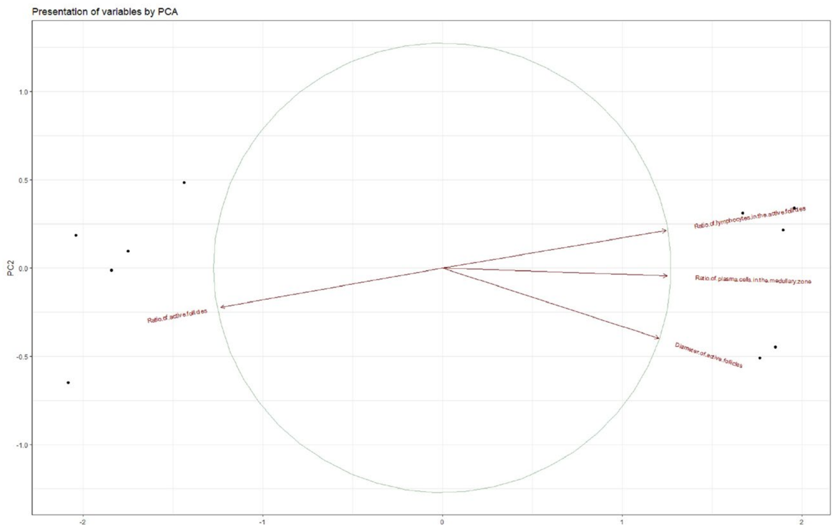

Fig 6. Principal component analysis (PCA) plot showing histological and cellular variables with the circle of correlation between quantitative characteristics

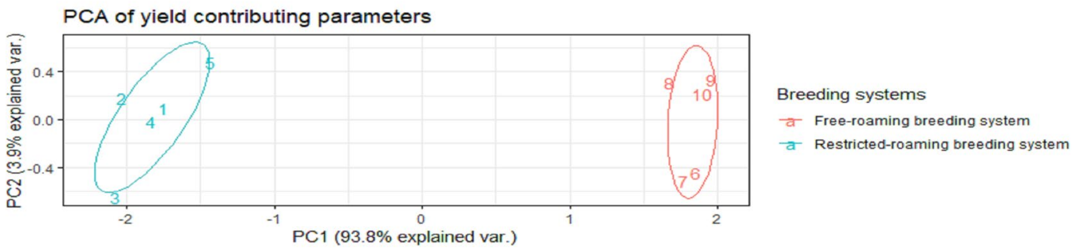

Fig 7. Biplot of the obtained observations from PCA on the projection plane of PC1 and PC2.

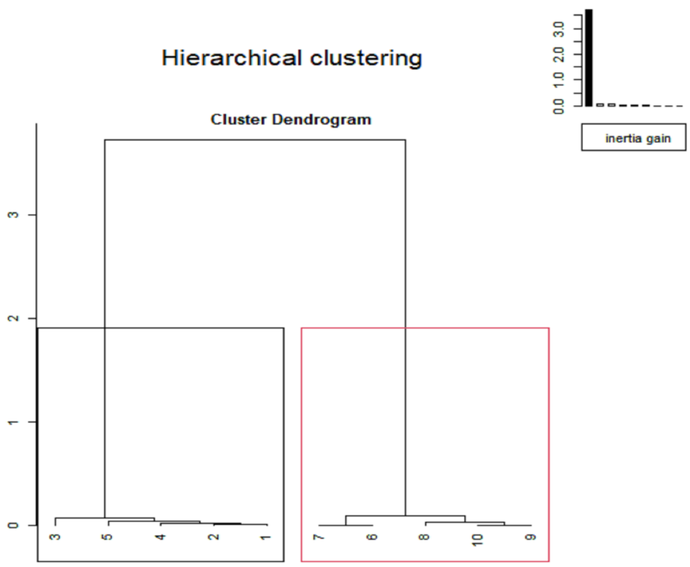

Fig 8. Classification dendrogram of dissimilarity in the dromedary's lymph node in the two different rearing systems.

et al., 2015). Both studies found that exposure to pathogens at an early stage of life leads to an increase in the number of active follicles and the production of more antibodies. These findings give significance to the obtained findings as they provide evidence that supports the relationship between exposure to pathogens and the structure of lymph nodes in dromedaries.

According to Cerutti *et al*. (2013), the variation in the ratio of plasma cells in the medullary zone of lymph nodes is due to the structure of this zone, which contains networks of reticular fibers where antibodies are fixed after being produced by plasma cells. This study highlights the importance of the structure of lymph nodes in regulating the ratio of plasma cells. A similar variation in the number of immune cells in animals under different breeding systems was found by Hussen and Al-Sukruwah (2022) who reported that dromedaries in intensive housing systems had an increased level of blood neutrophils and eosinophils. Additionally, the ratio of lymphocytes in the active follicles of dromedaries raised in a restricted-roaming system was found to be 63.96 ±1.87 %, while the ratio was higher, 74.9±2.52 %, in animals raised in a free-roaming system. This suggests that the animal housing system also plays a critical role in regulating the number and ratio of immune cells. These studies demonstrate that both the structure of lymph nodes and the animal housing system have a major impact on the composition and function of the immune system.

Also, Herman *et al*. (1972) revealed that during an immune response, the microcirculation within lymph nodes increases, leading to physiological hyperplasia. This variation may be beneficial due to the exposure to pathogens in animals living in free-roaming systems, as discussed by Grandin and Shivley (2015).

Adamo (2014) discovered that the practices used in the rearing system during animal housing play a significant role in modulating the morphology and cellular composition of the immune system. This study highlighted the importance of proper animal housing and rearing practices in maintaining a healthy immune system. Additionally, the study conducted by Gaashan *et al*. (2020) found that measurements of many immunological parameters in samples taken from animals under different breeding systems can vary greatly. These measurements can range from a normal baseline to extremely high levels. These findings by Gaashan *et al*. (2020) correspond directly with the conclusions drawn by (Adamo, 2014), which correspond directly with the findings from this study.

It is worth mentioning that these findings are in line with other studies that have reported the impact of rearing systems on the immune system of animals, Gaashan *et al*. (2020) reported that the newborns dromedaries raised in an extensive rearing system present a higher number of neutrophils, monocytes, and lymphocytes, than the other population of dromedaries raised in a restricted roaming system.

In this study, similarity and dissimilarity were utilized to refer to the degree of resemblance or variation in the histological features of the sampled individuals. The Euclidean distance metric was employed to compute the degree of similarity or dissimilarity between individuals based on their morphometric traits. The Euclidean distance is a widely recognized technique for measuring morphometric distances, also, the study's verdict was that the hierarchical agglomerative clustering (HAC) method demonstrated a similar population structure as the PCA analysis. Consequently, the results produced from the HAC method can be trusted for understanding the population structure.

Regarding the representation of the dendrograms, it was observed that the individuals of the first class are the dromedaries raised and living in a free-roaming livestock system, whereas the individuals of the second class are the dromedaries raised and living in a restricted-roaming livestock system. The dendrograms group similar individuals according to the studied parameters, taking into consideration the enormous number of variables surrounding the animals raised and living under different rearing systems such as the microclimate, the stressful factors, and the adaptation processes. It is important to note that these variables may lead to significant changes in the architectural structure of immune organs. Additionally, the lack of knowledge about normality intervals for measurable parameters such as histo-morphometric and cyto-morphometric results is the main reason for the discordance between researchers who are trying to establish whether their findings are normal or abnormal. This can lead to a sort of misdiagnosis concerning the histological and cytological examination of immune organs pathologies. This highlights the importance of considering the different variables that can affect the structure and function of immune organs, and the need for more research to establish normality intervals for measurable parameters in order to accurately evaluate the health of immune organs.

CONCLUSION

Livestock systems, which refer to the management and housing of domesticated animals, may potentially influence on the morphology (structure and function) of lymph nodes in dromedary camels, the immunogenesis process were highly active in the dromedaries living in a free-roaming livestock system. This suggests that the animals raised and living in a free-roaming breeding system are more resilient and able to fight off pathogens more effectively than the animals living under restricted-roaming livestock systems.

These findings suggest that the livestock system of dromedary camels may influence the size and function of lymph nodes, with traditional livestock systems potentially leading to larger lymph nodes and higher levels of immune cells. However, it is important to note that this study only examined a small number of animals, and further research would be needed to confirm these findings and to understand the mechanisms underlying any observed differences. Additionally, it is worth mentioning that the livestock system is just one factor that may affect the morphology of lymph nodes in dromedary camels, and other factors such as age, diet, and environment may also play a role. Therefore, it is crucial to take into account the potential influence of these other factors when studying the effect of livestock system on the morphology of lymph nodes in dromedary camels. This will help to provide a more comprehensive understanding of the factors that influence the morphology of lymph nodes in dromedary camels and how they affect the overall health and immunity of these animals.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the research and publication of this study.

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