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Study the Effect of Age on the Morphological, Histological and Immunohistochemical Structures of The Thymus in Sheep and Dogs

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Abstract

The thymus of twenty Awassi sheep and a street dog is used for this study. The age of animals was two months and one year in both species, and each age had five animals. The gland consisted of cervical and thoracic lobes in sheep, but in dogs have thoracic lobe only. The maximum value of length and weight was in puppies, which were (7.22±0.295cm and 17.760±0.3286g) respectively, while the value of the size was maximum in lambs (15.26±0.182 ml) at P ≤0.05 value. As the age of the animal aged, the thymus was regressed and became very small. The histological and histochemical characterized by a thin connective tissue capsule with collagen and a few reticular fibres, and the highest value of capsular thickness was in adult dogs (45.9±21.70 µm). The cortical thickness in the puppies had the highest value (124.7±49.01 µm), while the medulla thickness was maximum in the lamb (377.4±510.74 µm). The thymus of adult dogs lost the demarcation between the cortex and medulla and appeared as the lymphoid follicles surrounded by epithelial reticular cells and connective tissue with a thinner thickness in the cortex and medulla (15.1±8.04 µm and 36.6±8.06 µm), respectively), compared with adult sheep. T cells give a positive reaction to the CD3 antibody in both species; the highest density and strong intensity were in lambs. The macrophage gives positive staining to the CD68 antibody and appears more distributed and with strong intensity in the thymus of adult dog, while the sheep give negative reaction except in the medulla in adult sheep.

Keywords: Thymus, Morphohistological, Immunohistochemical, CD3 and CD68 Antibodies, Sheep, Dog.

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Introduction

The thymus was known to the ancient Greeks, and its name comes from the herb thyme (in Greek: θύμος), which became the name for a "warty excrescence", possibly due to its resemblance to a bunch of thyme (1). The thymus gland is the primary lymphoid organ in which the T lymphoid precursors mature and are programmed through a complex sequence of cell proliferation, selection, death, and maturation (2, 3). Morphologically, the thymus has a thoracic portion, which is located in the cranial mediastinum, and a cervical portion along the trachea (4). Histologically, in most species, the thin capsule surrounds each lobe and gives rise to trabeculae, which partially subdivide the thymus into incomplete lobules of varying size and direction(5), the peripheral area, called the cortex, appeared darker in color than the paler central area, called the medulla, which was occupied by globular multicellular formations and the Hassall's corpuscles (6). Much research has dealt with the study of the thymus in animals, but there was no research on normal dogs, especially in adults, and little research was conducted on the lamb. So this study focused on the thymus in sheep and dogs and compared them according to age to understand how environmental stress can affected on the structures of the thymus in both species.

Materials and methods

For this study, twenty animals were divided into groups. One group involved ten Awaasi healthy sheep of both sexes that were taken from Basra Modern Slaughterhouse in Basrah governorate (6). These animals were divided into two groups; each group included five animals. One group was two months old, and the other was one year old. Other samples were collected from ten healthy street dogs in Basrah governorate and divided into two groups according to age: one group with five puppies and the other group with five adult dogs. The samples from the dogs were collected after the animals were scarified by euthanasia (7). Morphological studies of the samples were carried out by measuring the length, weight and size of the thymus.

Histological and histochemical study

The collected sample was fixed with formaldehyde (formalin) 10% of (72 hours) to maintain the tissue as a normal state (9) and washing with tap water for (4-6hours) to remove any fixative from the tissues and then by passing it in grades or concentration of Ethyle alcohol (70%, 80%, 90%, 100% and 100%) for 2 hrs. The specimens were cleared by using two changes of xylene (50% xylene: 50%alcohol) and 100% xylene, then infiltrated and embedded in paraffin wax at (58-60)C°, twice for 3 hours, and the third change overnight to form good morphology (10), then trimming and sectioning with a microtome, mounting on the glass slide, and staining with different stains(hematoxylin and eosin stain, Masson's Trichrome stain, reticulin stain and periodic acid Schiff reaction according to (11).

Immunohistochemical study

The CD3 and CD68 Marker was used to detect the density of T lymphocytes and macrophages in the thymus and compared between the animals. Slides prepared by removal of paraffin were placed in a 56-60 ℃ oven for 15 min, then transferred to a xylene bath and two changes of xylene for 5 min. Then two changes of fresh absolute ethanol for 3 min, rehydrated, 90% ethanol for 3 min, and 80% ethanol for3min. Rinsed in running tap water and placed in PBS for 30 min at room temperature. The Microwave retrieval method was used. Enough drops of 3% hydrogen peroxide were used to inactivate Endogenous Peroxidase. Then, washed with PBS wash bath for 2 min. Primary Antibody for the CD3 and CD68 was added to the slid then the Secondary Antibody Reaction (Biotin/Streptavidin Detection) was used and applied the DAB chromogen to each 1ml of substrate buffer, finally Counterstaining was applied with Mayer's hematoxylin and mounted using aqueous mounting medium such as Faramount (12) and (13).

Statistical analysis

The (one-way ANOVA) program was conducted, and POS hoc test (Tukey´s HSD) was used to analyze the morphological and histological results and determine which specific group differed significantly from each other at $P \leq 0.05$ (14) and (15).

Result

The thymus gland in the lambs was lobulated organ and large, pale-pink in color and consisted of two parts, the cervical part that located in the neck region adjacent to the trachea ventrolatrally and connected to other part the thoracic part by short isthmus and appeared as scattered or fragments at the base of the trachea, the thoracic part it was represented the greater part of the thymus located in the cranial mediastinum reaching to the pericardium in front the heart and attached the chest at the level of 3-5 ribs. On the left side, it is connected to the cranial lob of the left lung, and on the right side, it is situated on the greater vessels of the heart.

When the sheep reached puberty, the two parts were regressed, and only the thoracic part remained in low development and appeared pale pink in color and smaller in size. In puppies was large, lobulated, flat, and soft, triangular in shape, and pale in color, extended from the thoracic inlet to the pericardium of the heart and contact the cranial lobe of the lung, the ribs at (1-5) and consist of two lobe the right and left lobe and the large part was positioned on the left surface to the pericardium than onto the right, and this study recorded there was no cervical portion in the thymus of dog. As the age of the dog advanced, the thymus underwent regression; it was small, with less lobulation, elongated in shape, and pink-red in color (Figure 1and Figure 2).

The statistical analysis revealed there were significant differences in the mean of the thymus length at level P≤0.05, the length in the thymus of puppies was the maximum value (7.22±0.295cm) and in the adult dog was the minimum value (2.18±0.311cm) compared to lamb and adult sheep (6.20±0.224cm and 3.86±0.462cm) respectively. The weight of the thymus was significantly increased in the puppies (17.760±0.3286 g), lamb (13.160±0.2608g), adult dog (4.160±0.5079C g) and adult sheep, which is have the lowest value (3.020±0.3834g) among the animals and the size of puppy's thymus appeared in the largest size (15.26±0.182ml) than lambs, adult sheep, and adult dog (12.50±1.817ml, 5.04±0.371ml, and 2.02±0.383ml), respectively, (Table 1).

The histological structures of the thymus were a lobulated organ that consisted of three regions: the capsule, cortex, and medulla (Figure 3). The thymus was surrounded by the thin connective tissue capsule mainly made of collagen and reticular fiber to provide the strong and supportive structures of the thymus and contained some lymphocytes, the capsule was giving rise to trabeculae that carry the blood supply into and away the thymus and consisted of connective tissue of collagen and reticular fiber to form the framework of the thymus, these septa divided the thymus into lobules partially these lobule separated at the outer margin and connected in the center to each other. The subcapsular region of the thymus, which appeared as a dark, densely staining layer containing an extensive population of small immature predominant cells, T lymphocytes. These cells had a round to oval nucleus with one or more nuclei during the dividing activity and contained prominent, strong basophilic cytoplasm. These cells were more active in the deep cortex and corticomedullary junction. (Figure4) .The medulla represented the contact region between the lobule and appeared with paler staining than the cortex, which consisted of large, mature T lymphocytes, less densely, with a higher amount of cytoplasm than in the cortex. The epithelial reticular cells are in various shapes and sizes with different stages of degeneration and keratinization, and macrophages are located in the corticomedullary region and housing the specialized structures that called the Hassall's corpuscles appeared rounded or elongated in shape, varies in size and their number was varies among the animal species and age and higher number in small lamb that adult sheep and in adult dog then puppies these structures consisted of a layers of epithelial reticular cells with different stages of degeneration and some of the keratinization contained a hallo concentric cyst involved cellular debris and lymphocyte and macrophage (Figure 4).

In adult animals (sheep and dogs), the thymus shrank and reduced in size and was infiltrated with fat tissue, which is significantly abundant in adult animals, especially in adult sheep. While in the dog, the thymus loses the characteristic appearance of lobulation, and the demarcation between the cortex and medulla is lost, taking the form of the lymphofollicular structures. The cortex was thin and irregular, and the lymphocytes were scattered and decreased in number. The medulla contained more prominent epithelial reticula cells that appeared in different shapes, such as irregular cysts or cords, and were infiltrated with connective tissue. (Figure 5).

The statistical analysis illustrated there were significant differences at P ≤0.05 value in the mean of the capsular thymic thickness, which was significantly increased in the thymus of adult dogs and became denser than in the lamb, adult sheep, and puppies (45.9±21.70 µm, 38.7±26.12 µm,26.5±11.38µm and 25.8±11.23µm) respectively, the cortical thickness in the young animals were significantly increased in the puppies (124.7±49.01µm), and decreased and became thinner in the adult dog (15.1±8.04µm) while, the thickness of medulla in the lamb was significantly increased than in other animals (377.4±510.74µm) (Table 2). Histochemically, the Masson´s Trichrome stain demonstrated the collagen fibers in the capsule and trabeculae, which appeared blue to green in color, and the amount of this fiber varies among the ages of sheep and dogs. It was more prominent and with high density in the sheep, particularly in adult sheep (Figure 6). While with the reticuline stain, the reticular fibers can be visible with a smaller amount than collagen fibers in the capsule and trabecular, and around the blood vessels, it was higher density in the lamb and puppies, and less prominent in the adult sheep and adult dog (Figure 7). The supporting framework of the thymus was based on the presence of reticulo-epithelial cells that provide source of energy from the glycogen that stored in the cytoplasm of these cells and give positive reaction with PAS stain that appeared magenta in color and the density of the glycogen were more in the puppies than lamb, adult sheep and adult dog which clearly visible in the capsule and connective tissue and in the endothelial cells of blood vessels (Figure 8).

Immunological study:

Both sheep and dog thymus give a positive reaction to the T cell CD3 antibody. The thymus cortex contained small, immature, densely packed T lymphocytes which appeared a brown color with different degrees of intensity. The T cells in the thymic cortex of lamb gives positive reaction with the high distribution and strong intensity more than in adult sheep and the puppies, while the adult dogs show the lowest distribution and intermediate intensity (Figure 9). In the medulla, the T cells showed positively stained cells to the CD3 marker and appeared with strong intensity in younger animals, particularly in the lamb, more than in adult animals, that showed intermediate intensity with a few distribution in adult dogs (Figure 10). In this study, the macrophages in the thymus gives positive reaction to the CD68 antibody in the cortex of the dogs and their density were clear visible in the adult dog with strong intensity when compared with the puppies, which appeared contained few number and intermediate intensity. In the medulla, the macrophage give positive reaction in the thymus of adult sheep with weak intensity, in contrast to the dogs that give intermediate intensity and more distribution in adult dogs than in puppies and adult sheep (Figures 11 and 12).

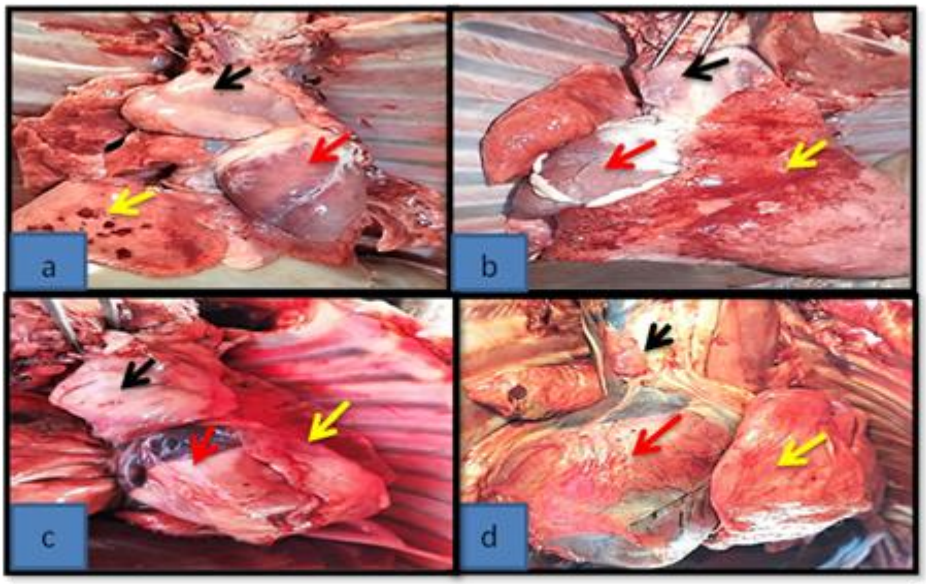

Figure 1: Morphological structures of thymus gland in : (a) Lamb (b) Adult sheep (c) Puppy(d) Adult dog, show: Thymus (black arrow), Heart (red arrow), Lung(yellow arrow) .

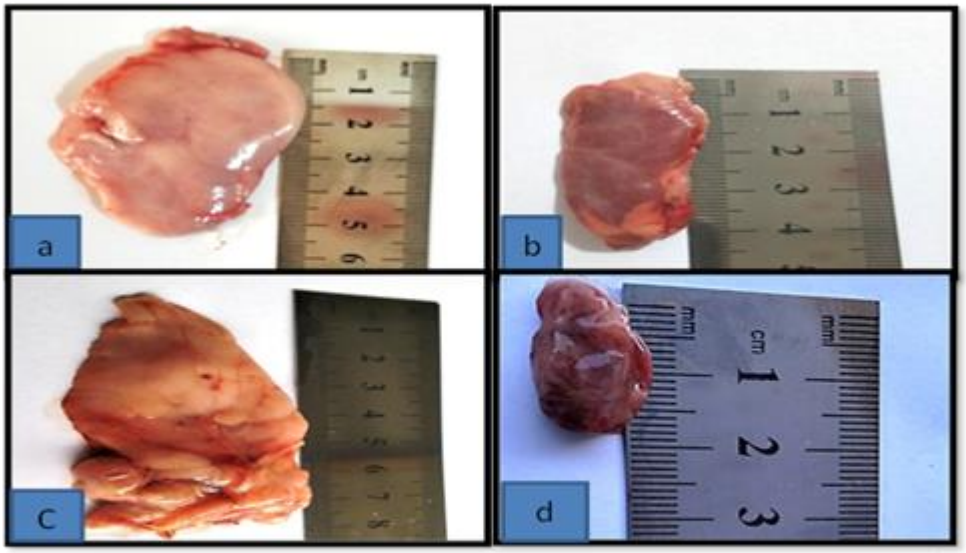

Figure 2: Photographic image show the length of thymus in (a) lamb, (b) adult sheep, (c) puppies and (d)adult dog.

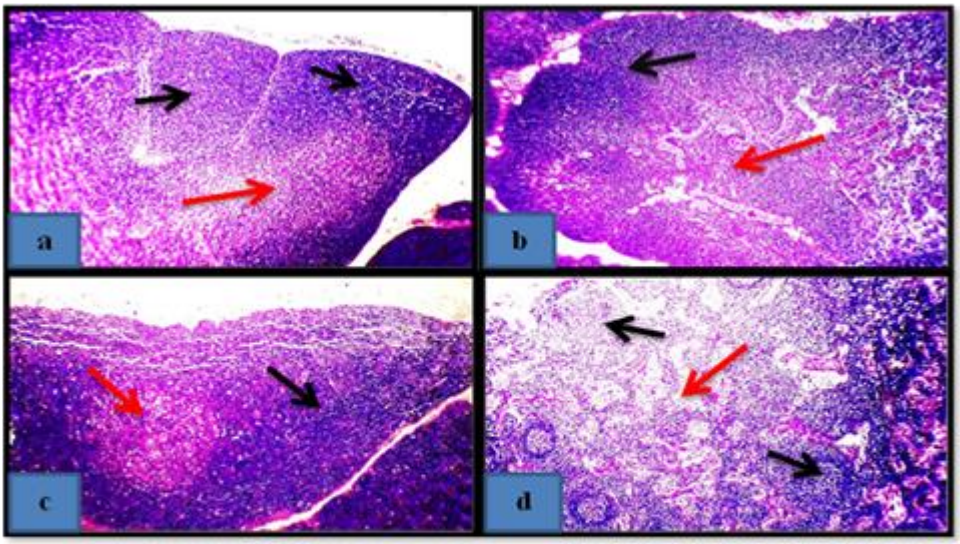

Figure 3: Morphohistological structures of thymus gland in (a) Lamb, (b) Adult sheep, (c) Puppy (d) Adult dog show : Cortex (black arrow), Medulla (red arrow).H&E. stain. 4X.

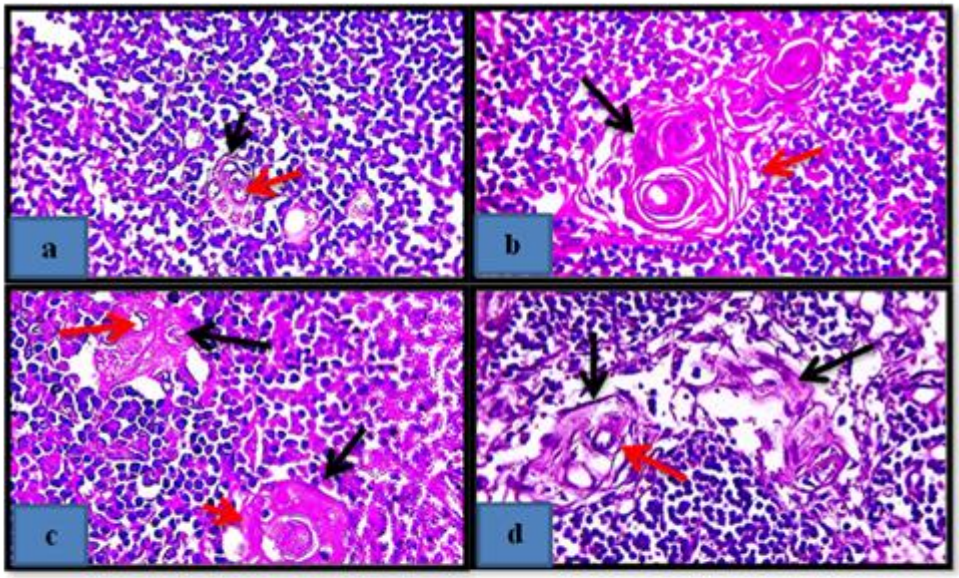

Figure 4: Morphohistological structures of medulla of thymus gland in (a) Lamb (b) Adult sheep (typical) (c) Puppy (d) Adult dog show : Hassall's corpuscle (black arrow), epithelial reticular cells (red arrow).H&E. stain. 40X.

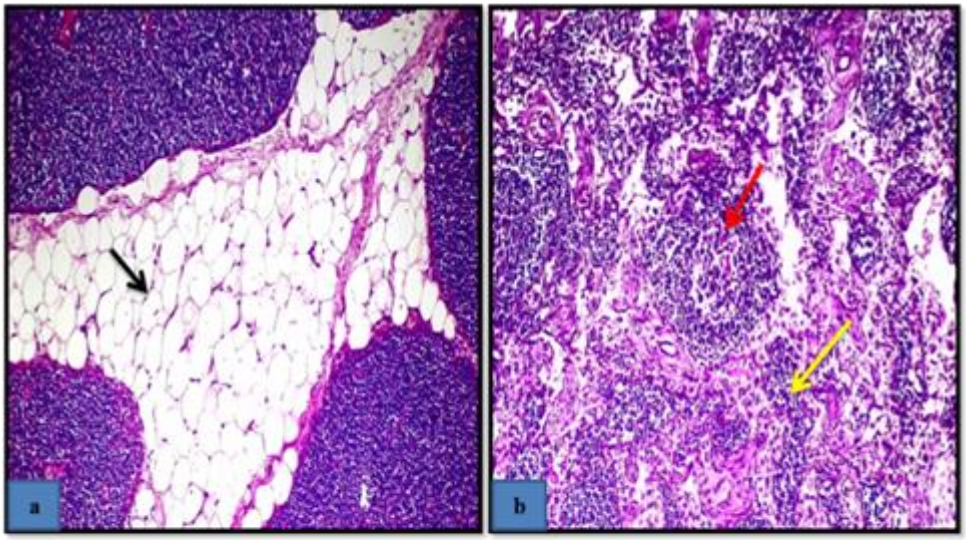

Figure 5: Morphohistological structures of thymus gland in (a) Lamb (b) Adult sheep (c) (d) Adult dog show : Cortex (red arrow), Fat tissue (black arrow), epithelial reticular cells (yellow arrow). H&E stain. 10 X.

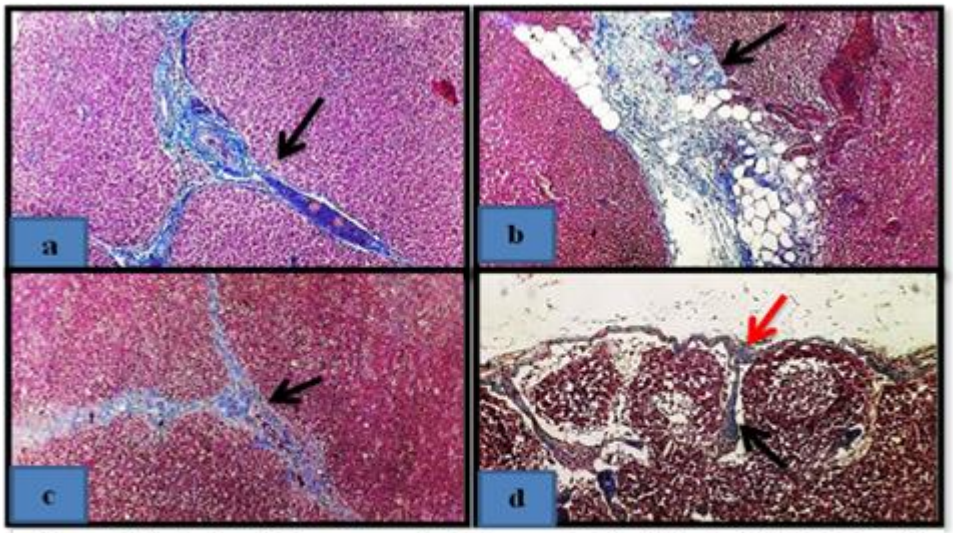

Figure 6: Histolochemical structures of of thymus gland in (a) Lamb (b) Adult sheep (c) Puppy (d) Adult dog show : blue collagen fibers in capsule (red arrow), Trabecula (black arrow), Masson's Trichrom stain. 10X.

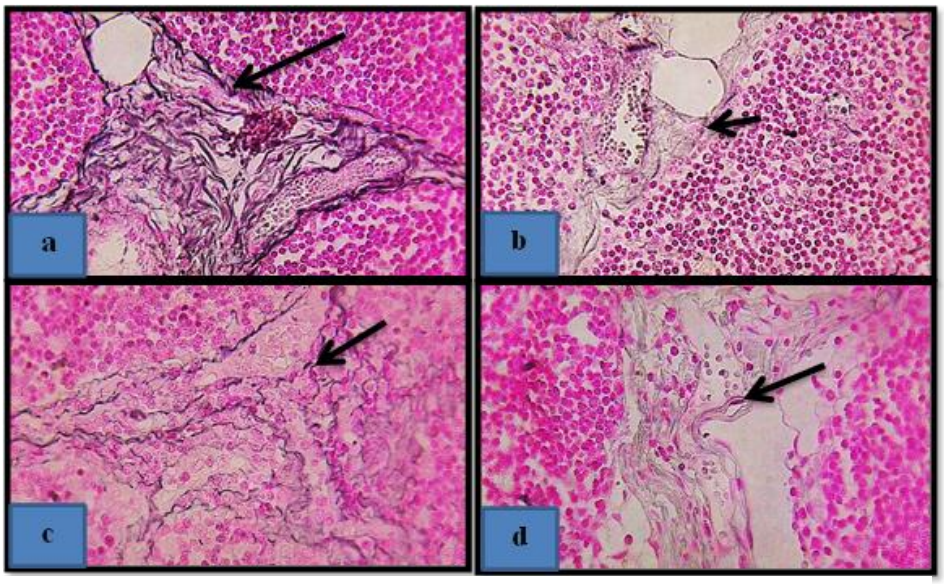

Figure 7: Histochemical structures of thymus gland in (a) Lamb (b) Adult sheep (c) Puppy (d) Adult dog show : black reticular fibers in Trabecula (black arrow) Reticulin stain. 40X.

Table (1): Mean of length, weight and size in the thymus in sheep and dog:

Animal/parameter	Length/ cm	Weight / g	Size/ ml
lamb	6.20±0.224A	13.160±0.2608B	15.26±0.182A
Adult sheep	3.86±0.462B	3.020±0.3834C	5.04±0.371C
Puppies	7.22±0.295A	17.760±0.3286A	12.50±1.817A
Adult dog	2.18±0.311C	4.160±0.5079C	2.02±0.383C

*A, B and C significant differences at the level P ≤0.05 between the age the animals.

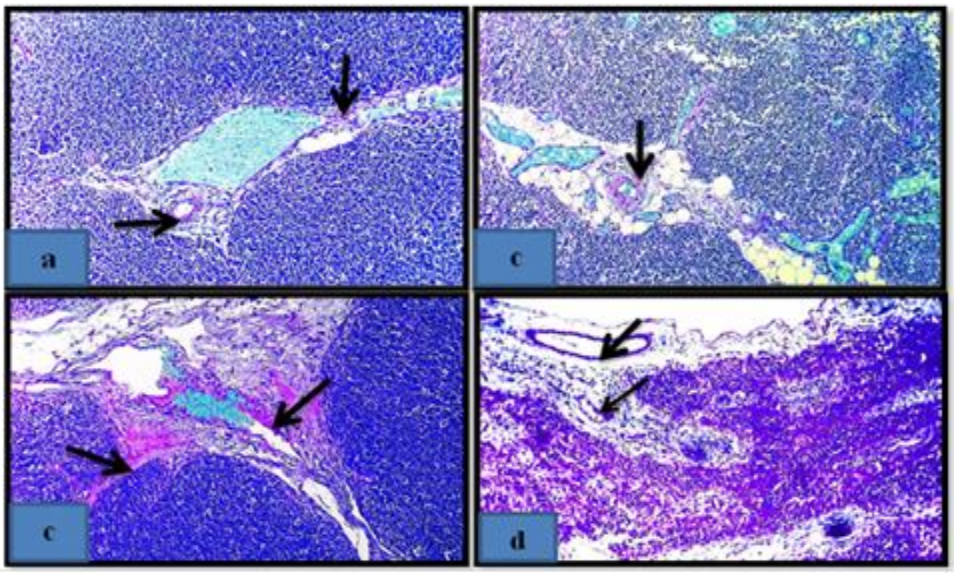

Figure 8: Histochemical structures of the thymus gland in (a) Lamb (b) Adult sheep (c) Puppy (d) Adult dog show the density of the carbohydrate deposition in in blood vessels with trrbeculae and capsule. PAS. Stain 10X.

Table (2): Mean of capsule, cortex and medulla of thymus in dog and sheep:

Species/ region	Capsule / µm	Cortex / µm	Medulla/ µm
lamb	38.7±26.12B	114.7±43.05 A	377.4±510.74A
Adult sheep	26.5±11.38C	78.1±22.67B	268.8±74.16B
puppies	25.8±11.23C	124.7±49.01A	180.0±67.54C
Adult dog	45.9±21.70 A	15.1±8.04C	36.6±8.06D

*A, B, C and D significant differences at the level P ≤0.05 between the ages of animals.

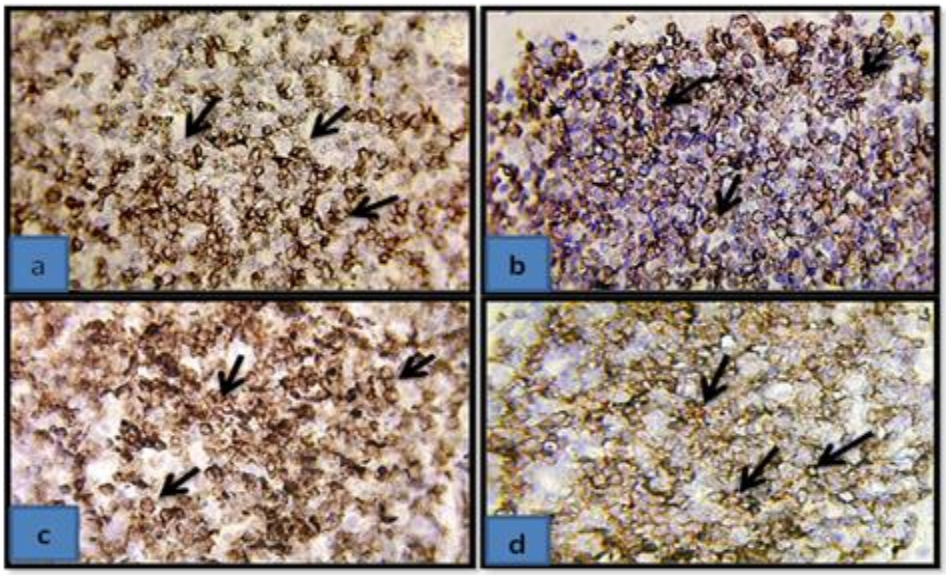

Figure 9: Immunohistochemical section show the density of the CD+ T cells staining of the cortex of thymus in (A) Lamb (b) adult sheep (c) puppy(d) adult dog. CD3 marker.40X.

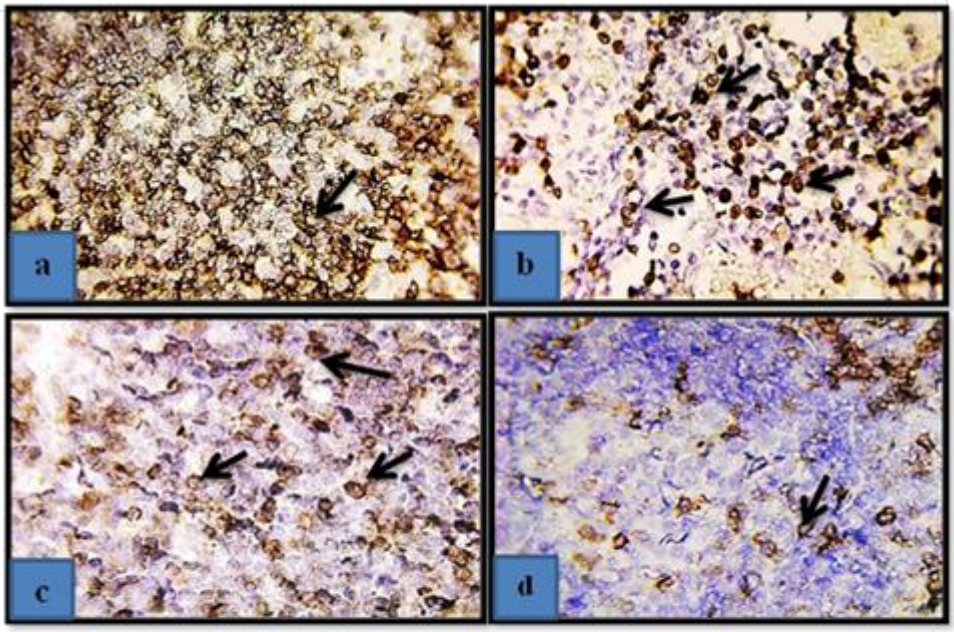

Figure 10: Immunohistochemical section show the density of the CD+ T cells staining of the medulla of thymus in (a) Lamb (b) Adult sheep (c) Puppy (d) Adult dog. CD3 marker.40X.

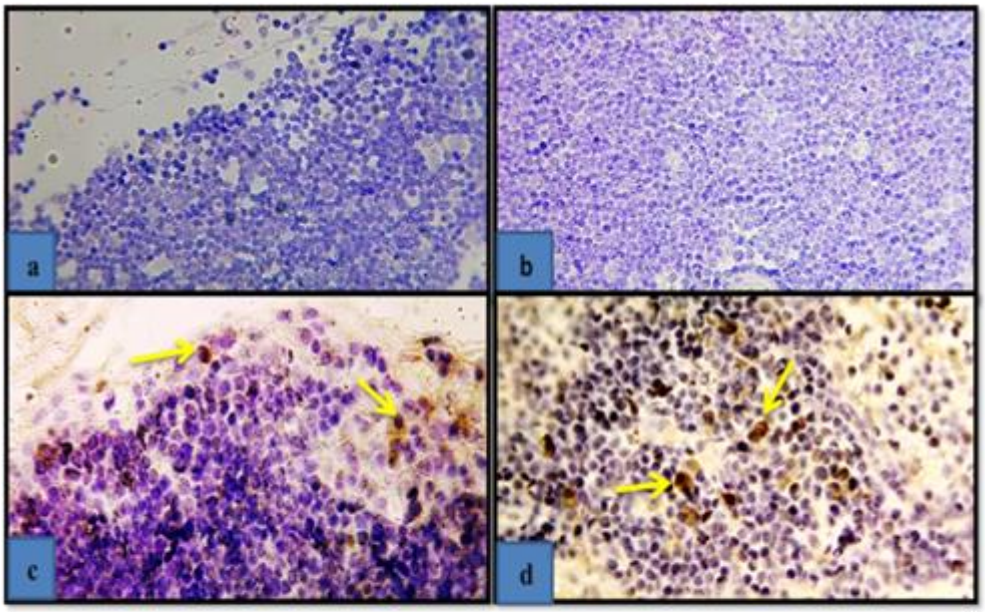

Figure 11: Immunohistochemical section show the density of the CD+ macrophage staining of the cortex of thymus in (a) Lamb (b) Adult sheep (c) Puppy (d) Adult dog. CD68 marker.40X.

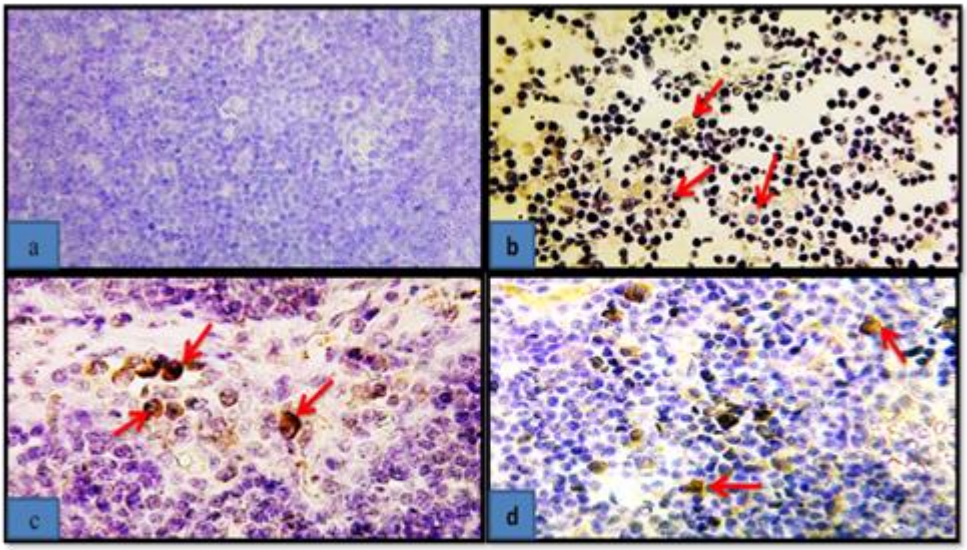

Figure 12: Immunohistochemical section show the density of the CD+ macrophage staining of the medulla of thymus (red arrow) in (A) Lamb (b) adult sheep (c) puppy (d) adult dog. CD68 marker.40X.

Discussion

Morphologically the thymus in lamb was consisted of two part the cervical part and thoracic part and connected to gather by short isthmus, the cervical part was the smallest scattered part on the trachea and the dorsal lobe was the largest lobe and located at the left at cranial mediastinum region this result agree with (16) in chicken that have many lobes on the neck and thoracic, (17), (18) in lamb and (19) in Bos taurus. The shape of the dorsal lobe was lobulated in shape, similar to findings (20) in cattle and (4) in dromedary camel, but some studies revealed there were intermediate lobes in the cattle and pigs (21), and this finding disagrees with our result. While in adult sheep, the lobulation had disappeared and became smaller, as described in (18) in lambs. The color of the gland was pink pale in color in lamb with 2 moth and changed to gray pink in adult sheep this finding coincide the result in (22) in sheep and (23) in goat that reported the thymus appeared pinkish at 4 months of age and changed to pale yellow with advancement of age while disagreement with (24) that noticed white to pale yellowish colored thymus in rabbits. The puppies thymus was appeared large, pale pink, lobulated, triangular in shape consist of the this result corresponded to the (20) and (25) and consist of the cervical part larger lobe that positioned to the left side of the precardium and right smaller lobe this agree with (26) and, (27) were mentioned the thymus in the dog was lack the cranial lobe, and agree with (26) in horse and dis agreement with (28) in goat that said the thymus in goat contained additional cranial lobe .so the thymus mas restricted in one organ and give it the larger size than in sheep this finding correspond to (29) in dog. The mean weight, length, and size were higher values in puppies than in other animals, and as the animals developed, these values were decreased, and the lobulation was regressed, and the color appeared more grey. This result agrees with (30) in dog, (18) in lamb and (28) in goat.

Histologically, In this research the surrounding thymic capsul and trabeculae that divided the thymus into incomplete lobule in younger animals this correspond (31) in camel, in both animals and ages the capsule was consisted of the collagen, reticular and few elastic fibers and the density of this fiber in adult sheep than other species and ages this similar (22) in sheep and dissimilar with (32) in goats that mentioned the thickness of capsule was increased as the animal was growing. Deeply stained cortex and densely packed population with immature small lymphocytes and the mean of thickness was maximum in the younger animal specially in puppies while the medulla populated with lightly packed large lymphocytes and the mean thickness was high in value in the lambs than other animals and ages this agree with (22) in sheep and (3) in different species, the cortex and medulla thickness was decreased in adult sheep and dog this came to agree with (20) and the hassall's corpuscles was vary in development and it's number during the animal developing with different size this finding related to finding (34). In adult dogs, the histological structures of the thymus undergo more involution, and the cortex appears very thin and contains adipose tissue surrounded by medulla with lymphoid tissue and connective tissue (3). The number

of Hassall's corpuscles located within the medulla varies and is more prominent in the lamb than in other animals. This is due to the age-related changes in the thymus during the involution process (24) and (34) in the rabbit and goat. Hiatochemically, The amount of collagen fibers that appeared more prominent in the capsule and trabeculea of the thymus in the adult sheep than other animals and the reticular fiber was more density in the lamb and puppies than adult sheep and dog and the amount of glycogen more visible in the reticuloepthelial cells that considered the source of energy in thymus and more prominent in the thymus of puppies this finding was corresponding to the (35)in goat, (36) in buffalo calves and (37) in goat. Immunohistochemically, in this research, the CD3 marker was used to detect the T cell population and density in the thymus, in which the T cells were located more densely in the cortex and less abundant in the medulla of the sheep and dogs, and this result was similar to (38) and (39) in equine. The lamb showed a higher proportion score and intensity score of T cell staining in the sheep than in the dog, and particularly in the lamb, this result was in agreement with (40), who said the T lymphocyte number was higher in young yaks than in adults. This results in the involution and regression of the thymus during adulthood, leading to decreases in the number of T lymphocytes and replacement with adipose tissue, similar to (41) and (42). For the detection of macrophages in the thymus, the CD68 has been used (43). In our results, the animals gave positive staining for the macrophage in the thymus cortex of dogs in both ages, but the sheep gave a negative reaction because the T cell population in the cortex was maintained by the thymic epithelial cells during the positive selection(44). The number of macrophages was more abundant with strong intensity in the medulla of adult dogs participating in thymocyte selection, engulfing, and degrading apoptotic thymocytes. This result was in agreement with (45), while in disagreement with (46) and (47), who mentioned that the number of macrophages was more abundant in the younger yaks.

Conclusion

In our study, the thymus of sheep consists of two portions: cervical and thoracic parts, while the dog thymus consists only of the thoracic part. The values of the length and weight were the highest in puppies, but the size increased more in lambs. Significant differences in the thickness of the capsule, cortex, and medulla and filled with fat tissue as the thymus involuted, the Hassall's corpuscles were more visible in lambs. The T cell in the cortex gave a positive reaction to the CD3 antibody and more populated in the cortex of lambs, while the cortex in the thymus of sheep gave negative staining to the CD68 antibody for macrophage, but the dog thymus gave a positive reaction with strong intensity in both cortex and medulla of dogs.

Conflict of interest

There is no need to declare a conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee

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دراسة تأثير العمر على التراكيب الشكلية, النسجية والكيمياء النسجية المناعية للغدة الصعترية في الاغنام والكلاب

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الخلاصة

في هذه الدراسة تم استخدام عشرين غدة صعترية من الاغنام العواسية والكلاب الضالة بعمر الشهرين وعمر السنة لكلا الجنسين وكل عمر تضمن خمسة حيوانات. امتلكت الاغنام فص عنقي وفص صدري بينما الكلاب احتوت على الفص الصدري فقط ،وكان اعلى قيمة لطول وحجم الغده في الجراء (0.295±7.2سم و 0.3286±17.760غم) على التوالي بينما كان اعلى معدل للحجم في الحملان (0.182±15.25ملم)عند مستوى $P \leq 0.05$. وكلما تقدم عمر الحيوان فان الغدة تتراجع وتصبح صغيرة الحجم. تتميز التراكيب النسجية والكيمياء نسجية بمحفظة من النسيج الضم يتكون من ألياف الكولاجين والقليل من الالياف من الألياف الشبكية، وكان اعلى سمك للمحفظة في الكلاب البالغة (21.70±45.9 مكروميتر) وسمك طبقة القشرة في الجراء كان الاعلى قيمة (49.01±124.7 مايكروميتر) بينما سمك اللب كان اعلى قيمة لها في الحملان(510.64±377.4 مايكروميتر). في الكلاب البالغة تفقد الغدة التمايز بين منطقة القشرة واللب وتظهر بشكل جريبات لمفية تحيط بها الخلايا الطلائية الشبكية ونسيج ضام وتكون ذات سمك (8.04±15.1 مايكروميتر 8.06±36.6 مايكروميتر) على التوالي عند مقارنتها مع الاغنام. اعطت الخلايا التائية تفاعل ايجابي للأجسام المضادة (CD3) في كلا الجنسين وكان اعلى كثافة وانتشار لها في الحملان. ان خلايا البلعمة اعطت تصبغ ايجابي مع الجسم المضاد (CD68) واعطت اكثر انتشار واعلى شدة في الكلاب البالغة بينما اعطت الاغنام تفاعل سلبي ما عدا منطقة اللب في الاغنام البالغة التي اعطت نتيجة ايجابية.

الكلمات المفتاحية : الغدة الصعترية، شكلية نسجية، كيمياء نسجية مناعية، أجسام مضادة سي دي ٣ وسي دي ٦٨، اغنام، كلاب.