

Immunohistochemical and Histomorphological Study of Postnatal Development of the cecal tonsils in Turkey (*Meleagris Gallopavo*)

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Summary

Forty normal turkeys divided into four age groups: 1 week, 1 month, 2 months and 7 months. The cecal tonsils were separated and prepared for histological and Immunohistochemical studies. At 1 week old the tonsil appear as lymphoid aggregation of the lamina propria submucosa around central fossula. No septae among follicles, no germinal centers observed. At 1 month old the cecal portion which included the tonsil easily separated from other parts due to an increase cecal wall diameter was increased. The tonsil composed of a lot of tonsil crypts. The tissue septae among the crypts observed. At 2 and 7 months old the tonsils bulging from the internal surface of cecum toward the lumen as a rough large mass white to pink in color, germinal centers were present in the lymphoid follicles, the cecal tonsil diameter is increase with ages. The lymphocytes diameter is increasing with age also the internal surface of tonsil was lined with simple columnar epithelium (intestinal mucosa). The dimensions of cecal tonsils and lymphocytes measurements and tonsil mucosa are significantly increase with age. (CD268) antibody revealed presence of mature B lymphocytes within the parenchyma of bursa at two and seven month's old turkey. CD8 no expressed at the first month age while different degrees of expression appeared at the other ages.

Keywords: Tonsils , turkey, mucosa, T lymphocytes, Immunohistochemistry.

Introduction

Gut associated lymphoid tissue (GALT) is considered the main constituent of mucosa associated lymphoid tissue and adjacent to lamina propria. GALT includes several types of cells: as special inducers, immunoregulators, and effectors that are distinct from those involved in systemic immune function(1). Unlike mammals, birds have no lymph nodes, so the initiation and development of immune response happens mainly in GALT and in the spleen. The cecal tonsil are similar to the Peyer's patches in structure containing central crypts, diffuse lymphoid tissues, and germinal centers (2). In the cecal tonsil, both T and B cells are present in the germinal centers, as are plasma cells expressing surface IgM, IgG, and IgA (3). The function of the cecal tonsil is unknown, but its uptake of orally administered carbon particles had been shown, suggesting a role in antigen sampling (4). The aim of this study was to provide information about the developmental changes of the turkey spleen and distribution of lymphocytes in the lymphatic organs in the important periods of turkey life that allow to

determination of the tonsils characters and range of changes in lymphatic organs to increase the immunity and served in planning and reprogrammed the time and mode of vaccination in Iraqi turkey farms..

Materials and Methods

Forty healthy turkeys of both sexes at four ages (1 week, 1 month, 2 months and 7 months) were used in the current study. They were selected from local farm in Diyala city, Iraq. The birds were housed in clean cages under strict hygienic conditions. Histotechniques were done at the laboratory of veterinary medicine collage, University of Diyala, Iraq. The cecal tonsils dissected and immediately fixed in Bouin's and neutral buffer formalin solution, Paraffin-embedding and sectioning 6-7 μ m by rotary microtome. The cecal tonsil were stained with Hematoxylin and Eosin (H&E) for routine histological examination(5). Other sections were used for Immunohistochemical studies using polyclonal antibody to B-cell activation factor receptor (BAFR) (CD268) produced in rabbit (cloud-clone corp. USA). Monoclonal antibody to T-cell (CD8) produced in mouse.

Results and Discussion

Morphological observations: Large cluster accumulations of lymphatic nodules easily detected as nodular masses like thickening of the cecal wall near the cecocolic junction. These tonsils very clear when dissection of the wall of cecum doing (Fig.1). At 1 week old there was thickening of the cecal wall that containing the tonsils (Fig. 2A). At 2 month old the cecal portion included the tonsil was easily separated from other parts due to the increased the cecal wall diameter (Fig. 2B). At 2 and 7 months old the tonsils bulging from the internal surface of cecum toward the lumen as a rough large mass white to pink in color (Fig. 3A,B).

Histological observations: The cecal wall composed of four histological layers: mucosa, sub mucosa, muscularis and serosa. Mucosa was simple columnar epithelium and protruded into the lumen as villi. Sub mucosa composed mainly of connective tissue contain lymphoid aggregations of cecal tonsils. Muscularis composed of two muscular layers inner circular and outer longitudinal and surrounded the cecal tonsils. Serosa was mesothelial layer (Fig. 4). At one week old turkey the cecal tonsils appear as aggregations of lymphoid masses in the lamina propria sub-mucosa surrounded the central fossula. No septae among lymphoid follicles were presents and no germinal centers were observed (Fig.5A,B). The cecal tonsil dimension was 59.1 ± 4.9 . The lymphocyte dimension was 0.08 ± 0.01 . The mucosa thickness were 2.7 ± 0.6 (Table,1). At one month bird, the cecal tonsil dimensions were 110.8 ± 2.7 . The tonsil composed of a lot of tonsil crypts. Each crypt composed of secondary follicles surrounded the central fossula. The tissue septae among the crypts were observed in this age (Fig.6 A,B) The lymphocytes diameter was 0.2 ± 0.01 . The mucosa thickness was 2.5 ± 0.1 (Table,1). At two months old the cecal tonsils measurements were 408 ± 77.5 . The lymphocytes diameters were 0.5 ± 0.02 . The mucosa thickness was 2.4 ± 0.03 . Germinal centers were presented in the lymphoid follicles (Fig.7A, B). At seven months old the cecal tonsils measurements were 641.0 ± 77.5 . The lymphocytes diameters were 0.3 ± 0.01 . The mucosa thickness was 12.5 ± 0.03 .

Germinal centers were presented in the lymphoid follicles (Fig.8A, B). The cecal tonsil diameter was increased with ages. The lymphocytes diameters were increased with age. The internal surface of tonsil was lined with simple coloumnar epithelium (intestinal mucosa). The dimensions of cecal tonsils and lymphocytes measurements and tonsil mucosa were significantly increased with age (Table, 1).

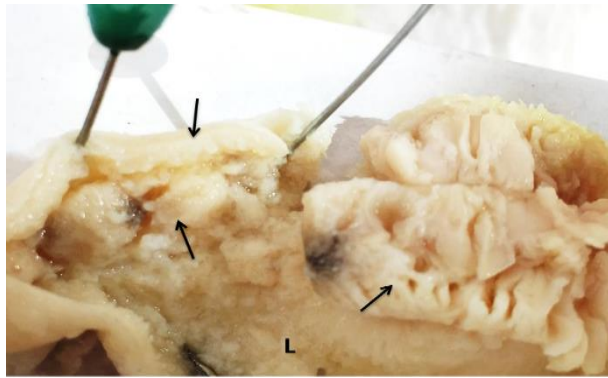
Immunohistochemical observations: CD8 is a marker of choice for the cytotoxic T cells and for T cells with suppressor activity. At one week old negative expression of CD8 (Fig.9A). At one month old CD8 show negative expression in sub-epithelial region (Fig.9B). At two and seven months old CD8 were showed positive expression (Fig.10A, B).

CD268 was a marker of choice for identification of mature B cells (plasma cells) distributions within the cecal tonsil parenchyma. At one week old CD268 was negative expression (Fig.11A). At one month old negative expression of CD268 in sub epithelial regions (Fig.11B). At two and seven months old positive expression of B cells (Fig.12A, B).

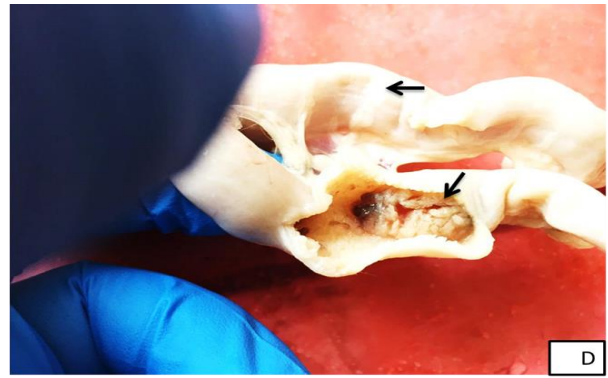
Table,1: Histological parameters of the turkey cecal tonsils in the four age groups.

Age	Cecal tonsil dimension	Lymphocyte diameter	Mucosa thickness
1week	59.1 ± 4.9^d	0.08 ± 0.001^d	2.7 ± 0.6^d
1month	110.8 ± 2.7^c	0.2 ± 0.01^c	2.5 ± 0.1^c
2months	408 ± 77.5^b	0.5 ± 0.02^b	2.4 ± 0.03^b
7months	641.0 ± 77.5^a	0.3 ± 0.01^a	12.5 ± 0.1^a

*Small letter:significant difference different at $p \leq 0.05$ or 0.01 .



Figure,1: Shape, color and location of the turkey cecal tonsils (arrow), cecal lumen(L).



Figure,3B: Shape and location of turkey cecal tonsils at seven months age (arrow).



Figure,2A: Shape and location of the turkey cecal tonsils at one week age (arrow).

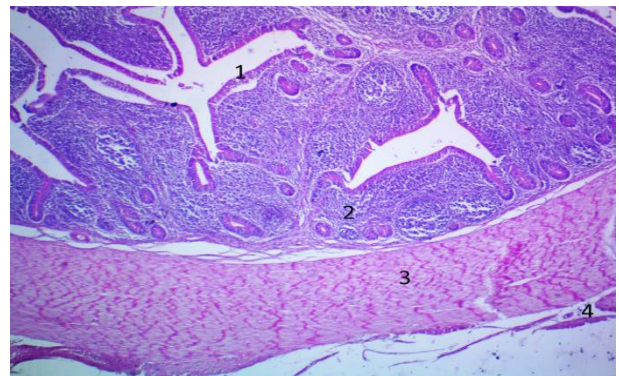


Figure.4: Histological cecal tonsil of the turkey cecal tonsil: four layers of the tonsil mucosa(1),submucosa(2) muscularis(3) and(4) serosa.(H&E stain 10x).



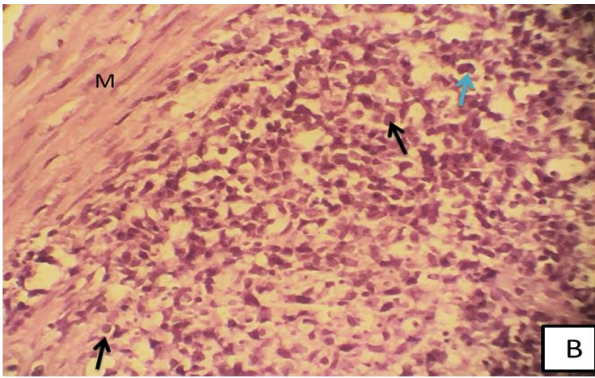
Figure,2B: Shape and location of turkey cecal tonsils at one month age (arrow).



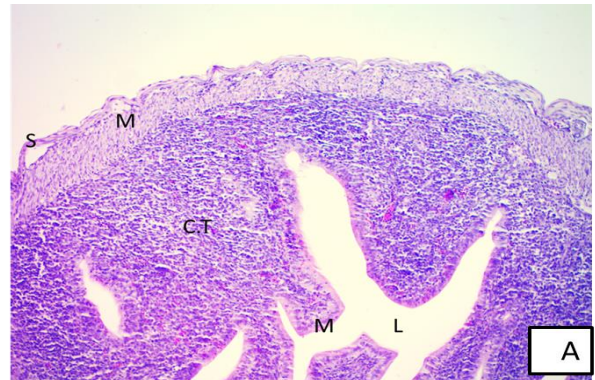
Figure,5A: Histological section of the turkey cecal tonsil at one week: cecal wall (arrow),cecal tonsil(C.T) surrounded the central fossula(C.F).(H&E stain 10x).



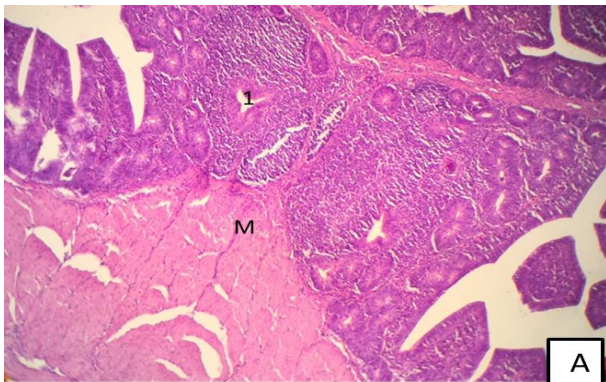
Figure,3A:Shape and location of turkey cecal tonsils at two month age (arrows).



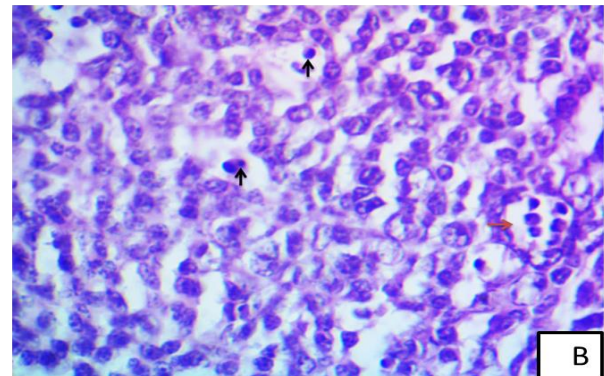
Figure,5B: Histological section of the turkey cecal tonsil at one week: cellular elements of the cecal tonsil: muscular layer (M), large lymphocytes (blue arrow) small lymphocytes (black arrows). (H&E stain 10x).



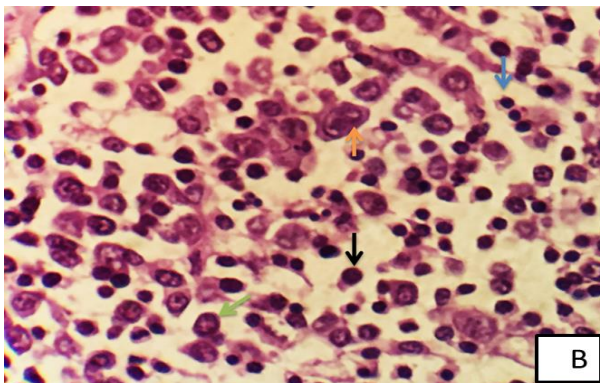
Figure,7A: Histological section of the turkey cecal tonsil at two months: muscular mucosa(M.M), central fossula(F), cecal tonsil(C.T), mucosa (M.),cecal lumen(L).(H&E stain 10x).



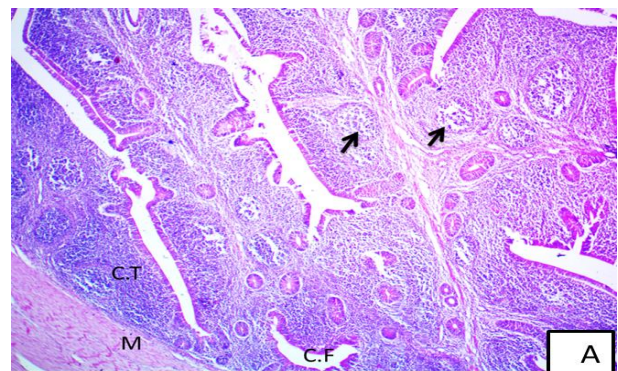
Figure,6A: Histological section of the turkey cecal tonsil at one month shows the muscular mucosa(M), central fossula (1), cecal tonsil(2).(H&E stain 10x).



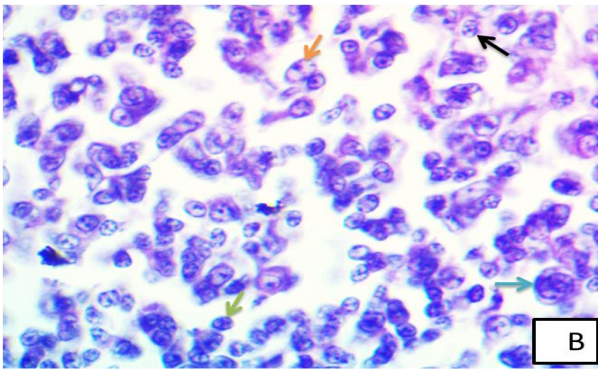
Figure,7B: Histological section of the turkey cecal tonsil at two months shows small lymphocytes(blue arrow) and large lymphocytes(brown arrow),plasma cell(green arrow),macrophage(black arrow).(H&E stain 100x).



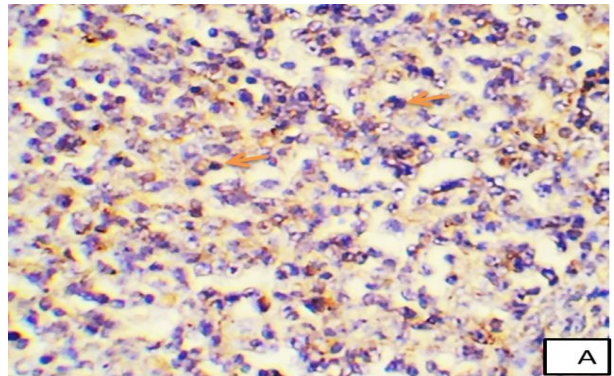
Figure,6B: Histological section of the turkey cecal tonsil at one month: small lymphocytes (blue arrow) and large lymphocytes (black arrow), plasma cell (green arrow), macrophage (brown arrow). (H&E stain 100x).



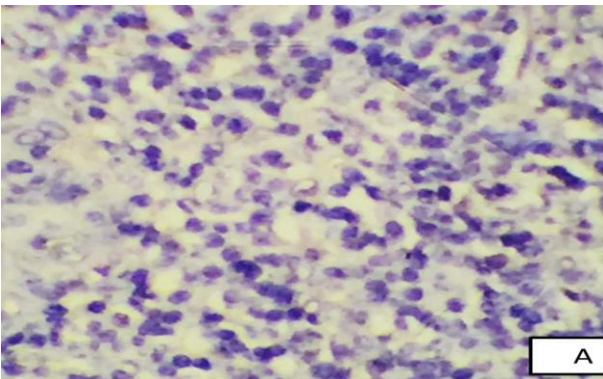
Figure,8A: Histological section of the turkey cecal tonsil at seven months: muscular mucosa (M), the central fossula(C.F), cecal tonsil(C.T), germinal center(arrows)..(H&E stain 10x).



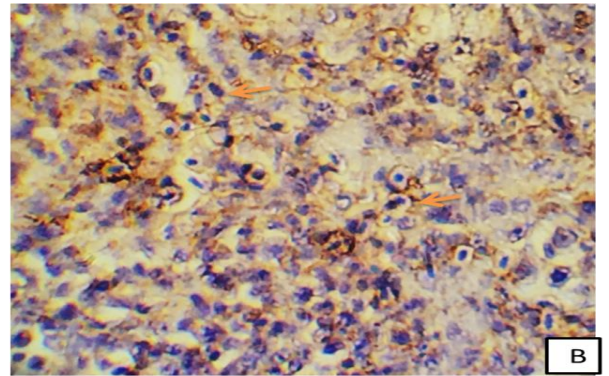
Figure,8B: Histological section of the turkey cecal tonsil at seven months: small lymphocytes(green arrow) and large lymphocytes(brown arrow),plasma cell(black arrow),macrophage(blue arrow).(H&E stain 100x).



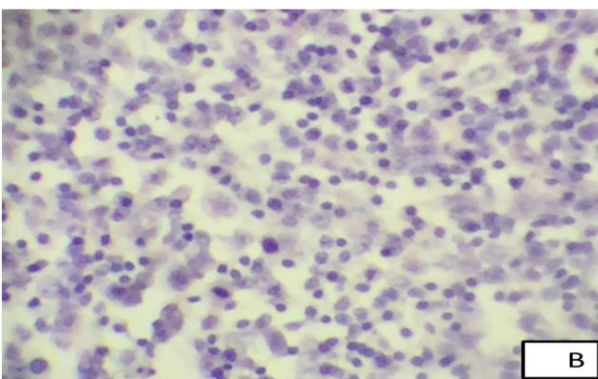
Figure,10A: Immunostaining of a section of the cecal tonsil at two months old turkey: positive reaction of T cells in cecal tonsil parenchyma (arrows). (Immunoperoxidase, 40X).



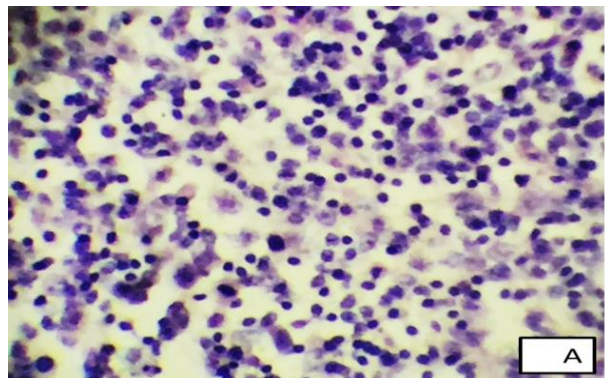
Figure,9A : Immunostaining of a section of the cecal tonsil at one week old turkey: negative reaction of T in the parenchyma of the tonsil. (Immunoperoxidase, 40X).



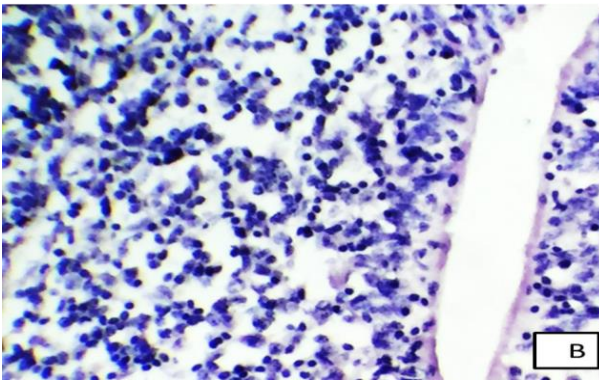
Figure,10B: Immunostaining of the cecal tonsil at seven months old turkey: positive reaction of T cells in cecal tonsil parenchyma (arrows). (Immunoperoxidase, 40X).



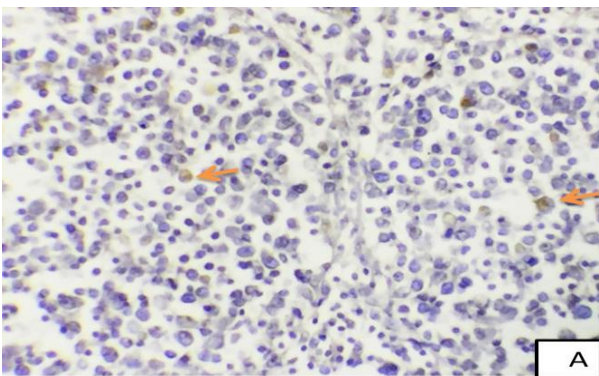
Figure,9B: Immunostaining of a section of the cecal tonsil at one month old turkey: negative reaction of T cells in cecal tonsil parenchyma (Immunoperoxidase, 40X).



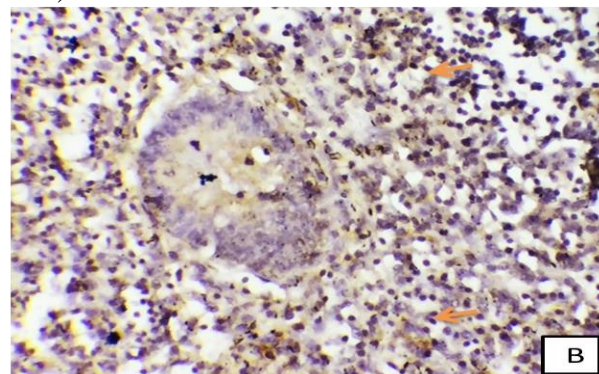
Figure, 11A: Immunostaining of the cecal tonsil at one week old turkey: negative reaction of B cell in the parenchyma of the tonsil. (Immunoperoxidase, 40X).



Figure, 11B: Immunostaining of the cecal tonsil at one month old turkey: negative reaction of B cell in the parenchyma of the tonsil. (Immunoperoxidase, 40X).



Figure,12A: Immunostaining of the cecal tonsil at two months old turkey: positive reaction of B cell cells in cecal tonsil parenchyma (arrows). (Immunoperoxidase, 40X).



Figure,12B: Immunostaining of the cecal tonsil at seven months old turkey: positive reaction of B cell cells in cecal tonsil parenchyma(arrows).(Immunoperoxidase, 40X).

At 1 week old the cecal tonsil appear as a thickening of the cecal wall. The tonsil is composed of four histological layers: mucosa which consists of simple columnar epithelium, submucos a connective tissue, muscularis of two layer circular and longitudinal and

connective tissue serosa. (6) The presence of four distinct histological layers are formed the Cecal tonsil: tunica mucosa, submucosa, muscularis and serosa. The presence and development of lymphoid tissue in the laminapropria submucosa make the other layers of cecal wall bulging toward the cecal lumen. At one week old CD268 showed negative expression, Which agreement with other (7),then referred to mature B cells positively expressed after 6 week post hatching. At this age the bird depend on maternal immunity and all lymphocytes are immature therefore showed negative expression. At 1 month old the cecal portion which included the tonsil easily separated from other parts due to the cecal wall diameter is increased. The tonsil composed of a lot of tonsil crypts. Each crypts composed of secondary follicles surrounded the central fossula, this result agrees with other findings (8) who registered that several tonsilar units (crypts) were composed the paranchyma of cecal tonsils. At this period the cecal tonsil developed and take the specialized form by arranging of the lymphocytes as nodules on each side of fossula. At the period between one week to one month old negative expression of CD8, this result agrees with others (9) who observed T lymphocytes were migrated to cecal tonsils after the first week post hatching chick. At this age the bird depend on primary lymphoid organs. At one month old negative expression of CD268 in sub epithelial regions. (7) registered the presence of positive expression of mature B cells at the 1st month post hatching. (10) At 4 weeks old broiler chicken, the B cells show positive expression in the lamina propria under the epithelium, the germinal centers and distributed within lymphatic nodules, lamina propria and core of villi. The current study applied on non-treated non vaccinated turkey bird therefore no expression of the B cells are observed. At 2 and 7 months old the tonsils bulging from the internal surface of cecum toward the lumen as a rough large mass white to pink in color,this result was previously found (11), who revealed that the tonsils are visible in the chicken, duck and goose as thickening of the internal wall of the cecum. Also the tonsils are located within the medial

wall of both caeca (7). The germinal centers are present in the lymphoid follicles. It was found that each tonsil contain germinal center composed mainly of small and large lymphoblasts (12). At two and seven months old CD8 are showed positive expression. It was observed that the T cells were positive expression in T-dependent areas of cecal tonsils of adult chickens was observed (13). (14) Chicken the CD8 show positive expression limited in the sub epithelial lamina propria of the caecal tonsil. Cecal tonsil were active in this age due to the fact that the primary lymphoid organs were enter the involution stage. At two and seven months old positive expression of B cells. This result was agree with the other findings (15), they referred to the presence of B cells in the cecal tonsils paranchyma.

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دراسة كيميائية مناعية نسيجية وشكلانية نسيجية تطورية للوز الاعورية في الديك الرومي بعد الفقس

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الخلاصة

أدرج أربعون ديك رومي طبيعي مقسمة إلى أربع فئات عمرية (1 أسبوع ، 1 شهر ، شهرين و 7 أشهر). تم جمع اللوز الاعورية وأعداد للدراسات النسيجية والمناعية. في الاسبوع الاول من العمر تظهر اللوز الاعورية على شكل تجمعات ليمفاوية من الصفيحة الحقيقية تحت المخاطية حول الحفرة المركزية. لا توجد حويصلات بين الجريبات ، لا توجد مراكز إنتاشيه . لوحظ. في عمر شهر واحد ان جزء الأعور الذي يشمل اللوز يمكن تمييزه بسهولة من الأجزاء الأخرى بسبب ازدياد قطر جدار الأعور . اللوز الاعورية تتكون من الكثير من الخلايا اللوزية. لوحظت الحويصلات النسيجية بين الخلايا. في الشهرين الثاني والسابع من العمر تبرز اللوزتين من السطح الداخلي للأعور نحو التجويف ككتلة كبيرة خشنة من اللون الأبيض إلى الوردي ، المراكز الانتاشية موجودة في الجريبات للمفاوية. قطر اللوز الاعورية يزداد مع زيادة العمر. يزيد قطر اللوزتين مع التقدم في العمر. السطح الداخلي للوز الاعورية يبطن بظهارة عمودية بسيطة (الغشاء المخاطي المعوي). أبعاد اللوزتين وقياسات الخلايا للمفاوية وسماكة الأغشية المخاطية اللوزية تزيد بشكل معنوي مع التقدم في العمر. كشف الجسم المضاد (CD268) وجود الخلايا الليمفاوية البائية الناضجة داخل متن الجراب عند عمر شهرين وسبعة أشهر في الديك لرومي. اما الجسم المضاد CD8 لم يعبر عنه في عمر الشهر الأول بينما ظهرت درجات مختلفة من التعبير في الاعمار الأخرى..

الكلمات المفتاحية: اللوز، الديك الرومي، الظهارة، الخلايا للمفاوية التائية، الكيمياء النسيجية المناعية.