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Avian caecal tonsils Anatomy and Histology, A species comparison in five Iraqi domesticated birds: Review article

Thekra F. Saleh[1*] Omar Y. Altaey[2*] Naziha S. Ahmed[3*]
*Dept. of Anatomy, college of Veterinary Medicine, University of Mosul
Corresponding Author Email :omaryonus@hotmail.com

Abstract

The anatomy and histology of caecal tonsils among different birds' species is vital to understanding the relationship between the physical structure and gut health. the current article intended to reviewing the morphological differences of cecum and caecal tonsils in five local domesticated birds An extensive search related to caeca and caecal tonsils anatomy and histology performed through researches and articles databases and internet indexes websites, then papers collected, analyzed and explained in this review Results revealed that caecum differ in shape, length, breadth, and symmetry among different birds' species. also, the current review summarized the histological and cellular differences like the thickness of caecal wall, length and shape of tonsillar villi, lymphoid tissue organization, and cellular distribution.In conclusion: Understanding of the anatomy and histology of caecal tonsil in different birds' species is crucial, due to the defense and protective role of caecal tonsils as apart of immune system and the morphological differences in birds may explain the immunological variation and the resistance of different bird types to some enteric infections. this works will provide a considerable assistance for biological and veterinary immunity researchers

Keywords: caecal tonsils, poultry, lymphoid tissue, veterinary anatomy

Introduction

Bird's immune system is complex, compact, and less polymorphic in comparison to mammals with the presence of histocompatibility genes providing great resistance to infection reflecting years of evolutions (1). Lymphoid system mainly divided into primary and secondary organs, the primary organs composed of thymus and cloacal bursa, where (T and B lymphocytes) develop, while the secondary components comprise of the lymphocytes aggregation that proliferate outside their primary locations including the spleen and all mucosal-associated lymphoid tissue such as the caecal tonsils.(2, 3). Large intestine in birds consists of pair of caeca and short smooth rectum located between ilium and cloacae (4) each caecum consists of three portions : proximal, middle, and distal, the wall of caecum contains lymphoid tissues mostly at the basal portion called the caecal tonsils . the caecal tonsils consist of specialized lymphoid tissue which is organized in diffuse or nodular patterns with or without a germinal center, these tissue composed of T and B lymphocytes aggregations (5) Caecal tonsils play a significant role in defense mechanism inside the intestinal environment against the invasion of external pathogenic infections and maintenance of gut health (6) and with birds diet habits and digestion (4). The previous studies focusing on the morphology of the caecum in a particular species of birds without information about the anatomy and histology differences among bird's species and studies were apart of local bird types, despite the paramount related importance

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Therefore, the current review intended to present the anatomical and histological differences details of caecal tonsils among five domesticated local birds. (chickens) Gallus gallus domesticus (7) mentioned that tonsils' follicles are located at the caecal pouches particularly at the proximal end and the caecal tonsil primordium appears at about tenth day of incubated eggs and lymphocytes appear by eighteenth day. (8) found that caecal tonsils develop in the late incubation age, where major developmental events take place after hatching, particularly after a while of hatching, both T and B lymphocytes types are formed, but the number of T lymphocytes larger than that of B lymphocytes. And after 6-7 weeks later, the number of B lymphocytes increases significantly to outstrip the number of T lymphocytes, However, the caecum extend from the area between the ileum and the rectum and connected with the terminal iliac well particularly at the ileocaecal ligament. (2). (9) believes that each caecum is connected with the rectum by an ostium caeci. and at the base of each caecum the muscular sphincter is evident. Ceca are usually fingerlike in shape, resembling simple lateral branches of the intestine, as paired of approximately equal length, with separated lateral or ventrolateral openings into the colon (figure 1). Morphological observations has been described that caecum composed of three regions: proximal, with well-developed villi that are shorter and less in number than those which were found in the middle region; mid-jejunum, with elongated folds and small villi similar to those found in the middle region; and the distal, with transverse and longitudinal folds and small villi similar to those found in the middle region. (figure 1). (10). (11) referred that caecal tonsil in chicken is made up of four different layers of tissue, namely called tunics: t. mucosa, t. submucosa, t. muscularis, and t. serosa. The tunica mucosa contain multiple projections (villi) and Lieberkühn crypts, which are both surrounded by epithelial mucosae and above lamina propria mucosa. each crypt of Lieberkühn is surrounded by centered rings of the lymphoid cells. with Inside the lamina propria, mucosa and submucosa, a network of blood capillaries and connective tissue fibers. (figure 3). (12) noticed that lamina propria is extensively infiltrate with lymphocytes, whereas, a region of intense lymphocytic infiltration with both primary and secondary lymphoid nodules in the lamina propria and submucosa characterized by the presence of germinal centers. Each germinal region has a mixed of lymphocytes and plasma cells. Plasma cells are also present around lymphocytic aggregations in interfollicular portions (figure 2) (figure3). (13) reported that Lamina propria is full of lymphocyte, macrophage and mast cells and the Lymph nodules at the base of lamina propria contain big germinal centers. (14) also mentioned that The caecal tonsil is comprised of multiple tonsillar units that are made up of secondary lymphoid follicles with intermittent T cell areas positioned round the center and fossula branches into many crypts with close connections towards the caecal lumen. (9) noticed that the crypts of Lieberkühn surrounded by a lymphoid epithelium with presence M cells distributed throughout the columnar epithelial cells. M cell are (microfold cells its specialized intestinal epithelium present overlying lymphoid follicle perform transport function for luminal surface pathogens to the sub epithelial macrophage). The various tonsillar units are isolated from each other and from neighboring units by septa of connective

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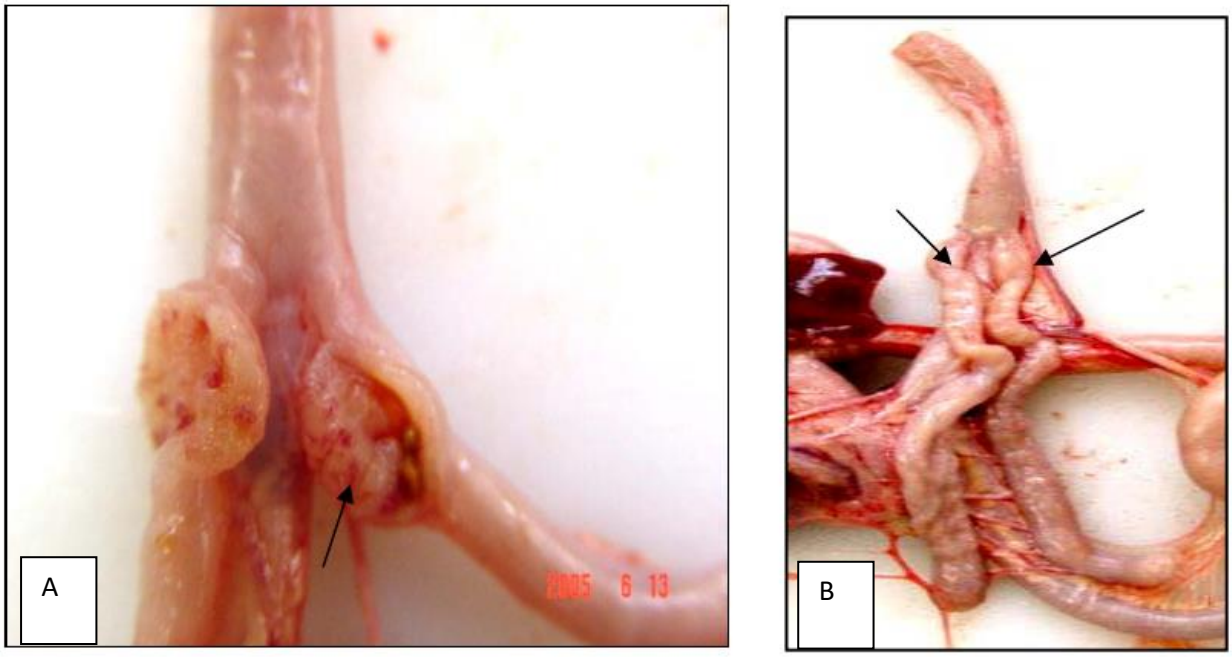
tissue. (figure 3). (8) referred that Lymphoid tissue can also be found in the walls of the blind tips of such caeca as scattered lymphoid tissue and lymphoid follicles inside the lamina propria mucosa and the submucosa, which are



nclosed by a lymphoid epithelium usually consisting of columnar epithelial

Figure (1): Photograph: (A) shows the caecal tonsils (arrows). (B) shows the same caecal tonsil areas from inside (arrow) (11)

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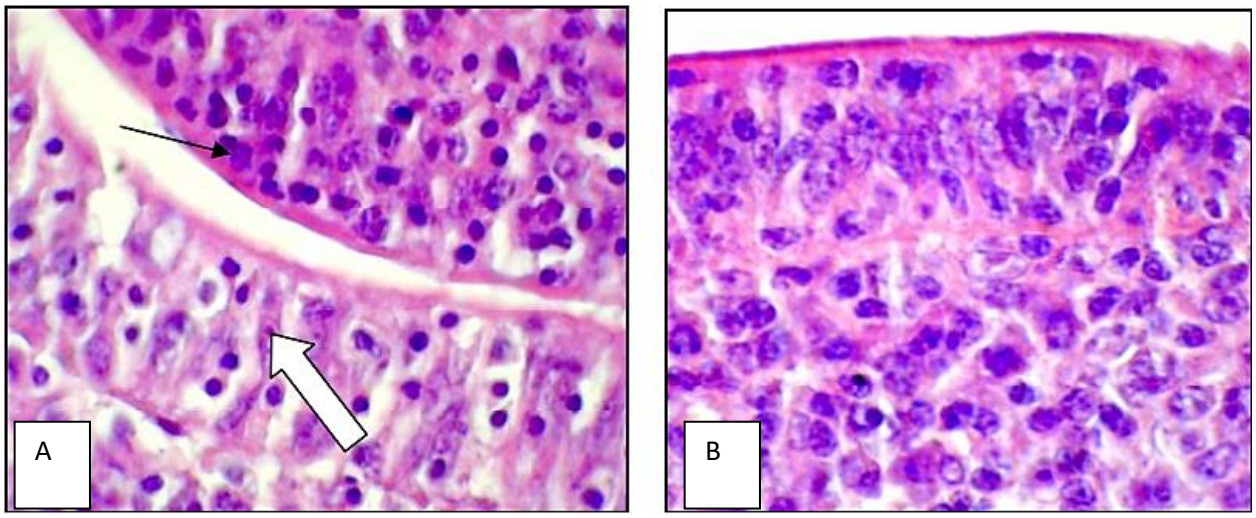
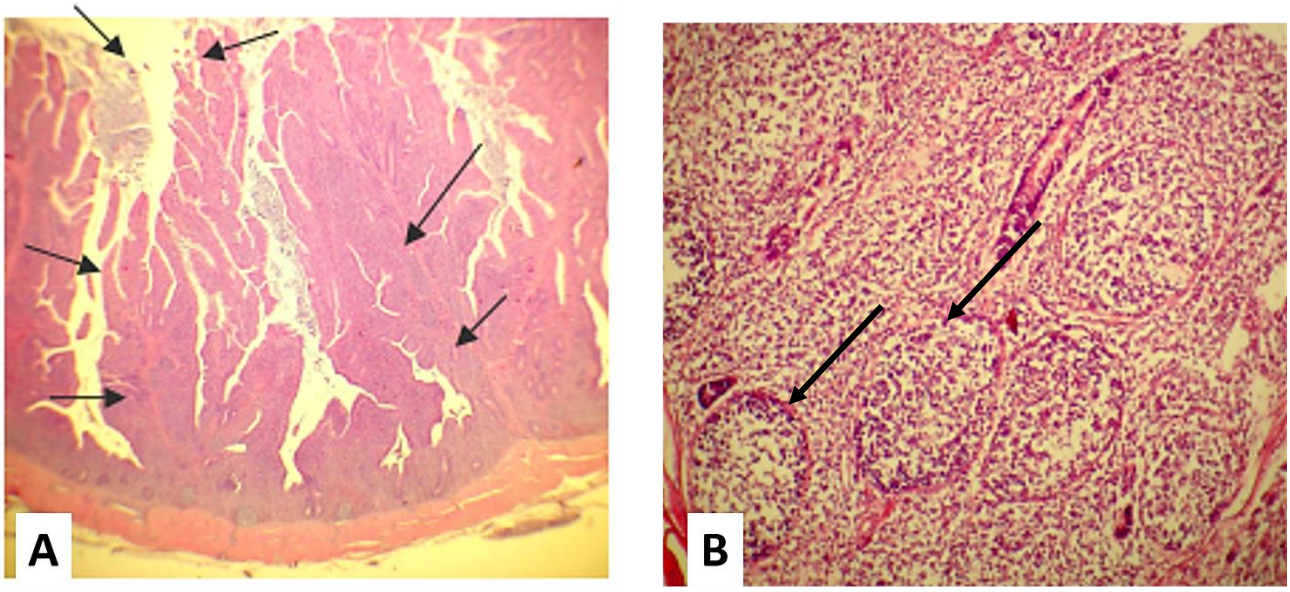


Figure (2): Photomicrographs show (A) the caecal tonsil of 4 month old white leghorn chicken H&E, 10x ,(B) Lymphatic follicles of the caecal tonsils in a 5-month-old white leghorn H&E, 200x. (11)

Figure (3 photomicrograph show (A) one caecal tonsil of 4 month of age white leghorn chicken



limited by (arrows) , H&E, 10x (B) lymphatic nodules of cecal tonsils in 5 month of age white leghorn chicken (arrows) , H&E, 30x(11). Turkey (Meleagris gallopavo)

The caecal tonsils are located on the basal part of the tubular caeca. Its appear as thick elevations on the surface of the caecal wall and become more pronounced with age (4) . (15) found that caeca in turkey are two long blind sacs structure, and the left caecum is slightly longer than the right one. Both arise from the ileorectal junction and accompanies the ileum and extend along its most length and are situated on the left side of the coelomic cavity. It consists of three parts include; proximal part (base) which is narrow lumen and thick wall, and dark pink, while the middle part (body) wider diameter, green-gray in color, while the distal part with curved end and thin wall is elongated and slightly bent, both caeca opened

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opposite to each other**.** (3) described the histological features of the turkey caecum in the first post-hatching day and mentioned that the caecal wall is composed of 4 layers, these layers are : tunica mucosa, tunica submucosa, tunica muscularis, and tunica adventitia. Tunica mucosa is composed of simple columnar epithelium with secretory goblet cells interspersed among the columnar cells, those epithelia is extending into high longitudinal folds of intestinal villi whose crypts communicated with the developing Lieberkühn glands. In lamina propria-submucosa layer, there are accumulations of lymphoid cells that base on the tunica muscularis, these lymphoid aggregations are composed of large lymphocytes that formed the earliest caecal tonsils (figure 4) . Otherwise, in turkey with 140-days old, the tonsil comprised of lymphatic nodules with low cellular density. There are obvious wide intercellular spaces among cells within the nodules. The nodules at the apical regions toward the caecal lumen tended to disintegrate and the internodular spaces initially occupied by diffused accumulations of lymphocytes which are dominated by intervening connective tissue (3).(figure 4)**.** (1, 16) noticed that the caecal tonsil is composed of several tonsillar units that consist of secondary lymphoid follicles with interspersed T cell regions located around a central fossula. This fossula divides into multiple crypts that connect directly the caecal lumen. The crypts are covered with a lymph-epithelium with numerous M cells dispersed amongst the columnar epithelial cells. similar to that found in chickens (figure 4) ,whereas, (10, 17) reported that the epithelium mucosae of the tonsils in turkey covered with simple columnar epithelium with goblet cells while the lymphatic nodules and diffused lymphatic tissues and are observed in the lamina propria-submucosa layers. (16) found that tunica muscularis of the caecal tonsil in turkey comprised of smooth muscle fibers devoid of lymphoid accumulation which bit different than chickens' tonsils

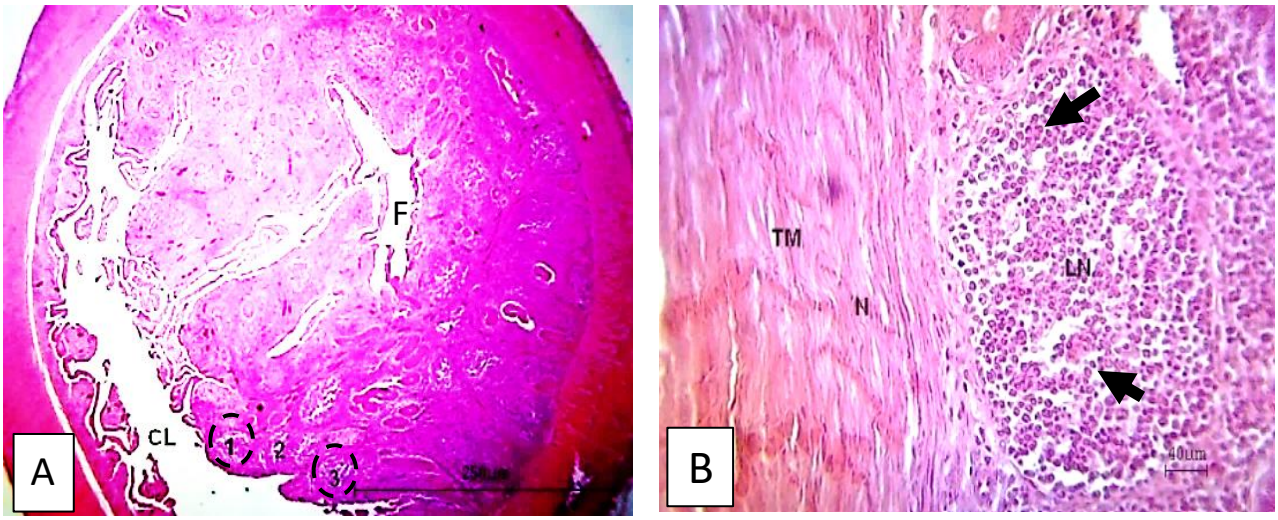

Figure (4): Photomicrograph (A) shows the of the caecal tonsil at day 56 , Note the proximity of lymphatic nodules, 1,2, and 3 to the mucosa. CL: caecal lumen F: fossula. X4, H&E (B) the caecal tonsil at day 126 showing the muscular layer, TM of the caecal wall and a lymphatic nodule LN ,lymphocytic aggregations (arrow) , nuclei of muscle cells N X40.H&E(3)

Ducks (Anas platyrhynchos domesticus)

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(18) found that ducks have well developed two caeca shaped as long blind sac with quite even diameter on the whole length. They are situated opposite to each other along the ileum, and directed towards the head, opening situated in the zone of the small intestine transition caecum is a long and cylindrical in shape,

(19) reported that caeca in ducks' is asymmetrical and the caecal length were 90.66 mm for left caecum and 78.43 mm for right caecum also the average weight of those portions was 0.47g and 0.42g, respectively.

(20) found that caecal villi are lined with simple columnar epithelium and numerous goblet cells and M cells and the crypts of Lieberkühn (intestinal glands) are very prominent at the base of these villi. The lymphatic tissue appear as a diffuse aggregations of lymphocytes which are distributed usually in the lamina propria - submucosa layer, these aggregations of lymphocytes consider not atypical lymphatic nodules and has no germinal centers in compare to other type of birds , and only diffuse sub epithelial immune cells filled the lamina propria layer of duck's caeca (figure 5)

(18) also noted that of ducks' caecal tonsils showed that lymphatic tissues organization differ from other fowls which is non-follicular and its totally diffused and the muscular layer is thicker. Also mentioned that the lamina propria showed intestinal crypts of Lieberkühn and the mucosa of the caecum carried tooth-shaped villi (figure 5)

(20) noted in his results that the caecal villi of ducks is significantly shorter than that of quails. and the thickness of the ceacal wall significantly higher in ducks incompare to quails (table2). And explain that these types of differences due to food nature and feeding habits

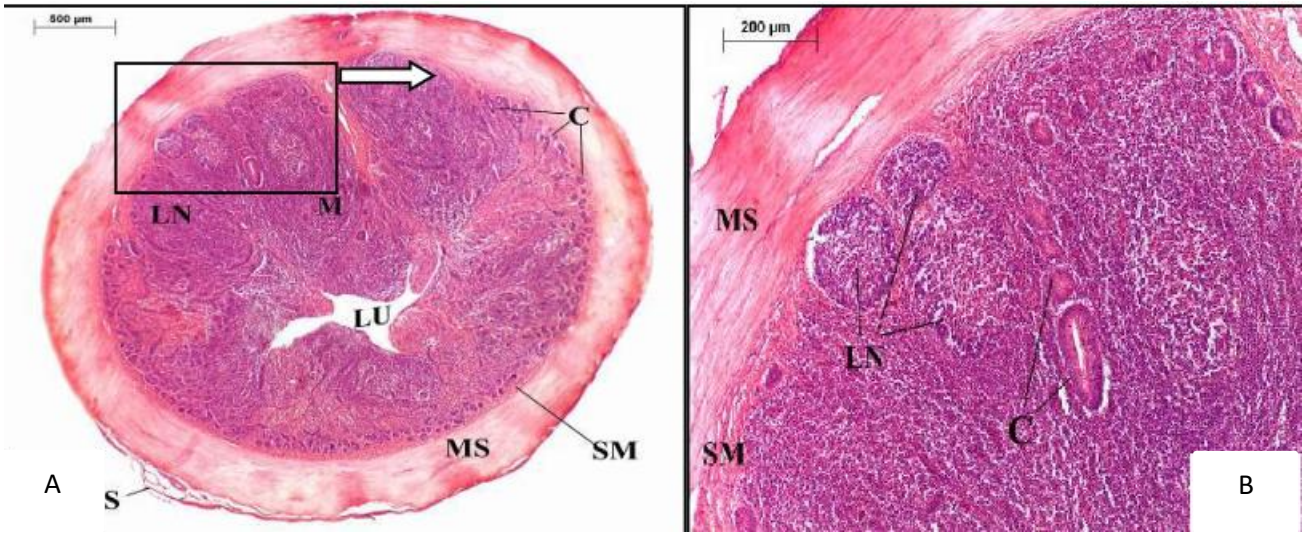

Figure (5): (A) Photomicrograph of section in proximal area of the beginning of the duck caeca showing serosa(s), muscularis(MS), submucosa(SM),mucosa(M), lymph nodes(LN), lumen (LU) and crypts of Lieberkühn(C) H&E. 4x (B) howing serosa(s), muscularis(MS), submucosa(SM), mucosa(M), the lymphatic groups (LN), the lumen (LU) and crypts of Lieberkühn(C) H & E. 10x (20)

Pigeon (Columba livia)

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(21) reoprted that caeca in pigeon composed of paired diverticulated as bud like rudimentary organs. The lamina propria-submucosa of the caecum is heavily infiltrated with diffuse lymphatic tissue.

Moreover, (22) reported that the pigeons' caecal wall is a thick with a very narrow lumen and heavy infiltration by diffuse lymphatic tissue in addition to lymphatic follicles which fill the whole thickness of the caecal wall and lamina propria - submucosa contained many diffuse and nodular lymphatic tissue.(figure 6)

(23) noted that the caecum of pigeons considered as a rudimentary organ with a very narrow lumen, this fact suggested that the caecum has no role in microbial fermentation of the food which happens in other domestic birds, the caeca expand caudally mostly from ileum's distal section and the proximal part of the colon, producing an ileo-caeco-colon connection, and looks as two buds extending from the ileocaecal connection.(figure 6)

Morphologically caecum is divided into three regions, proximal part which has a thick wall and narrow lumen because of the presence of lymphatic tissue and lymph nodules in its wall, represent the caecal tonsils, the middle part, and the distal part (24)

(25) reported that the proximal portion of the cecum contained prominent villi, where the lamina propria is infiltrated by enormous amounts of diffuse and nodular lymphatic tissue. and also the submucosa of this portion.

The caecal epithelium composed of simple columnar epithelium together with the goblet cells caecal tonsils present only in the proximal part of caecum and doesn't reach the middle and the distal part (23).Furthermore, (21) found that wall of the caecum is very thick in proximal part of caecum due to the highly lymphocytic aggregation in its wall and the lamina propria-submucosa is heavily infiltrated by diffuse lymphatic and nodular tissue . the caecal villi is short dome like in shape and lacked of goblet cells with numerus M cells with enfolded groups of lymphocytes and occasionally surrounded by macrophages and dendritic cells.

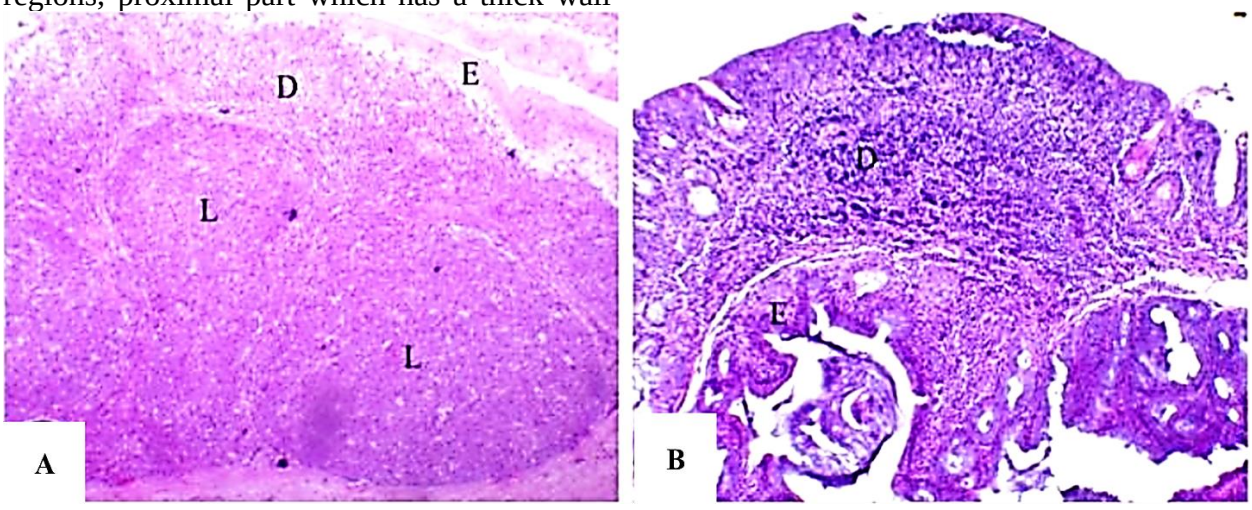

Figure (6): Photomicrograph (A) showing the cecal epithelium (E), lymphoid nodules (L) and diffuse lymphatic tissue (D) in the submucosa
(B) showing epithelium (E), lymph nodules (L.N) and diffuse lymphatic tissue (D) in the sub mucosa,10x H&E(21)

Quail (Coturnix japonica)

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The caecum in quail is composed of two long blind tubes branching off the ileocaecal connection and the caecum in general long composed of three regions like other galliform birds which are the proximal, the middle, and the distal part , The proximal portion is short in length with a small lumen and a stiff outer wall while the middle part of the cecum the long with a wide lumen and thin wall and the distal part is short and a pointed .(23)

(26) mentioned that the proximal portion of cecum composed of lymphoid and non-lymphoid compartment and the non-lymphoid part has typical intestinal layers the epithelium mucosae composed of simple columnar and gobbles cells and lamina properia filled with crypts of Lieberkuhn and there's a minimal sheath of muscularis mucosa sapertae between lamina properia from tunica sub mucosae followed by thick tunica mascularis layers and the cecum in general covered by tunica serosa from outside . (figure 7)

(27) found that both mucosal and muscular layer thickness continuously increase with age and reached its highest thickness around puberty and the M-like cells are present in large number at the caecal epithelium mucosae and mentioned that villi are absent in this part of the caecum.

(28) found that the quail's caecal tonsils (lymphoid portion) consist of 3 or 4 tonsillar follicles. A follicle included tonsillar crypts that were open at the fossula and open to the caecal lumen. Lieberkühn crypts of proximal caecum also grown into tonsillar crypts. The fossula is found at different depths and is surrounded by epithelium lymphoid tissues containing germinal centers. (figure 8)

(26) noted that the villi's crypts epithelial cells are most often small and lack goblet cells. In certain instances, follicle associated cells surround the lymphoid tissue. The follicle associated cells seems to be either cubical or squamous in shape, and it lacks goblet cells. There are a few epithelial cells are having dark cytoplasm in this segment.

(16) reported in his results there are no goblet cells in the lymphoid associated tissue. The lymphatic nodules have germinative centers. Based on these data, it is possible to conclude that the lymphoid tissue constituting the caecal tonsil, as well as the shape of the lymphoid tissue associated epithelium, are same in different bird species. the connective tissue of the villus, the lamina propria, the surrounding area of the intestinal glands, the submucosa, lymphoid tissue, and the tunica muscularis (figure 8)

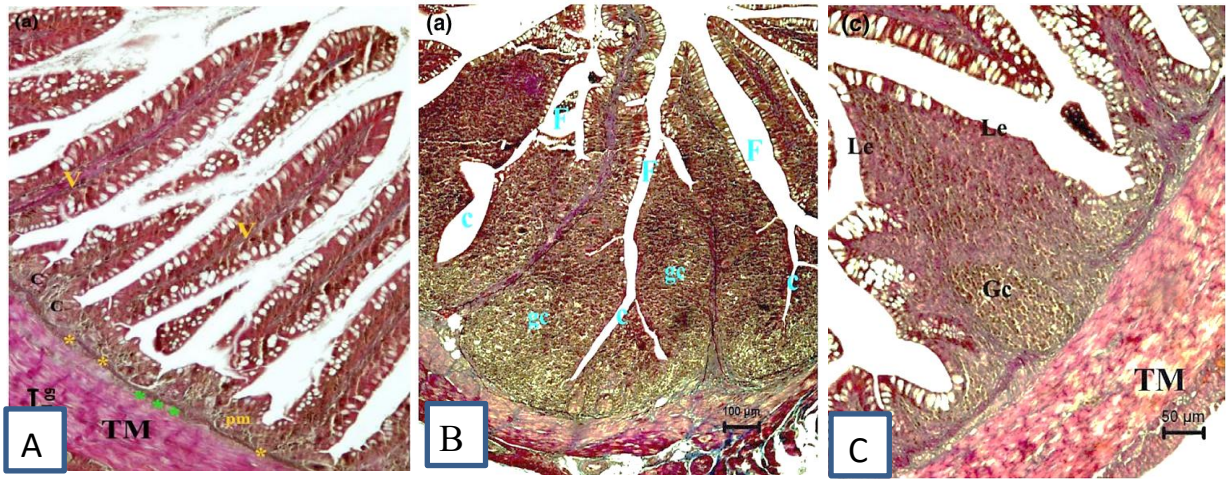

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Figure (7):Photomicrographs (A) show epithelial layer consisting of simple columnar epithelium with striated border and goblet cells, villi (V), lamina muscularis mucosae (yellow asterisks), submucosa (green asterisks), tunica muscularis (TM) and tunica serosa are found in the structure of the proximal , (B,C) Histological appearance of the caecal tonsil in the proximal caecum of quail, which is composed of tonsillar follicles (encircled with dashed line), is observed. tonsillar crypts (c) the fossula (F) , germinal centres (gc) , PAS method (A) crossman's three-staining technique(B) .4x (A,C) 10x (B) (26)

Findings of Species comparison

The macroscopic anatomy of caeca in all birds appear as a pair of blind sacs extend backward along the terminal part of small intestine , both caeca open directly at the junction between ilium and rectum , the caeca looks finger-like sac in chicken, turkey and quail and cylindrical tube-like in duck and small bud-like in pigeons, the caecal length also varies among bird types it's long in turkey and chicken and moderate in duck and quail and very short in a pigeon in addition to the caecal weight differences among studied birds (table 1)Furthermore, the caecal lumen is ranged from a very narrow lumen in pigeons and quail to a wide diameter lumen in turkey, duck, and chicken. the thickness of the caecal wall also varies significantly among bird types which are thick in chickens, pigeons, and ducks while the thickness of the caecal wall is moderate in quail (table 2). the symmetry between the left and right caecum also differs from one bird type to another, the left and right caecum are approximately symmetrical in chicken and quail but it's asymmetrical in turkey, ducks, and pigeon .Histologically, caeca are composed of four distinct layers: tunica mucosae, tunica submucosa, tunica mascularis, and tunica serosa, the mucosal layer sends tiny projections called villi which are finger-like in chicken and turkey and tooth-like in duck and quail, the length of villi significantly different among birds which are long in chicken and quail and short in the pigeon (table 2).

Villi were lined with epithelium comprised of simple columnar cells and numerous goblet and M cells. Lamina properia filled with crypts of Lieberkühn which are prominent at the base of the villi in all the birds, the lymphocytic tissue organization in caecum present in the form of nodular and diffuse aggregations which were distributed usually in the lamina propria mucosae and submucosal layer, the typical lymphatic nodules (follicles) with germinal centers present very clear in chicken, turkey, and pigeon while absent in duck and quail .Whereas diffuse lymphocytic aggregations present in all birds as both large and small groups of lymphocytes, the diameter of the caecal lymphoid nodules in chicken, pigeon, and turkey was 248.36±55.69 µm and 160.88± 33.80 µm and 641.0±77.5 µm respectively. lymphoid tissue in ducks distribute as a sheet or as small cellular groups present within lamina properia and less extent in tunica submucosae

The caecal muscular layer also varies among birds, the tunica mascularis is thin in quail and turkey and devoid of lymphocytic infiltration while it's thick in pigeons, duck and chicken and lymphocyte infiltrated deeper within muscular layer.Authors suggested that differences of the anatomy and histology of caecum and caecal tonsils among bird species is result of different diet habits and size differences of digestive system among birds

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Table 1: Morphometrical parameters of the caeca in quail, chicken, turkey , duck and pigeons (19,24,29,30)

Species	Caecal weight (g)	Left caecum length(cm)	Right caecum length (cm)
Chicken (29)	11.35 ±0 .21	13.14±0.44	13.15±0.21
Turkey (30)	14.29 ±0 .24	24.250 ±2.28	23.750 ±2.42
Duck (19)	0.47 ± 0.14	9.07 ± 14.0	7.84 ± 13.09
Pigeon (24)	0.38±0.04	0.36±0.05	0.38±0.06
Quail (24)	0.53±0.10	8.13±0.26	8.13±0.26

Table 2: Histometrical parameters of the caeca in quail, chicken, duck and pigeons (20)

Species	Height of villi (µm)	Thickness of wall (µm)
Quail	177.11± 4.69c	96.88±4.62a
Chicken	246.48± 26.03d	147.57± 9.27c
Duck	130.67± 6.77b	114.44± 5.20b
pigeon	98.32± 6.38 a	164.11 ± 9.23 d

One-way Anova and Duncan post-hoc association test
Different letter in each column indicate significant differences at $p \leq 0.05$

Conclusion

Understanding of the anatomy and histology of caecal tonsil in different birds species is crucial , due to the defense role of caecal tonsils as apart of immune system , the anatomy of the ceacum differ among birds in the shape, length , thickness of caecal wall and symmetry furthermore, the differences in the histological layers , shape of villi and lymphatic tissue distribution and organization , the morphological differences in birds' caecal tonsils may explain the immunological variation and the resistance of different bird types to some enteric infections. the current review clarify and summarize authors works to provide considerable assistance for biological and veterinary immunity researchers

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