Comparative Histological and Ultrastructural Studies on the Rectal Caeca of Three Birds

Samia M. Abd El-Wahab, Abdel Razik H. Farrag, Ragaa M. El Deeb and Shimaa A. Eltatawy

Department of Zoology, Faculty of science, Al-AZhar University, Cairo, Egypt.
Pathology Department, Medical Division Research, National Research Centre, Cairo, Egypt.
Department of Zoology, faculty of science, Ain Shams University, Cairo, Egypt.

ABSTRACT

Caeca are outpouches of the alimentary canal originating at the junction of the small and large intestine. The histology and ultrastructure of these caeca in birds reveal a considerable specific variation, which likely reflects functional differences among avian species. Each caecum divides into three regions or zones; proximal, middle and distal. In this work, a comparative study by light and transmission electron microscopy is carried out on the three different caecal regions of quail, duck and owl. The histological results show the presence of caecal tonsils in the beginning of the proximal region of quail caeca only. These tonsils are consisting of the aggregated masses of lymphocyte, forming a multiple nodules and encapsulated by muscle fibers. However the duck caeca were devoid of caecal tonsils and all caecal regions contained aggregation of lymphoid nodules while, in the owl caeca the lymphoid tissue found only in the proximal zone. The presence and shape of villi, plicae circulars and crypts were explained in detail. The ultra structure of the caecal regions of the each bird explained how the proximal perform its absorptive function more than the middle and distal regions through the presence of different organelles such as microvilli and mitochondria. Histology and ultra structure of the caeca in the three birds revealed some important facts which may be differ from that established in the past.

Key words: Rectal caeca - Histology – TEM - Aves.

Introduction

Avian large intestine consists of paired caeca and a short straight colon joined to ileum and cloaca. The quail is a Galliformes bird and duck is an Anseriformes and both of them have big economical benefits. The owl is Strigiformes bird which play an important ecological role in the environment. The histology of the caecum has been regarded as being similar to that of ileum and colon, although recently major differences in absorption pattern between the three caecal zones have been discovered (Dantzer, 1989). The aim of this work is to give a general configuration of the mucous membrane at the light and transmission electron microscopic levels and a more detailed description of the ultra structure of the epithelial cells of the three caecal regions.

Material and Methods

Animals:

In the present work, birds from three different feeding habits were selected. Five adult healthy birds of each sex were obtained from 1- a quail farm in Kaffr Elshikh, Coturnix coturnix (common quail), 2- a duck farm in Cairo, Cairina moschata (Muscovy duck) and 3- trapped alive from caves in Abo-Rwash area of Giza pyramids, Athene noctua (little owl). The specimens were sacrificed and rapidly dissected (Fig.1 A, B and C).

For histological studies, the caecal tissues removed immediately after sacrificing the birds and divided into 3 equal segments: proximal (closest to ileocaecal junction), middle, and distal (blind

Corresponding Author: Samia M. Abd El-Wahab, Department of zoology, faculty of science, Al-AZhar University, Cairo, Egypt. E-mail: abdelwahabs@hotmail.com
end). Caecal samples were fixed in the Bouin’s fixative for 24 hours, dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin wax. Sections were then cut at 6 µm thickness and stained with Harris Haematoxylin and Eosin (Gridley, 1960). Slides were examined and photographed with a photomicroscope (Leica microscope) for histological investigation.

For transmission electron microscope studies, all caecal samples: proximal, middle, and distal were:

1- fixed in 4% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH7.3) for 4 hours, after two rinses in the same buffer for a period of 20 minutes followed by post fixation in osmium tetroxide OsO₄ for 2 hours.

2- washed three times for 30 minutes in the same buffer (sodium cacodylate buffer).

3- dehydrated in ascending grades of ethanol; from 10 to100%, 10 minutes in each one except the finely one 100% for 30 minutes for three changes (each one for 10 minutes).

4- Cleared in propylene oxide for two changes in 10 minutes.

5- Put in equal volumes of propylene oxide and Epon 812 for one hour at room temperature.

6- Pour off half the (propylene oxide- epon) mixture in a waste bottle and add 2 volume of Epon 812 for 3 hours at room temperature.

7- The samples put in a pure Epon 812 resin overnight.

8- The samples embedded in Epon 812 resin mixture and polymerized in the oven at 60 °C for 24 hours.

9- Semi- thin sections cut from these blocks by using Reichert-Jung Ultra cut ultra microtome, stained with toluidine blue and examined by the light microscope.

10- Ultra thin sections obtained from selected blocks are mounted on copper grids and double-stained with uranyl acetate and lead citrate.

11- Sections were examined and photographed by electron microscope (JEOL- JEM 2100) in National Research Centre, Dokki and in the Electron Microscopy Unit of the Regional Center for Mycology and Biotechnology, Nasr City, Cairo, Egypt.

**Fig. 1:** Photographs of the (A) common quail, (B) Muscovy duck and (C) little owl.

**Results:**

I- Light microscopy:

a- The proximal zone:

The proximal zone in adult quail begins with caecal tonsil that differs histologically from the remaining of this zone.

The caecal tonsil is consists of aggregated masses of lymphocytes that forming a multiple nodules and encapsulated by muscle fibers. Few crypts of Lieberkühn were entrapped within the caecal tonsil. As population of lymphocytic nodules was increased, a marked reduction in the number of entrapped crypts. Some crypts were either lost their arrangement totally or partially resulted in cluster of cells (Fig. 2).
Fig. 2: A photomicrograph of a transverse section of the caecal tonsil zone of the quail caeca showing muscle fibers (MF), muscular layer (ML), lymph node (LN) and crypts of Lieberkühn (C) (H&E. stain) (Scale bar: 500 μm).

While in duck, there is no caecal tonsil but the beginning of the proximal zone appears much like the caecal tonsil. This beginning formed of serosa, muscularis, submucosa and mucosa which built up of aggregated masses of lymphocytes forming a multiple nodules and supported by connective tissue; trabeculae. The lumen nearly obstructed and numbers of crypts of Lieberkühn are noticed (Figs. 3a and 3b).

Fig. 3a: A photomicrograph of a transverse section of the beginning of proximal zone 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. stain) (Scale bar: 500μm).

Fig. 3b: A photomicrograph of a magnified part of figure (3a) showing serosa (s), muscularis (MS), submucosa (SM), mucosa (M), lymph nodes (LN), lumen (LU) and crypts of Lieberkühn (C) (H&E. stain) (Scale bar: 200 μm).

The remaining of the proximal zone in both quail and duck consists from external to internal of serosa, muscularis that formed of two layers: thin longitudinal and thick circular muscle layers. The layer of submucosa is very thin connective tissue followed by mucosa (Figs. 4 and 5).

While the owl, proximal region consists of: serosa, muscularis that formed of one layer of circular muscle fibers followed by submucosa, muscularis mucosa and mucosa. There is no any organized lymphoid tissue but number of lymphocytes appears inside lamina properia (Figs. 6 and 7).

Number of villi is found in mucosal layer of owl while, they are little in quail and duck. There are a great number of crypts of Lieberkühn in quail, duck and owl. The proximal region of owl only shows presence of number of plicae circulares (Figs. 7, 8a and 8b).

b-The middle zone:

The wall of middle zone appears thinner than that of the proximal zone with large lumen in all studied birds. It consists of serosa, muscularis, submucosa, muscularis mucosa and mucosa in quail and owl (Figs. 9 and 11) but in duck, there is no muscularis mucosa (Fig. 10).
**Fig. 4:** A Photomicrograph of a transverse section of the proximal zone of the quail caeca showing longitudinal muscle layer (LML), circular muscle layers (CML), villi (V), lumen (LU) mucosa (M), lymph node (LN) and crypts of Lieberkühn(C) (H&E. stain) (Scale bar: 500 μm).

**Fig. 5:** A photomicrograph of a transverse section of the proximal zone of the duck caeca showing serosa(s), muscularis (MS), mucosa (M), lymph nodes (LN), lumen (LU), villi (V) and crypts of Lieberkühn (C). (H&E. stain) (Scale bar: 500 μm).

**Fig. 6:** A photomicrograph of a transverse section of the proximal zone of the little owl caeca showing serosa (s), muscularis (MS), submucosa(SM), mucosa(M), villi(V), plicae circulaires (PC), lumen (LU) and crypts of Lieberkühn(C)(H&E. stain) (Scale bar: 200 μm).

**Fig. 7:** A photomicrograph of a magnified part of a transverse section of the proximal zone of the little owl caeca showing submucosa(SM), villi(V), lumen (LU) lamina properia(LP) and crypts of Lieberkühn(C)(H&E. stain) (Scale bar: 50 μm).

**Fig. 8a:** A Photomicrograph of a magnified part of figure (4) showing villi (V), goblet cell (GC), lamina properia(LP) and lumen (LU) (H&E. stain) (Scale bar: 50 μm).

**Fig. 8b:** A photomicrograph of a magnified part of figure (5) showing muscularis (MS), submucosa (SM), lumen (LU) villi (V) and crypts of Lieberkühn (C). (H&E. stain. (Scale bar: 200 μm).
In quail and duck, the muscularis layer is composed of longitudinal and circular muscle layers but, in owl it is formed of one circular muscle layer only. The height of villi is decreased in owl and absent in quail while, in duck the villi are blunted. The mucosa is heavily infiltrated with lymphatic tissue as well as lymphoid nodules in duck but, the lymphatic tissue is absent in quail and owl. Numerous crypts are found in quail and duck but little in owl (Figs. 12, 13 and 14). The plicae circulars in middle zone appear only in quail and duck (Figs. 12 and 13) while absent in owl (Fig. 14).

c- The distal zone:
The wall of the distal appears thinner than that of the proximal and middle zones with large lumen in the three birds and composed of the same layers as in middle zone of each previous bird (Figs. 15, 16 and 17).
The crypts are absent in owl while, in quail and duck they are found in a huge number. The height and number of plicae circulars appear decreased in quail and duck and the lymphatic tissue become moderately infiltrated the mucosa of this zone in duck (Figs. 18, 19 and 20). Blunted villi also observed in this region in duck (Fig. 19).
II– Transmission electron microscopy:

a-The proximal zone:

The proximal zone explains the mucosal epithelia, which composed of the columnar epithelia and numerous goblet cells in all birds. In quail caeca the cytoplasm of the columnar cells is moderately electron dense and the nucleus is heterochromatic with prominent nucleolus. The columnar epithelial cells in owl have moderate electron dense cytoplasm and basal oval shaped irregular nuclei. Prominent small nucleolus in addition to scattered heterochromatin in between the nuclear pores and patchy chromatin pattern are observed in the nucleus. The apical part of the columnar epithelial cells in owl bears numerous microvilli toward the lumen, giving the brush border appearance and the strands of microvilli fibrillary roots run clearly from the microvilli down into the body of the cell. While in quail the microvilli beaded in form; sometimes they appear wrinkled with a vesiculated apex. The latter condition observed especially in the microvilli bordering of goblet cells. In the duck caeca the columnar epithelial cells have moderate number of short apical microvilli appear as protrusions from apical plasma membrane of these cells and the strands of microvilli fibrillary roots run from the microvilli down into the body of the cell clearly (Figs. 21, 22 and 23).

Great number of mitochondria with different shapes include rod and rounded forms with transversal cristae appears in proximal zone of the three birds. Small and large interaepithelial lymphoid cells are seeing in duck caeca.

The narrow intracellular spaces between the adjacent epithelial cells can also observe in duck and owl caeca. The walls of the adjacent epithelial cells of owl proximal region connected by junctional complex of tight junction, at the apical of cells and desmosomes, deep to the tight junction (Figs. 24 and 25).
b-The middle zone:

The middle zone of the owl caeca shows the elongated columnar epithelial cells with moderate electron dense cytoplasm in addition to presence of many goblet cells filling with mucous secretion in all birds. In quail and owl caeca numerous microvilli showing decrease in height than that of proximal zone and forming the brush border are present with its fibrillar roots. While the duck caeca have a number of short apical rootles microvilli (Figs. 26, 27 and 28).

Fig. 24: TEM micrograph of the proximal zone of the duck caeca interaepithelial lymphoid cells (L). (Scale bar: 2 μm).

Fig. 25: TEM micrograph of the proximal zone of the owl caeca showing caecal epithelial cell (CE), nuclei (N), mitochondria (M), desmosomes (D) and brush border (BB). Scale bar: 5 μm.

Fig. 26: TEM micrograph of the middle zone of the quail caeca showing epithelial cells with microvilli (MV), microvilli fibrillary roots (MR), goblet cell (GC) and mitochondria (M). (Scale bar: 2 μm).

Fig. 27: TEM micrograph of the middle zone of the duck caeca showing epithelial cells with rounded-shaped nucleus (N), a prominent nucleolus (NU), moderate number of short microvilli (MV) and goblet cells (GC) with mucous secretion. (Scale bar: 2 μm).

Fig. 28: TEM micrograph of the middle zone of the owl caeca showing epithelial cells with microvilli (MV), microvilli fibrillary roots (MR), nuclei (N), mitochondria (M), Golgi apparatus (G), endoplasmic reticulum (ER), and tight junction (TJ). (Scale bar: 5 μm).

The mitochondria of duck are rounded in shape while it is rounded or rod in quail. Scattering numerous mitochondria with rounded, rod and finger print shapes found supranuclearly filling the cytoplasm of the owl epithelial cells. Some interaepithelial lymphoid cells are seeing in duck caeca. The intracellular spaces between the lateral plasmalemma of the adjacent neighboring cells appear in
quail caeca and become narrow in owl caeca. The walls of the adjacent epithelial cells apically connected by tight junction and interdigitations of lateral plasmalemma between neighboring cells are clearly observed in owl caeca.

c - The distal zone:

The distal zone of the quail caeca under electron microscope shows presence of columnar epithelial cells with irregular elongated heterochromatic nucleus, in which large prominent nucleolus are present and in duck caeca the epithelial cells show presence of basal irregular oval-shape nucleus with prominent small nucleolus. While in owl caeca the mucosal cells containing basal oval-shaped irregular nucleus with a basal prominent nucleolus larger than that of the proximal and middle zones and the heterochromatin in between the nuclear pores observed. The intracellular spaces between the adjacent epithelial cells become wider than that of the middle zone of duck caeca. The apical part of these cells bears in quail caeca a numerous microvilli, which appear with a vesiculated apex and some mucus vesicles are visible at the apices of the microvilli. The strands of microvilli fibrillary roots run from the microvilli down into the body of the cell clearly. While the apical plasma membrane of the epithelia showed some dome shape rootlets apical protrusions in duck caeca (Figs. 29, 30 and 31).

![Fig. 29: TEM micrograph of the distal zone of the quail caeca showing epithelial cells with microvilli (MV), microvilli fibrillary roots (MR), Golgi apparatus (G), granules (GR) and mitochondria (M). (Scale bar: 2 µm).](image1)

![Fig. 30: TEM micrograph of the distal zone of the duck caeca showing epithelial cells (EC) with oval-shaped nucleus (N), goblet cells (GC) with mucous secretion, mitochondria (M), Interaepithelial lymphoid cells (L) and basal membrane (BM) are also seeing (Scale bar: 2 µm).](image2)

![Fig. 31: TEM micrograph of the distal zone of the owl caeca showing nuclei (N), nucleolus (NU), different mitochondrial shapes (M) and lymphocytes (L) between epithelial cells. (Scale bar: 10 µm).](image3)

In this zone a huge number of rounded shape mitochondria appear in all examined birds in addition to the necklace shape in owl only (Fig. 32).

![Fig. 32: TEM micrograph of the distal zone of the owl caeca showing nuclei (N), nucleolus (NU), necklace and other mitochondrial shapes (M) and some vacuoles (V) in the epithelial cells. (Scale bar: 2 µm).](image4)
Discussion

The present investigation was proposed to study, in a comparative manner, the histological and ultra structures of the caeca in a granivorous bird, common quail (Coturnix coturnix); omnivorous bird, Muscovy duck (Cairina moschata) and carnivorous bird, little owl (Athene noctua). The caecum is exposed to continuous and constant invasion of bacterial or non-bacterial antigens of extra caecal origin, since it receives the back flowing urine from the urodeum of the cloaca through the colon. It is evident that the proximal zone of the caecum is concerning about the surveillance against foreign microorganisms more than the middle and distal zones. Therefore the presence of lymphoid tissues could play a highly important role in immunological surveillance against foreign microorganisms. 

Caecal tonsils, on which nearly half of the lymph nodules are accumulated, are major lymphoid tissue in the avian cecum (Kitagawa et al., 1998 and Hamedi et al., 2013).

In the recent study the histological examinations of the proximal zone of quail caecum recorded the presence of the caecal tonsil which is the most immunologically mature lymphoid organ in birds Akter et al. (2006) at the beginning of this zone.

It was histologically formed of the aggregated masses of lymphocytes that forming a multiple nodules and encapsulated by muscle fibers. In which some crypts were either lost their arrangements totally or partially, probably this may be due to the pressure from developing lymphocytes. Such results agree with that reported by (Akter et al. 2006; Usha Kumary et al., 2009 and Hamedi et al., 2013).

On the other hand, the caecum of the duck were not beginning with caecal tonsil as in quail caecum but the proximal zone begin with built up of aggregated masses of lymphocytes forming a multiple nodules and they have some supporting connective tissue; trabeculae. This structure is resemble to the caecal tonsil structure and agree with the finding that recorded by Kitamura et al. (1976).

The proximal zone of the owl also showed the presence of number of lymphocytes in lamina properia but there is no accumulation of lymphoid nodules as in quail and duck. This finding is in accordance with that of Tyto alba in (Ismail, 2000).

The proximal zone of the three birds in this study also showed presence of great number of long villi which leaving only a rather small lumen. These findings agree with all caecal researches such as (Chen et al., 2002; Potter et al., 2006; Meyer et al., 2009; Usha Kumary et al., 2009; Zaher et al., 2012; Svihus et al., 2013; Kadhim et al., 2014 and Hussein and Rezk, 2016).

Movement of material in the caeca and the lower digestive tract has been extensively reviewed and the researchers showed that, the antiperistaltic movements propel material from the proximal towards the distal end of the caeca and contribute to filling the caeca and mixing the contents, while peristaltic movements contribute to mixing and emptying of the caeca (Svihus et al., 2013).

The muscularis layer in our research is the most well developed layer in the caeca especially in the proximal zone. This layer can improve the anti peristaltic and peristaltic movements of caeca. Such suggestion reported by (Hamedi et al., 2013).

The middle and distal zones showed the presence of number of plicae circulars in quail and duck which is believed to increase the surface area or it may have a blocking function when the caecal contents are emptied. These results agree with that reported by (Chen et al., 2002; Zaher et al., 2012 and Svihus et al., 2013).While in the owls only small villi were present and there are no plicae circulars and this result is in accordance with that reported by (Potter et al., 2006 and Meyer et al., 2009).

The middle and distal regions of the duck caeca showed presence of blunted villi and this finding is agree with that of fowl in (Hodges, 1974) and of red Jungle fowl in (Kadhim et al., 2010). On the other hand, there are no villi in the middle and distal regions of the quail caeca and this result is similar to the result observed Chen et al., (2002); Usha Kumary et al. (2009) and Zaher et al., (2012).

Usha Kumary et al. (2009); Kadhim et al., 2010 and Zaher et al., (2012) also explain that the middle and distal regions of caeca contain great number of crypts and this result is homologous to the recent study in quail and duck caeca. While there are no crypts in the distal region of owl caeca and this result is in agreement with that of (Potter et al., 2006 and Meyer et al., 2009).

Kitagawa et al. (1998) and Hamedi et al., (2013) stated the presence of highly developed lymphoid tissues in the proximal and distal regions and also suggest that the distal region is an
important site for immunological surveillance in the caecal host defence system as the caecal tonsils. Such result is in accordance with that of our results of the distal region of duck caeca.

Because of the fermentation and other bacterial or chemical processes that have been shown to occur in the caeca, it is logical to conclude that the caecal epithelium could be a site for primary absorption of nutrients or for re-absorption of electrolytes or amino acids derived from the urine (strong et al., 1990).

Our results depending on the transmission electron microscope observations in the proximal region explained presence of microvilli that longer than that of the middle and distal regions in the three birds and these results is similar to that of (Dantzer, 1989; strong et al., 1990; Ferrer et al., 1991; Usha Kumary et al., 2009 and Svihus et al., 2013).

The surface area increase due to microvilli could be related to both active and passive transcellular transport, with greater surface areas permitting more rapid absorption of material from the caecal lumen (strong et al., 1990).

The proximal region was the only region that had significantly greater microvillous surface area than the other regions, so that the middle and distal regions of the caeca in the three birds may be capable of less absorption than the proximal portion and this idea is in accordance with several researches (strong et al., 1990; Ferrer et al., 1991 and Majeed et al., 2009).

The proximal zone in the duck caeca showed the beaded microvilli. Dahm et al., (1980) thought that contractile filaments, known to be present in the microvilli and in the terminal web of epithelial cells, cause the formation of the beaded microvilli. While the microvilli in the distal region became as short, stubby protrusions like that of quail and fowl in (strong et al., 1990).

The ultrastructure results explained the presence of many goblet cells in the caeca of the three birds and it found mainly in the proximal region more than the middle and distal regions. This result is similar to that of (Dantzer, 1989; strong et al., 1990 and Usha Kumary et al., 2009).

Goblet cells tend to be concentrated toward the proximal end of the caeca. The goblet cells are probably responsible for mucous secretions that may lubricate the caecal contents, and their concentration in the proximal region may facilitate passage of material into or out of the cecum. The proximal and middle regions are roughly equal in their proportion of goblet cells, and the distal portion has a lower proportion. These results are exactly similar to that found in (strong et al., 1990).

The ultrastructure results of this investigation are also explain the presence of numerous mitochondrial shapes include rod and rounded in addition to necklace shape that found in owl and these findings are in accordance to that found in (Dantzer, 1989).

Mitochondria are probably related to the energy requirements of active transport across the apical membrane, which could be facilitated by the greater density mitochondria near the apical surface. An increase in the mitochondrial number could allow a greater rate of active transport. However, the large volume of mitochondria among caecal regions suggests that active transport could be an important mechanism in all caecal regions.

In the present study different types of cellular junctions like tight junction and desmosome were found in all caecal regions of the owl and it serve as a permeability barrier for paracellular transepithelial fluid movement. This control function is important because the primary transport of sugars, amino acids, and small peptides in intestinal epithelia occurs through this paracellular channels (Dantzer, 1989).

The intracellular spaces between the lateral plasmalemma of the adjacent neighboring cells also appear in this study and it increased in the distal region than the proximal and middle regions in the caeca of both quail and duck. While in owl explained the presence of inter digitations between the adjacent cells especially in the proximal zone and all these factors could allow greater exposure to luminal contents for absorption.

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References


