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Gross and histological investigations on the cecum of the black-crowned night heron (*Nycticoarx nycticorax*)

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Abstract

This study was aimed to investigate the gross and the histological structure of the ceca in the black-crowned night heron *Nycticoarx nycticorax*. Ten captured birds were used in this study. Grossly, the cecum in the night heron was a single left unilateral tube-like (intestinal) structure. It was located at the junction between the small and large intestine, with its pointed free end (the apex) directed cranially. The cecal base possessed a well-developed muscular ring, which may act as a sphincter. Histologically, the mucosa at the proximal region was lined with simple columnar epithelium with few goblet cells. The lamina propria contained a well-developed accumulated lymphoid nodules which either covered with small villi or intermediate villi. The lamina propria and submucosa were infiltrated with the lymphocytes and contained lymphatic nodules extended into the sub mucosa. The cecum possessed a certain pattern of the distribution of lymphatic nodules in the distal rather than that of the proximal regions. The mucosal epithelium at the distal part was hardly observed and the submucosa consisted of dense masses of lymphatic nodules and inter-follicular lymphatic tissues. These extended deeply in the submucosa and contained germinal centers of lymphatic tissues found either deeper and/or closer to the muscular wall, or higher in the lamina propria. The lymphatic nodules and inter-follicular tissues were therefore seemed almost fulfill the entire lumen of the distal part. In conclusion, it was advocated that the ceca of the black crowned night herons may have an important function in immunological responses of the bird against different microorganisms.

Keywords: avian ceca, herons, cecal lymphocytes, *Nycticoarx*

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Introduction

The avian ceca classified according to Naik (1962) followed by McLelland (1989) into five histological types, as follows: the intestinal at which the ceca are long and histologically similar to the small intestine, as in galliforms (fowl, quail), ratites (ostrich), diving birds (loons, Grebe) and cuckoos. Glandular, which has long and well-developed ceca with an abundance of goblet cells and capable of profuse secretary activity, as in owl. Lymphoid that have small ceca with many lymphocytes

aggregations, which present in pelican, heron, eagles and sparrow. Vestigial, as in penguin while it usually absent in parrots, swifts and hummingbirds.

The Black-crowned night heron *Nycticorax nycticorax* is the world's most widespread heron and it has been considered as a one of the most important bioindicator species (Kim and Koo, 2007). For these, it provides a model for examining the lymphoid type commonly found in heron.

While there are several studies on the birds ceca, including, chicken (Dantzer, 1989, Ferrer et al., 1991, Verma et al., 2019), guinea fowl (Ilugn et al., 2018), pied crow (Igwebuike and Eze, 2010), common pigeon (Udoumoh et al., 2016) and quail (Hamedi et al., 2013, Rajathi, 2017, AbuAli et al., 2019), very few studies was found concerning the lymphoid type as in the cattle egret *bubulcus ibis* (Hussein and Rezk, 2016). Moreover, the available literatures provide very little information regarding the cecum in the black-crowned night heron. Therefore, the current work was aimed to investigate the macro and micro structure of cecum in the black night heron.

Materials and methods

The study was conducted under the strict adherence to the principles of care and use of animals in research, teaching and testing (Olfert et al., 1993), following ethical approval by The College of Veterinary Medicine, University of Diyala. Ten mature (6 males: 4 females) black-crowned night heron *Nycticoarx nycticorax* were captured by mist net near Diyala's River. The body weight were recorded then the birds euthanized with overdose of ketamine 25mg/kg B.W and xylazine 5mg/kg B.W injected through the alar vein of mature bird (Hess, 2005) with insulin syringe. All measurements were taken instantly after body dissection for all ceca. Thereafter, each cecum was dissected from the abdominal viscera and its length was taken, and then weighed. Transverse parallel interrupted sections were performed at equal intervals along the length of the ceca and immediately placed into the 10% neutral buffered formalin (NBF) for 24h to ensure optimum fixation. Finally, all tissue samples were processed by routine methods, embedded in paraffin wax and then sectioned at 5µm, and stained with routine staining (H&E) and PAS stain (Bancroft and Stevens, 1982). Measurements of the epithelial tissue thickness were calculated and calibrated using a Fiji J software. These measurements were performed on each photomicrograph and the data were recorded as mean ± SEM.

Results

Gross anatomy

The cecum in the black crowned night heron was a single left, short tube like structure, it was located at the junction between the ileum and the large intestine (colon) with their pointed apex directed cranially (Figure 1). This single cecum was fingerlike looking much similar to a simple unilateral extension of the intestine. It also possessed a well-developed basal ring of circular muscle forming a sphincter. All measurements including, mean ± SE of body mass, the whole length of intestine, small intestine, rectal cloacal length and cecal length are presented in Table 1.

Histology:

The histological data was found into two different forms: proximal region and distal (tip) region. The mucosal epithelium at the **proximal part** was showed lining with simple columnar epithelium with few goblet cells. The lamina propria contained a well-developed grouped of lymphoid nodules were either covered with small villi adjacent to distal region or intermediate villi adjacent to intestine (Figure 2). These later were almost similar to those of mucosal small intestine and contained cells called goblet cells which appeared scattered with few numbers and contained different sizes. The lamina propria and sub mucosa were observed infiltrated with the lymphocytes and mast cells. It was also found some nodules extended into the submucosa adjacent to the intestinal glands (Figure 2). However, the cecum had a certain pattern of distribution of lymphatic nodules in the distal rather than that of the proximal regions. The mucosal epithelium at the **distal part** was hardly observed and showed that the submucosal epithelium were consisted of dense masses of lymphatic nodules and many lymphocyte infiltration. These were extended to the submucosa and showed diffuse inter-follicular lymphatic tissues (Figure 2). The lymphatic tissues contained germinal centers which was found either deeper and closer to the muscular wall, or higher in the lamina propria. The lymphatic nodules and inter-follicular tissues in addition to reticular fibers were therefore seemed almost fulfill the entire lumen of the distal part. These differences were therefore revealed between the mucosal epithelium from proximal and distal cecal parts. The tunica muscularis showed there was no much differences in the pattern of tunica muscularis around the cecal entrance (proximal part) and/or apex (distal part). The serosa was observed ramification by the blood supply. Additionally, the morphometric analyses of the epithelial thickness presented in Table 2 did not show much differences in the epithelial thickness between proximal and distal region. The same results indicated in the thickness of tunica muscularis around the cecal entrance and/or apex.

Discussion

Histomorphological analyses indicated that black crowned night heron possessed a left single, short and not well developed ceca with a unique structural epithelium characterized generally by lymphoid type. This finding is similar to that of McLelland (1989), described as lymphatic type. However, it is contrary to most avian ceca which are paired and of roughly equal length, with separate lateral or ventro-lateral openings into the colon or rectum (Clench, 1999, Scanes and Pierzchala-Koziec, 2014). In comparison with other cecal birds, the pointed cranial end not well developed as appeared in others birds such as chicken (Ferrer et al., 1991), geese (Chen et al., 2002, Kushch et al., 2019), turkey (Nnadozie et al., 2019) and quail (AbuAli et al., 2019). The ceca in the current study were small and/or not well developed in all examined birds. However, the current finding similar to what reported in pigeons except that pigeons have paired not well developed ceca (Udoumoh et al., 2016). It can therefore be assumed that ceca tend to be well-developed in herbivores, omnivores, and birds that feed on molluscs (Clench and Mathias, 1995), but Goudie and Ryan (1991) found that they are variable in insectivores and carnivores and small in piscivores and graminivores. Additionally, there are a strong a correlation between cecal mass and diet: herbivores have larger ceca than carnivores, and omnivores are intermediate. These suggested that cecal size is might be influenced by dietary proclivities and

forces, with herbivorous species having long ceca, whereas faunivorous species having short ceca (Hunt et al., 2019, Abadi et al., 2019). From the current study ceca appeared fingerlike in shape, looking much similar to simple unilateral extensions of the intestine. This result differs from the ceca of most of avian species ceca that described ceca as a simple tubular organs such as fowl, geese, duck and quail (Dantzer, 1989, Ferrer et al., 1991, Zaher et al., 2012, Denbow, 2015). Also, this result contrary to sacculated and/or have diverticula that found in some species such as rheas, ostrich, kiwis, screamers and some *tinamous*, (McLelland, 1989). These results provide further support for the hypothesis that the type of the food play an important role in cecal development and adaptation. Hence, the birds feed on diet rich in cellulose would be expected to have well-developed caeca, whereas those feed on grains, fruits, fishes, and nectar would be expected to have less cecal development (DeGolier et al., 1999, Hunt et al., 2019, Xia et al., 2019, Grond et al., 2020). It can thus be suggested that not well developed ceca found in night herons could be possible due to the types of the food which is mostly fishes (Clench, 1999). The ceca of night herons was found to have a well-developed basal ring of circular muscle which might forming a sphincter not a flap valve. These outcomes are contrary to that of (McLelland, 1989, Clench and Mathias, 1995, Denbow, 2015) who found that the digestive tract of most birds contains a pair of out pocketing that project from the proximal colon at its junction with the small intestine. From their bases, ceca usually extend on either side of the ileum and are loosely attached to it by mesentery and the ileocecal ligament (Clench, 1999). Histologically**,** the proximal part was showed that the mucosa were consisted of a well-developed aggregated lymphoid nodules were either covered with small villi adjacent to distal region or intermediate villi adjacent to intestine. These observations suggest that there is a clear correlation between the structural cecal epithelium and its function (Józefiak et al., 2004). The current findings revealed the presence of different pattern of lymphatic nodules in the distal than that presence in the proximal regions. These results are contrary to Akter et al., (2006) who demonstrated that most of the nodular lymphatic tissues of cecal chickens were found in the proximal part. Hence, it can be suggest that the distal region may be the most important region for immune responses, because the large numbers of lymphatic nodules presences in this region. In addition, the heavily accumulations of lymphatic nodules and inter-follicular tissues that seemed almost fulfill the entire lumen of the distal part was attributed to the presence and accumulation of lymph nodules in this part as it is shown by Hussein and Rezk (2016) in *cattle egret*. These lymph nodules were appeared close to the base of lamina propria and contained large germinal centers, make them seemed closer to each other. These differences were therefore revealed between the mucosal epithelium from proximal and distal ceca. It is therefore apparent that these morphological changes are an evidence for different functions between the two compartments (Hunt et al., 2019). These results disagree with the finding of (Zaher et al., 2012) who found that the cecum wall is thinner than other intestinal segments, encompasses lymphatic tissues mostly appears at ilocecal junction creating cecal tonsil. Additionally, Alshamy et al. (2018) stated that the mucosal ceca is similar to that of the intestinal mucosa, with lesser goblet cell and fewer glands. It is likely to hypothesize that these morphological features of the ceca in night herons might have an important function in immunological responses against different microorganism. Based on the current findings, it can be affirmed that the heavily lymphatic nodules are mostly situated in the lamina propria and submucosa of the distal cecal part than they are in their proximal part. In conclusion, it was advocated that the cecal epithelium in black crowned night herons may has an important function in immunological responses of the bird against different microorganism.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author's contribution

Authors are equally contributed for the manuscript.

Table 1. Biometric data of the intestine in black night herons.

Body mass(gm)	Total intestine length (cm)	Small intestine length (cm)	Large intestine length (cm)	Cecal length (cm)	Ceal/ intestine (%)
816±13.75	127.76±0.26	118.45±0.25	9.31±0.24	1.07±0.03	0.84±0.02

Table 2. Measurements the epithelial thickness of the cecal regions in black night herons.

Cecal regions	Thickness of wall (µm)	Lumen diameter(µm)	Height villi (µm)	Lymphatic nodules (µm)
Proximal	269±5.5	2128±28.5	427±11.8	-
Distal (apex)	248±3.3	2508±38.3	-	1.44±0.05

Figure 1: Photographs of mature black crowned night herons illustrates anatomical position ventral view. A) Shows anatomical of the (i) intestine, (l) liver, and (g) gizzard. B) Illustrates a single short not well developed (c) ceca appeared as a fingerlike in shape has (t) top and (m) middle region and its attachment between (il) ileum and (co)colon.

Figure 2: Photomicrographs illustrate the histological features of the ceca in black night herons. (A) Shows a cross section in the proximal region, whereas (D) represents the distal region, (red arrows) indicates mucosal glands, while (yellow arrow) shows lymphatic nodules. B) Proximal region shows goblet cells (black arrows) and mucosal glands (red arrows), while in (E) represents distal region filled with lymphatic nodules (yellow arrow). PAS stain in proximal region (C) shows mild to moderate mucopolysacharides reaction while in distal region (F) did not show.(A, D, H&E stain; bar= 200 µm), (B, E, H&E stain; bar= 50 µm), (C, F, PAS stain; bar= 50 µm).

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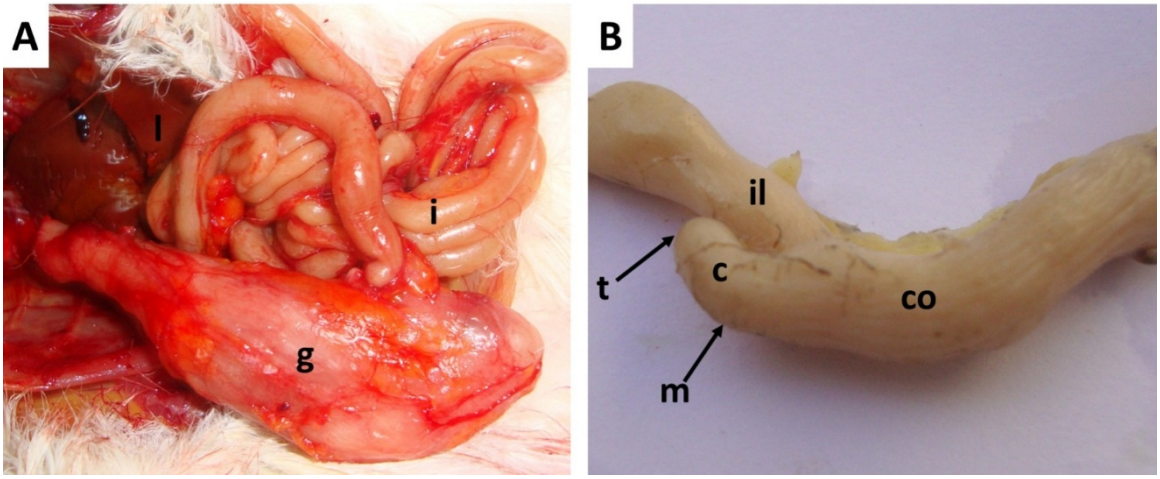

Figure 1.

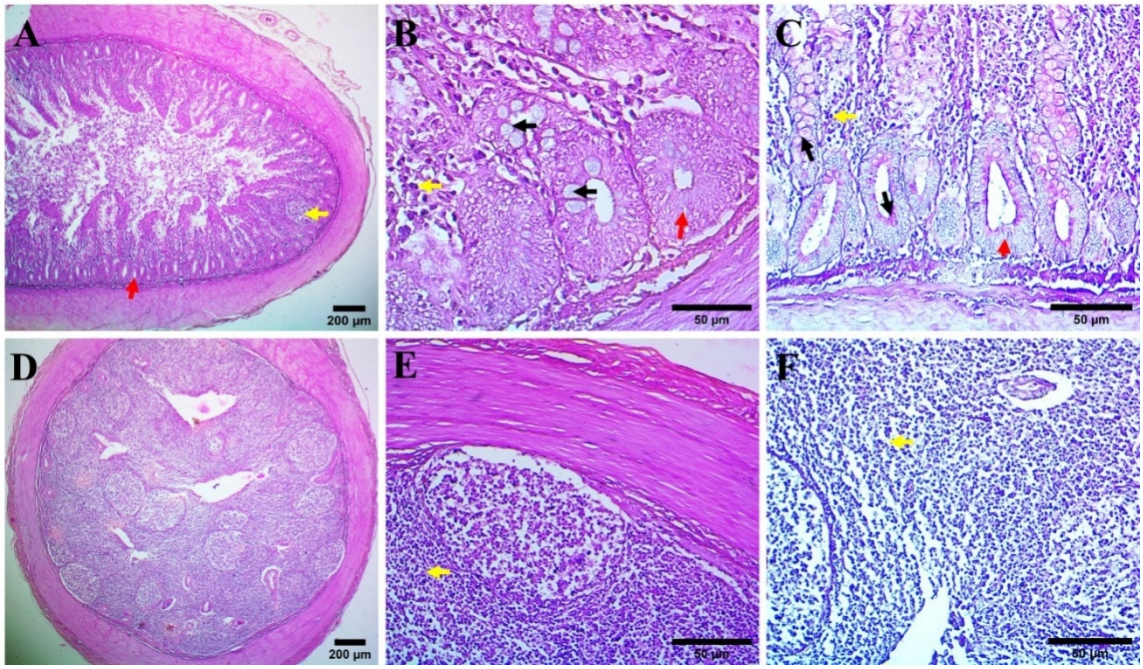

Figure 2.