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HISTO-MORPHOLOGICAL STUDY OF IMMUNE CELLS IN CAECUM OF DIFFERENT POULTRY BIRDS

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ABSTRACT
The present research was carried out to have comparative study of the histological architecture of the caecum of different poultry birds. The study was carried out on six numbers of adult birds of quail, chicken and duck of both sex of 4-6 wks, 3-4 months and 8-10 months of age respectively without any detectable abnormalities. The mucosal surface of quail caecum showed highly developed flat areas and ridges pattern of villi whereas in chicken and duck, the mucosa was having more prominent tooth-shaped villi. Significantly ($p < 0.05$) greater villi heights (in μm) was observed in chicken (246.48 ± 26.03) than the quail (177.11 ± 4.69) and duck (130.67 ± 6.77). The thickness of the caecal wall (in μm) was significantly ($p < 0.05$) higher in chicken (147.57 ± 9.27) followed by duck (114.44 ± 5.20) and quail (96.88 ± 4.62). Diffuse sub epithelial immune cells of chicken and duck caecum fill the lamina propria layer. Distinct lymphoid nodules surrounded by capsular connective tissues were noticed deep inside the villous core in chicken only. The results of the present study could be helpful to understand the affection of immune tissue due to diseases and abiotic factors as the caecum plays a vital role in immunological surveillance against foreign microorganism.

INTRODUCTION

Small body size, early maturity and fast growth are attributed for economical viability of poultry farming round the globe. Optimum health of poultry birds is directly proportional to the economic return. But, infectious diseases and heat stress in summer shake the financial backbone of farmers as diseases and abiotic stress factors affect the immune system resulting in disorganization of the histological architecture of concerned organs, immune suppression, poor production and mortality (Hussan *et al.*, 2009). The role of researchers, pharmaceutical agencies and feed industries are supporting economic return of the farmers. Practice of immunization, feeding of ingredients in a need based manner, scientific rearing conditions and use of disease resistant stock are not adequate to reduce loss at farmer's hand and to make poultry farming sustainable among alternative agricultural avenues. So, there is a need to focus the research on alternative management of infectious diseases and heat stress in the interest of farmers. Since, immune system plays a pivotal role in maintaining optimum health by protecting from infectious diseases and adverse abiotic factors; well understanding of such system in avian species becomes essential for the development of novel and effective control strategies.

The caecum, a peripheral lymphoid tissue, the largest part of avian large intestine is an intestinal out-pocketing of gut. Besides, participation in cellulose digestion and liquid absorption (Trampel and Duke, 2004), it acts as a defence organ by producing immunoglobulin in large mass of diffuse and nodular lymphatic tissue in lamina propria and sub mucosa (Firdous and Lucy, 2012; Mary, 2006). The immune response elicited by lymphoid tissue of caecum (caecal tonsil) is regulated by either infection or by environmental factors. Also the caecal tonsils and lymphatic tissues in caecal wall depend on the activity of immunologically important primary lymphoid organs like bursa of fabricious and thymus (Rezaian and Hamedi, 2007; Kajiwara *et al.*, 2003). So, well maintained immune defence system with enormous lymphoid nodules throughout the mucosa provides protection against caecal environment as the caecal lymphatic nodules of different poultry birds are the sites for both immune responses and medicinal therapies.

Now a days, rise in temperature due to global warming and frequent infections due to erratic climatic factors results in significant immune suppression and huge mortality in broiler

chickens (*Gallus domesticus*) (Al-Ghamdi, 2008). On the other hand, the other two poultry birds, i.e. duck (*Anas platyrhynchos*) and quails (*Coturnix coturnix japonica*) show very less mortality. In respect to the above immunological points of view, the comparative histology of the caecum of the poultry birds appears interesting and literature on its comparative histology with micrometry analysis in different poultry birds is meagre. Therefore, the present work was carried out to understand the difference in histological architecture including its lymphoid tissue (tonsils) of the caecum of these poultry birds.

MATERIALS AND METHODS

Ethical approval

The present study was conducted with prior approval of the Institutional Animal Ethics Committee of the College (No. 433/ CPCSEA/ OVC). The quail and chickens were procured from Central Poultry Development Organization (CPDO) and ducks from Central Avian Research Institute (CARI), Bhubaneswar, Odisha.

Sample collection

The study was carried out on adult birds of either sex, six each from quail, broiler chicken and ducks of 4-6 weeks, 3-4 months and 8-10 months of age respectively. The birds had neither developmental nor any other systemic/ functional disorders. All the birds were killed by cervical sub-luxation method and the intact caeca (right and left) were collected carefully through ventral abdominal dissection. Only the apical part of caecum was collected as specimen tissues after complete washing with normal saline and those were devoid of any gross pathological lesions.

Preparation of tissues for histological study

The preparation of tissues for histological study was done by the method described by Giri *et al*. (2013) with slight modifications. Briefly, the representative tissue samples obtained from the birds were fixed in 10% buffered neutral formalin (BNF). After routine tissue processing, the tissues were cleared in xylene, followed by paraffin embedding to prepare paraffin blocks. The tissue blocks were then cut by semi motorized rotary microtome (Leica RM 2245) to obtain 5- 6 micron thick serial paraffin sections. The sections were mounted on clean, grease free, albumenized glass slides. Then the tissue sections were subjected to routine haematoxylin and eosin (H & E) staining for histo-morphological study as per the method described by Bancroft and Gamble (2007). Photographs from the selected fields were taken under low (×10) and high (×40) magnification.

Micrometry study and Statistical analysis

The micrometry was performed by using LAS (Leica Analysis Software) to record different histometrical parameters (in μm) like height of the villi, thickness of the muscular wall (Tunica muscularis) and size of lymphatic nodules as per the method described by Mandal *et al*. (2013). The data was subjected to statistical analysis according to Snedecor and Cochran (1994) particularly analysis of variance (ANOVA) and least significant difference (LSD) were made using Microsoft's MS 2000 Data Analysis package.

RESULTS

The mucosal surface of quail caecum (Fig. 1) showed highly developed flat areas and ridges pattern of villi as compared to those observed in chicken and duck (Fig. 3 and 5). In chicken and ducks, the caecal mucosa had more prominent, tooth-shaped villi. The chicken had significantly ($p < 0.05$) longest villi (246.48 ± 26.03) μm than those of quail (177.11 ± 4.69) μm and duck (130.67 ± 6.77) μm. Also thickness of caecal wall (in μm) was significantly ($p < 0.05$) highest in chicken (147.57 ± 9.27) followed by duck (114.44 ± 5.20) and quail (96.88 ± 4.62) (Table 1). Villi were lined with epithelium comprised of striated simple columnar cells and numerous goblet cells. Crypts of Lieberkuhn (intestinal glands) were very prominent at the base of the villi in all the birds. In all cases, the lymphatic tissue was elucidated in the form of nodules as well as diffuses aggregations which were distributed usually in the lamina propria part of the mucosa and even in sub mucosa layer. But lymphocytic aggregations and distinct lymphoid nodules with germinal centres were absent in duck and quail. In case of chicken, typical caecal tonsils were observed (Fig. 3 and 4). Diffuse sub epithelial immune cells filled up the lamina propria layer of chicken and duck caecum (Fig. 3 and 6). Even distinct lymphoid nodules surrounded by prominent nodular capsular connective tissue were noticed deep inside the villous core (Fig. 3 and 4). In all the poultry birds' lymphatic nodules and other diffuse lymphatic aggregations, both large as well as small varieties of lymphocytes were observed. The length of long and short diameter of chicken caecal lymphoid nodule was 248.36 ± 55.69 μm and 160.88 ± 33.80 μm respectively. We were unable to generate the respective data for rest two varieties of poultry birds as because there was no significant lymphoid nodule in their case.

DISCUSSION

The present findings on histological features of the caecal mucosa are comparable with those observed by Strong *et al*. (1990).The mucosal surface of caeca in quail showed highly developed villous ridges pattern and flat areas than those in chicken. The domestic fowl had significantly greater villi heights than those of quail and duck. Villous height in caeca of broiler chicken is almost similar to those observed by Mandal *et al*. (2011). But, there was no report regarding height of caecal villi and thickness of caecal wall in quail and duck so far. Present observation on caecal lymphatic tissue was in agreement with the findings of Ruhma *et al*. (2003) and Imilio *et al*. (2004), who reported that lymphatic nodules were most commonly distributed in the epithelium, lamina propria and sub mucosa of the apical part of each caecum because the caecum of chicken receives the back flowing of excreta through the rectum from the urodeum part of cloaca. In case of duck caeca,

Table 1: Histometrical parameters of the caeca in quail, chicken and duck

Species	Height of villi (μm)	Thickness of wall (μm)
Quail	177.11 ± 4.69^{b}	96.88 ± 4.62^{a}
Chicken	246.48 ± 26.03^{c}	147.57 ± 9.27^{c}
Duck	130.67 ± 6.77^{a}	114.44 ± 5.20^{b}

Superscripts indicate significant variation ($p < 0.05$) between groups within the column.

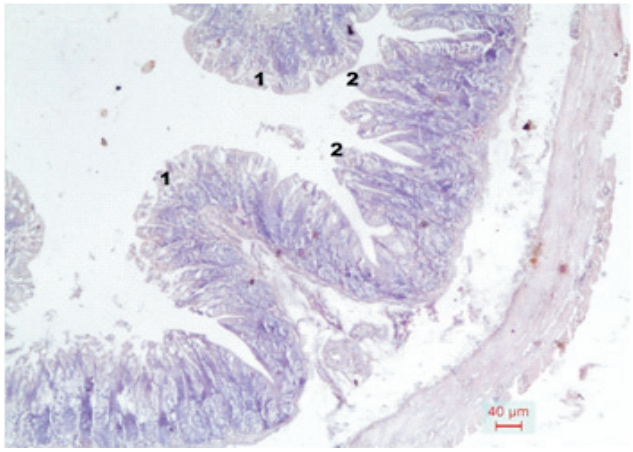

Figure 1: Photomicrograph of caecum of quail showing mucosal folds/ ridges (1), arrangement of villi (2) (H & E stain x10).

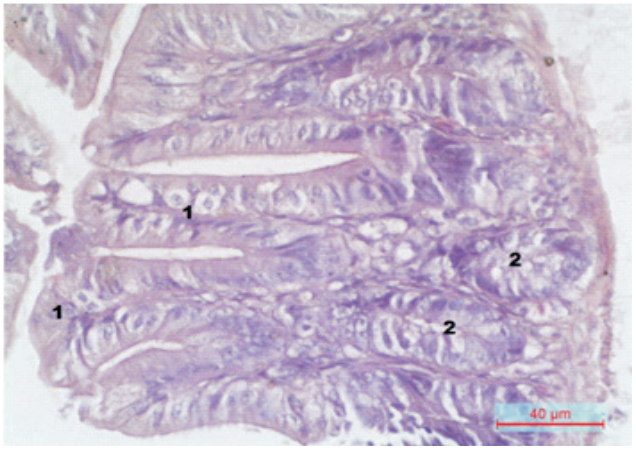

Figure 2: Photomicrograph of caecum of quail showing villi (1) & crypts (2) (H & E stain x40).

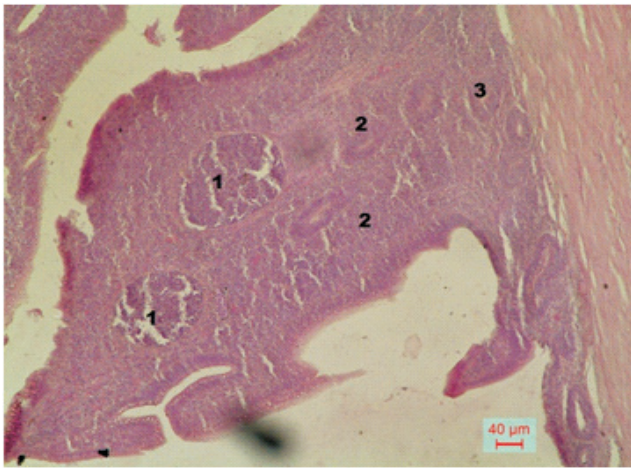

Figure 3: Photomicrograph of caecum of broiler chicken showing lymphatic nodules (1), diffuse lymphatic tissue (2) inside villi & lamina propria (3) (H & E stain x10)

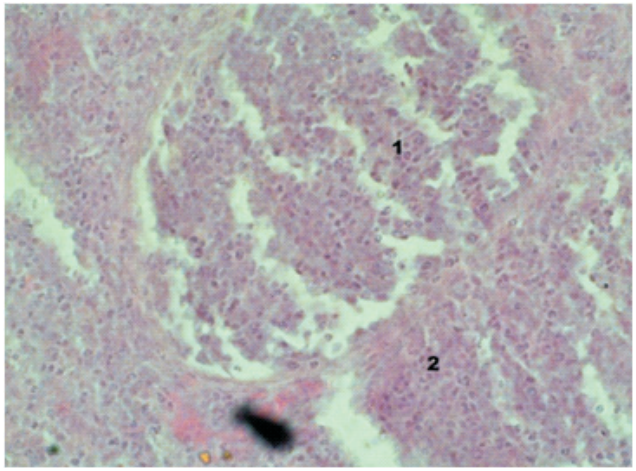

Figure 4: Photomicrograph of caecum of broiler chicken showing lymphocytes in lymphatic nodules (1) & adjacent diffuse lymphatic tissue (2) (H & E stain x40).

results of the present study corroborate well with that of Das and Biswal (1967) who described that the lymphocytic aggregation and distinct lymphoid nodules with germinal centres were absent. However, in the present study characteristic finding was the presence of diffuse lymphatic tissue in lamina propria layer of caecum in duck. A typical caecal tonsil could not be located unlike in domestic fowl and lamina propria showed tubular intestinal crypts of Lieberkuhn and the mucosa of the caecum carried tooth-shaped villi as earlier reported by Firdous and Lucy (2012). In our result it was well established that the height of villi of duck was significantly lesser than that of quail. However the wall thickness was significantly highest in chicken followed by duck and quail. This type of variation in villi height and wall thickness may be due to feeding habit of respective birds. Immunity of birds is directly proportional to height of villi and thickness of wall as because the sub-mucosa of villi as well as lamina propria, located in between base of villi and caecal wall, contains lymphocytes. So concentration of lymphocytes leads to degree of immunity to birds.

Now a day, poultry birds are receiving more attention around the globe, because of huge economical profits owing to their small size, early maturity and fast growth (Hamedi *et al.,* 2013; Sedqyar *et al.,* 2012). But, these are most susceptible to various parasitic diseases, thus limiting the poultry industry. During periods of stress especially physiology of birds depends vitally on the species involved, the caecal environment, and the ecological conditions under which they harbour. Most of the parasites harbour inside caecum and this organ play a vital role for providing immunity inside the caecal environment as it contains lymphatic nodules and immune-competent cells (Umar *et al.,* 2014).

The occurrence of diffuse lymphatic tissue in the lamina propria throughout the caecal wall in growing and adult birds highlights the immunological surveillance against the caecal luminal contents and thus helps in maintaining the caecal microenvironment. Therefore, the present study could be helpful to understand the affection of immune tissue due to diseases and abiotic factors as the caecum plays a vital role in development and maintenance of immunity.

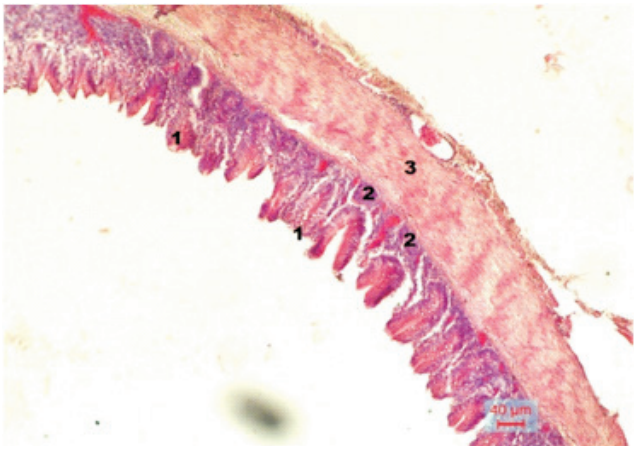

Figure 5: Photomicrograph of caecum of duck showing mucosa studded with villi (1), lamina propria (2) & caecal wall (3) (H & E stain x10)

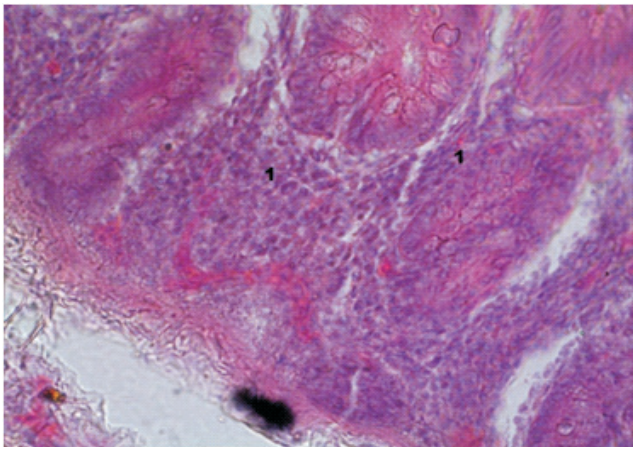

Figure 6: Photomicrograph of caecum of duck showing diffuse lymphatic tissue in lamina propria (1) of the mucosa (H & E stain x 40)

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