



Original Research Article

Histological and Ultrastructural studies of Caecal tonsil in Chicken (*Gallus domesticus*)

T.A.Kannan^{1*}, Geetha Ramesh², S.Ushakumari², G.Dhinakarraj³ and S.Vairamuthu⁴

¹Centre for Stem Cell Research and Regenerative Medicine, India

²Department of Veterinary Anatomy and Histology, India

³Director, Translational Research Platform, India

⁴Central Clinical Laboratory, Madras Veterinary College, Chennai, India

*Corresponding author

ABSTRACT

Keywords

Histology,
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Light and electron microscopic studies on caecal tonsil were done in layer chicken of various age groups ranging from day-old to forty weeks. The caecal tonsil revealed two types of lymphoid aggregations (germinal centre). The first type had an incomplete capsule and the second type was found encapsulated with connective tissue. The capsule of the germinal centre consisted of many layers of flattened reticular cells separated with an intercellular substance. The germinal centre consisted of lymphoblasts, lymphocytes of various sizes, reticular cells, plasma cells, mast cells and macrophages. In forty week-old birds, the lymphocytic population was observed to be comparatively reduced and more number of fibroblasts and collagen fibres were noticed. The caecal tonsil also had M cells with short and irregular microvillus.

Introduction

The alimentary tract is incessantly invaded by foreign antigenic or harmful substances and their lumina are also sites for the proliferation of both beneficial and disadvantageous bacterial flora (Mead, 1989 and Kitagawa *et al.*, 2000). Caecal tonsil, the immunodefence mechanism of the caecal environment regulates the proliferation of microflora in the caecum continuously and also prevents the invasion of extra caecal microorganisms. This is brought about by organization of enormous lymphoid nodules throughout the caecal mucous membrane forming tonsils (Kitagawa *et al.*, 1998).

A little work was done about the light and ultrastructural studies of the caecal tonsils in Chicken. Hence, the present study was designed to explore the details of caecal tonsils in the layer chicken of different age groups.

Materials and Methods

Caecal tonsils for light and transmission electron microscopic studies were collected from six different age groups such as day-old, four, eight, twelve, twenty and forty weeks. Six birds were used in each age group.

For light microscopic study, tissue pieces were collected from caecal tonsils and processed as per the standard paraffin embedding technique (Bancroft and Stevens, 2007). Tissue sections were cut at 3–5 micron thickness and used for the routine Haematoxylin-eosin staining method (Singh and Sulochana, 1978) and Masson's trichrome method for collagen and muscle fibres (Luna, 1968).

For electron microscopic study, small pieces of caecal tonsils (1-2 mm thickness) were collected and prefixed at 3 per cent glutaraldehyde and stored at 4°C. Subsequently, the tissues were washed, three changes (each 30 minutes) in cold sodium cacodylate buffer solution (pH 7.4) and post fixed in 1 per cent osmium tetroxide for two hours at 4°C. The tissues were then dehydrated in ascending grades of alcohol (50, 70, 80, 90, 95 per cent and absolute ethyl alcohol), propylene oxide: epoxy resin mixture and embedded in Epon-araldite mixture. Semi thin (1 micron) sections were stained by toluidine blue. Ultra thin sections (600 Å to 900Å) were prepared on Leica ultracut microtome, mounted on uncoated copper grids and stained with saturated solution of uranyl acetate and lead citrate. The ultra thin sections were examined under Phillips (Teknai-10) computer augmented transmission electron microscope operated at 60-kilowatt ampere (KVA).

Results and Discussion

Light microscopy

Two types of germinal centres were observed in all the age groups of chicken. The first type had an incomplete capsule and was noticed near the muscular layer of the caeca. This may represent a specific site of uncommitted cell proliferation and differentiation (Olah and Glick, 1975;

1979). Plasma cells of the caecal tonsil, in general, are found deep in the tunica propria. The location of plasma cells and plasmablasts in the caecal tonsil suggest that their lymphoid precursors could originate from the first type of germinal centre.

The second type of germinal centre located closer to the surface epithelium of the villi of the caecum and was encapsulated with connective tissue. Hence, it is presumed that the development of second type of germinal centre may depend upon antigenic influence which passes from the lumen to the germinal centre through the surface epithelium or via the vascular system (Olah and Glick, 1979).

The cellular components of the germinal centre of the caecal tonsils include lymphoblast, lymphocytes and primitive reticular cells (Plate-1). Plasma cells, mast cells and macrophages were also present (Glick *et al.*, 1981, Gomez *et al.*, 1998 and Kitagawa *et al.*, 1998).

Electron microscopy

The capsule of the germinal centre of the caecal tonsil of chicken consisted of many layers of flattened reticular cells separated with an intercellular substance. The cytoplasm of the reticular cells showed a number of smooth surfaced vesicles (Plate-2). The cell processes were seen with filamentous substance with small groups of scattered ribosomes (Olah and Glick, 1979).

The germinal centre consisted of reticular cells, large and small lymphocytes and macrophages (Olah and Glick, 1975). The structures of the reticular cells were similar to that of the reticular cells of the capsule. The number of reticular cells in the periphery of the germinal centre was higher than the central area (Kitagawa *et al.*, 2000).

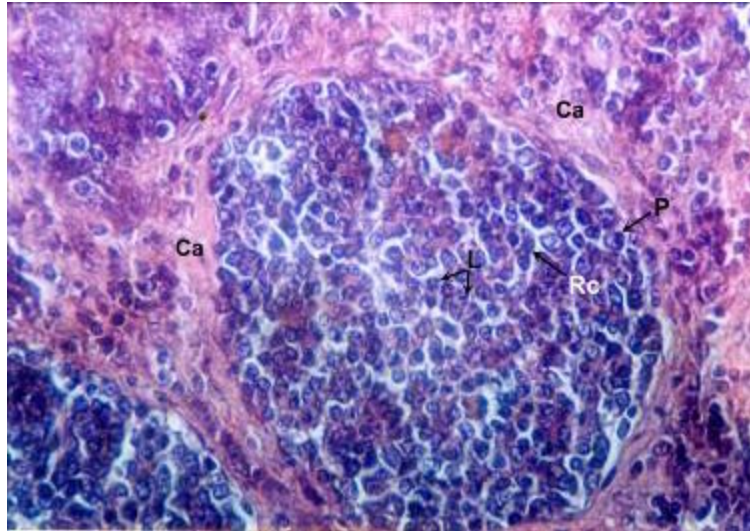


Plate.1 Photo micrograph of caecal tonsil of a twenty week-old chicken showing the cellular components of the germinal centre H&E x 630
Ca - Capsule L - Lymphocyte
P - Plasma cell Rc - Reticular cell

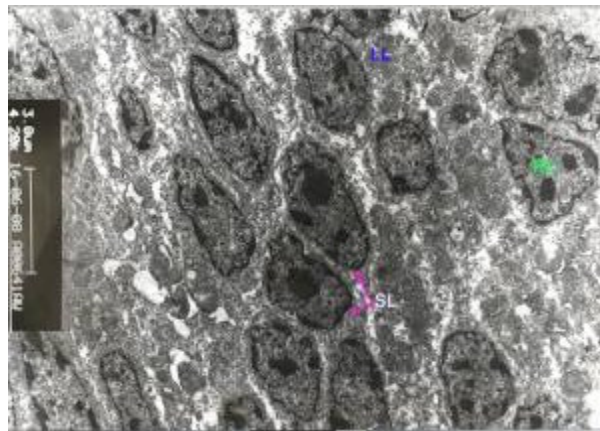


Plate.2 Transmission electron micrograph of caecal tonsil of a day-old chick showing the capsule of the germinal centre x 8900
Ca - Capsule N - Nucleus of reticular cell
Rc - Reticular cell V - Vesicle

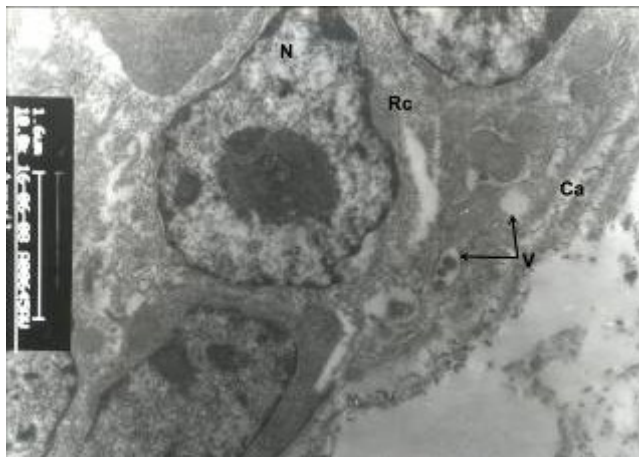


Plate.3 Transmission electron micrograph of caecal tonsil of a day-old chick showing the cellular components of the germinal centre x 3700
LL - Large lymphocyte; SL - Small lymphocyte; Rc - Reticular cell

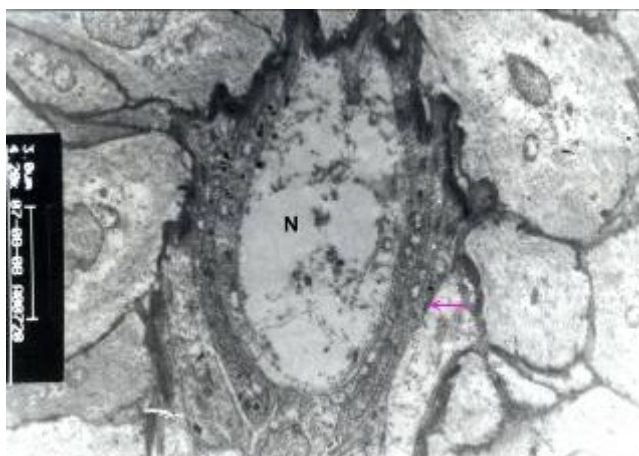


Plate.4 Transmission electron micrograph of caecal tonsil of an eight week-old chicken showing the M - cell (arrow) x 4200 N - Nucleus of M- cell

The large lymphocyte had a small amount of cytoplasm with ribosomes and few mitochondria. The nucleus was found to be heterochromatic and a well developed nucleolus. The small lymphocytes were round cells which had numerous ribosomes in their cytoplasm. A patchy chromatin pattern was observed in the nucleus (Plate-3). The other cellular components of the germinal centre included fibroblasts, plasma cells, macrophages and mast cells in all the age groups. In forty week old-birds, the lymphocytic population was observed to be comparatively reduced and more number of

fibroblasts and collagen fibres were noticed.

The caecal tonsil of the chicken also had M cells which were found associated with the surface epithelium of the villi of the caecum (Jeurissen *et al.*, 1999). These M cells were found to have short and irregular microvilli and were observed to be connected with the neighbouring epithelial cells by desmosomes. Some of the M cells were columnar shaped and some of them were found to be dome-shaped. The M cells possessed darker cytoplasm but the density was found to be less. The cytoplasmic

processes were found to project at the apical surface (Plate-4). Numerous small vesicles with a few ribosomes, rough endoplasmic reticulum and mitochondria were present within the cytoplasm. The nucleus was larger and possessed less heterochromatin with a nucleolus. The distal part of the villi of the caecum showed many small irregular cavities which corresponded to the lymphatic space of the villi (Bye *et al.*, 1984; Uchida, 1987).

The caecal tonsil revealed two types of lymphoid aggregations (germinal centre). The first type had an incomplete capsule and the second type was found encapsulated with connective tissue. The capsule of the germinal centre of the caecal tonsil consisted of many layers of flattened reticular cells separated with an intercellular substance. The germinal centre consisted of lymphoblasts, lymphocytes of various sizes, reticular cells, plasma cells, mast cells and macrophages.

The large lymphocyte had a small amount of cytoplasm with ribosomes and few mitochondria. The nucleus was found to be heterochromatic with a distinct nucleolus. The small lymphocytes were round with numerous ribosomes in their cytoplasm. A patchy chromatin pattern was observed in the nucleus. In forty week old-birds, the lymphocytic population was observed to be comparatively reduced and more number of fibroblasts and collagen fibres were noticed. The caecal tonsil of the chicken also had M cells with short and irregular microvilli and was observed to be connected with the neighbouring epithelial cells by desmosomes. They were associated with the surface epithelium of the villi of the caecum.

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References

- Bancroft, J.D., Stevens, A. 2007. Theory and practice of histological techniques. Churchill Livingstone, London.
- Bye, W.A., Alen, C.H., Trier, J.S. 1984. Structure, distribution and origin of M-cells in peyer's patches of mouse ileum. *Gastroenterology*, 86: 789–801.
- Glick, B., Holbrook, K.A., Olah, I., Perkins, W.D., Stinson, R. 1981. An electron and light microscopic study of the caecal tonsil: the basic unit of the caecal tonsil. *Dev. Comp. Immunol.*, 5: 95–104.
- Gomez, D.M.M., Fonfria, J., Varsa, A., Jimenez, E., Moreno, J., Zapata, A.G. 1998. Appearance and development of lymphoid cells in chicken (*Gallus gallus*) caecal tonsil. *Anat. Rec.*, 250: 182–89.
- Jeurissen, S.H.M., Wagenaar, F., Janse, E.M. 1999. Further characterization of M-cells in Gut-Associated lymphoid tissues of the chicken, *Poult. Sci.*, 78: 965–72.
- Kitagawa, H., Hiratsuka, Y., Imagawa, T., Uehara, M. 1998. The apical caecal diverticulum of the chicken identified as a lymphoid organ. *J. Anat.*, 192: 293–98.
- Kitagawa, H., Shiraishi, S., Imagawa, T., Uehara, M. 2000. Ultrastructural characteristics and lectin-binding properties of M cells in the follicle associated epithelium of chicken caecal tonsils. *J. Anat.*, 197: 607–16.

- Luna, L.G. 1968. Manual of histological staining methods of the armed forces institute of pathology, 3rd edn. Mc Graw Hill Book Co., New York. Pp. 95.
- Mead, G.C., 1989. Microbes of the avian caecum: types present and substrates utilized. *J. Exp. Zool. Suppl.*, 3: 48–54.
- Olah, I., Glick, B. 1975. A description of germinal centers and a secretory cell in the chicken caecal tonsil. Manuscript.
- Olah, I., Glick, B. 1979. Structure of the germinal centre in the chicken caecal tonsil: light and electron microscopic and auto radiographic studies. *Poult. Sci.*, 58: 195–210.
- Singh, U.B., Sulochana, S. 1978. A Laboratory Manual of Histological and Histochemical Techniques, Kothari Medical Pub. House, Bombay. pp: 28, 43, 52, 53.
- Uchida, J. 1987. An ultra structural study on active uptake and transport of bacteria by micro fold cells (M cells) to the lymphoid follicles in the rabbit appendix. *J. Clin. Electron Microsc.*, 20: 379–94.