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IMMUNOHISTOCHEMICAL STUDY OF THE POSTNATAL DEVELOPMENT OF LYMPHOID TISSUES AND MUCOSA OF BROILERS

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ABSTRACT

The experiment was carried out in the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh. A total 15 (fifteen) day-old "Cobb-500" chickens (broilers) of both sexes were purchased from "Kazi Farm Ltd." Mymensingh. For immunohistochemical staining purpose, the bursa of Fabricius, cecal tonsils and ileum was collected from postnatal broiler chickens after sacrificing by cervical subluxation method. Sample (n=3) was collected every 7 days interval starting from day 3 up to 32 days of age. Immunohistochemical study revealed that in the bursa of Fabricius, the Igs positive cells (IgA, IgG and IgM) were found principally beneath the capsule, around the follicles and in the cortex and also medulla. In all stages of development (from day 3 to day 32) IgM positive cells were more followed by IgG and IgA positive cells. In the cecal tonsils, Igs positive cells (IgA, IgG and IgM) were distributed within the lymphatic nodules, lamina propria and in the core of the villi. Early in the postnatal development (at day 3), no IgA positive cells were found in the cecal tonsils and IgM positive cells were more than IgG positive cells. At day 32, IgA positive cells were more than IgG and IgM positive cells. With the advancement of age, the Igs positive cells (IgA, IgG and IgM) were increased significantly. In the early postnatal development (at day 3) no immunoglobulin positive cells were found in the ileum. From the later stage (at Day 11), Igs positive cells (IgA, IgG and IgM) were found distributed in the lamina propria, around the intestinal glands and in the core of the villi of ileum. Immunoglobulin positive cells (IgA, IgG and IgM) were increased gradually with increasing age and IgA positive cells were more than IgG and IgM positive cells at day 32.

Key words: Lymphoid tissue, mucosa, Immunoglobulin

INTRODUCTION

Poultry industry is the most rising sector in Bangladesh. In our country establishment of a poultry farm and in continuing its production using proper management, various diseases (such as viral, bacterial, mycoplasmal, protozoal and nutritional deficiency diseases) are the major constraint. Among them, some diseases affect the immune system and cause disorganization of the histological structure of the system concerned leading to poor production of meat and egg and even mortality rate of the chickens increased significantly. The chickens have been used as experimental animals for the studies of immune system (Khan *et al*., 1998), because the chicken is a useful model for research into the basic features of immunology. The mechanisms of adaptive immunity in chickens have two components: one is the bursa- dependent system, i.e. the cloacal bursa (of Fabricius) with germinal centers and plasma cells in various tissues; this is responsible for humoral immunity (HI). The other is the thymus-dependent system i.e. the thymus and scattered collections of lymphocytes that are related to cellular immunity (CMI). The cell mediated immunity (CMI) in the mucosal lining of the respiratory tract, gastrointestinal tract and urogenital tract playing a vital role in defending the chickens and animals from exogenous harmful antigen including bacteria, mycoplasma and viruses (Befus *et al*., 1980). The CMI is caused by the natural killer (NK) cells and T lymphocytes subsets, and the humoral immunity is carried out by plasma cells containing different classes of immunoglobulins (IgA, IgG and IgM). These cells are abundant in the lamina propria of the mucosal organs (gastrointestinal tract, urogenital tract and respiratory tract) of the chickens, where they are acting as an immunosurveillance (Janeway *et al*., 1988). These cells also move into the lumen through the micropore of the basal lamina of the tract concerned and kill the pathogenic antigens by their natural killing activities (Tschopp and Nabholz, 1990). The generation and activities of these immunocompetent cells (plasma cells containing IgA, IgG and IgM and T lymphocytes) against a particular disease in the chickens can be enhanced by vaccination program. Thus, to know the immune mechanism of vaccine, the present research was designed to understand the development and frequency of plasma cells containing different classes of immunoglobulins (IgA, IgG and IgM) in the lymphoid organs (bursa of Fabricius and cecal tonsils) and mucosal organ (ileum) of chickens in the different ages of postnatal development using indirect immunoperoxidase method

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MATERIALS AND METHODS

The experiment was carried out in the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

Chickens

A total 15 (fifteen) day-old "Cobb-500" broiler chickens of both sexes were purchased from "Kazi Farm Ltd." Mymensingh. The chickens had no developmental disorders and detectable diseases that may influence in the distribution of lymphocytes and plasma cells containing different classes of immunoglobulins (IgA, IgG and IgM) in lymphoid organs (bursa of Fabricius, cecal tonsils) and mucosal organ (ileum) of chickens in their postnatal development.

Management

The chickens were reared in litter system in the poultry shed of the Department of Medicine, Faculty of Veterinary Science, BAU, Mymensingh. Biosecurity of the poultry shed was maintained strictly. Optimum temperature, lighting and ventilation were maintained in the brooder. To avoid the stress of transport, vitamin-C with water was supplied on the first day. For the first day, the chickens were maintained on suji (coarse flour of wheat) which was then replaced with a commercial ready feed (Paragon Poultry Feed, Mymensingh) and at day 4, BCRDV vaccine was administered through intraocular route for the prevention of Ranikhat Disease.

Collection of sample

For immunohistochemical staining purpose, the bursa of Fabricius, cecal tonsils and ileum was collected after sacrificing the chickens through cervical subluxation method. Sampling was done every 7 days interval starting from day 3 up to 32 days of age. Samples were collected from three chickens on each occasion.

Preparation of samples for immunohistochemical studies

For immunohistochemical studies the samples were collected, cut into pieces and then fixed in the "Bouin's fluid" for 12 hours. The selected samples were dehydrated in a series of ascending grades of alcohol (70%, 80%, 95% and 100%), cleared in several changes of xylene, and infiltrated with different grades of melted paraffin in the oven. The tissues were then embedded in paraffin and finally the sections were cut at 6 µm thickness using sliding microtome (MIC 509, Euromex, Japan). After sectioning, the sections were floated on luke-warm water in a floatation bath at 37^0C for stretching then the sections were attached on cleaned glass slides using eggs albumin and dried on a hot plate of slide warmer boxes. The sections were then deparaffinized first in several changes of xylene followed by rehydration in a series of descending grades of alcohol (100%, 95%, 80% and 70%) and finally. The histological sections were stained using indirect immunoperoxidase method as described earlier (Khan *et al*., 1997) to understand the development and frequency of plasma cells containing different classes of immunoglobulins (IgA, IgG and IgM) in the lymphoid organs (bursa of Fabricius and cecal tonsils) and mucosal organ (ileum) of chickens in the different ages of postnatal development.

Antibodies

The antibodies for detecting Igs-positive cells used in this experiment were normal rabbit serum (Biosource, Camarillo, California, USA), goat anti-chicken IgA (Bethyl Lab, USA), goat anti-chicken IgG (Bethyl Lab, USA), goat anti-chicken IgM (Bethyl Lab, USA) and HRP-conjugated rabbit anti-goat IgG (Bethyl Lab, USA).

Immunohistochemistry

The tissues were fixed in Bouin's fluid and were embedded in paraffin according to the conventional method. Paraffin sections of 6 µm in thickness were immunostained by the indirect immunoperoxidase method. In brief, after endogenous peroxidase was inhibited with methanol and hydrogen peroxide (H_2O_2), the sections were overlayed with 2% normal rabbit serum (Biosource, Camarillo, California, USA) diluted with 0.01M phosphate buffered saline (PBS) for 2 hours, followed by incubation with goat anti-chicken IgG (1:1000) (Bethyl Lab. Inc. USA), goat anti-chicken IgA (1:1000) (Bethyl Lab. Inc. USA) or goat anti-chicken IgM (1:1000) (Bethyl Lab. Inc. USA) for 18h at 4^0C. After brief washing with phosphate buffered saline (PBS), sections were treated with 1% peroxidase-conjugated rabbit anti-goat IgG (1:1000) (Bethyl Lab. Inc. USA) for 1h at room temperature. The positive reactions for different classes of Igs were revealed by treating the sections with 0.2 mg 3, 3′-diamino-

benzidine (DAB)-tetrahydrochloride dehydrate (AppliChem, Darmstadt) per ml of Tris-hydrochloride (0.05 M, pH 7.6) containing 0.03% H_2O_2 and then counterstained few dips with hematoxylin.

Histoplanimetry

The immunopositive cells (Igs-positive cells) in the lymphoid tissues (bursa of Fabricius and cecal tonsils) and mucosa (ileum) of different ages of chickens were counted in 20 fields at a magnification of 40 according to Weibel (1969) and their relative frequency per 0.1 mm^2 was calculated using ocular micrometer.

Counting of Igs-containing plasma cells

The Igs-positive cells in the cecal tonsils and ileum were counted in the lamina propria and in the core of the villi, where the Igs-positive cells were uniformly distributed. Distributions of Igs-positive cells in the lymphoid follicles were omitted for counting purpose. Photographs from the selected specimens were prepared and placed in the paper for better illustration of the results (Fig. 1).

RESULTS AND DISCUSSION

The bursa of Fabricius, thymus and spleen are considered as major lymphoid organs of chickens, which were very small at hatching. Their size increase gradually with advancement of age and at about 15 weeks of age the size of lymphoid organs attained a peak (Khan *et al*., 1998). In the bursa of Fabricius, the Igs positive cells (IgA, IgG and IgM) were found principally beneath the capsule, around the follicles and in the cortex and also medulla.

Table1. The frequency of Igs positive cells in the bursa of Fabricius of postnatal development of different ages of broilers (mean±SD)

Igs	Days				
	Day 3	Day 11	Day 18	Day 25	Day 32
IgG	62±1.99	88.52±0.58	102.00±1.15	100.00±2.31	135.00±1.73
IgA	36±2.31	79.00±1.15 b	85.00±2.89	115.00±1.15	118.00±1.15
IgM	95±2.85	135.00±1.15	149.00±0.58	165.00±1.15	219.00±1.15

Table 2. The frequency of Igs positive cells in the cecal tonsils of postnatal development of different ages of broilers (mean±SD)

Igs	Days				
	Day 3	Day 11	Day 18	Day 25	Day 32
IgG	15±2.15	62.00±0.58	95.00±1.15	118.00±1.15	122.00±0.58
IgA	0.00±0.00	80.00±1.15	112.00±1.15	115.00±1.73	140.00±0.58
IgM	65±1.95	90.00±0.58	101.00±1.15	112.00±1.15	135.00±0.58

In all stages of development (from day 3 to day 32) IgM positive cells were more followed by IgG and IgA (Table 1). These findings were similar with the findings of Honjo and Hirota (1993) stated that most of the bursal lymphocytes were IgM positive, a small population of bursal cells were IgG positive and a few cells were IgA positive. These findings were also embryologically supported by Rose (1979) observed that shortly after stem cells first appear in the bursa (about the 12^{th} day of embryogenesis), they express surface IgM, and about the time of hating IgG followed by IgA. Seeding out of the immunocompetent B-lymphocytes to the peripheral lymphoid tissue occurs in the same sequence, i.e. IgM bearing cells, IgG bearing cells and IgA bearing cells.

Table 3. The frequency of Igs positive cells in the ileum of postnatal development of different ages of broilers (mean±SD)

Igs	Days				
	Day 3	Day 11	Day 18	Day 25	Day 32
IgG	0.00±0.00	55.00±2.89	62.00±0.58	70.00±1.15	105.00±1.15
IgA	0.00±0.00	65.00±1.73	134.00±1.15	140.00±0.58	147.00±1.15
IgM	0.00±0.00	70.00±2.89	75.00±2.89	94.00±0.58	125.00±2.89

In the cecal tonsils, Igs positive cells (IgA, IgG and IgM) were distributed within the lymphatic nodules, lamina propria and in the core of the villi, these findings were similar with the findings of Khan *et al.* (2008) and Islam *et al.* (2008). Early in the postnatal development (at day 3), no IgA positive cells were found in the cecal tonsils and IgM positive cells were more than IgG positive cells, these findings were with the findings of Jeurissen *et al*. (1989) stated that in 5 days-old chickens, cecal tonsils were microscopically visible and consisted of large HIS-C7 positive leukocyte infiltrates. The leukocytes included B-cells with membrane bound IgM, IgG or IgA and some IgM and IgG plasma cells. At day 32, IgA positive cells were more than IgG and IgM positive cells these findings were similar with the findings of Khan *et al*. (2008) and Islam *et al*. (2008). With the advancement of age, the Igs positive cells (IgA, IgG and IgM) were increased (Table 2), these findings were similar with the findings of Jeurissen *et al*. (1989) stated that in older chickens, the size of cecal tonsils and the number of IgM, IgG and IgA plasma cells gradually increased.

In the early postnatal development (at day 3) no immunoglobulin positive cells were found in the ileum. From The later stage, Igs positive cells (IgA, IgG and IgM)were found distributed in the lamina propria, around the Intestinal glands and in the core of the villi of ileum, these findings were similar with the findings of Khan *et al*. (2008). Immunoglobulin positive cells (IgA, IgG and IgM) were increased gradually with increasing age and IgA positive cells were more than IgG and IgM positive cells at day 32 (Table 3), these findings were similar with the findings of Islam *et al*. (2008).

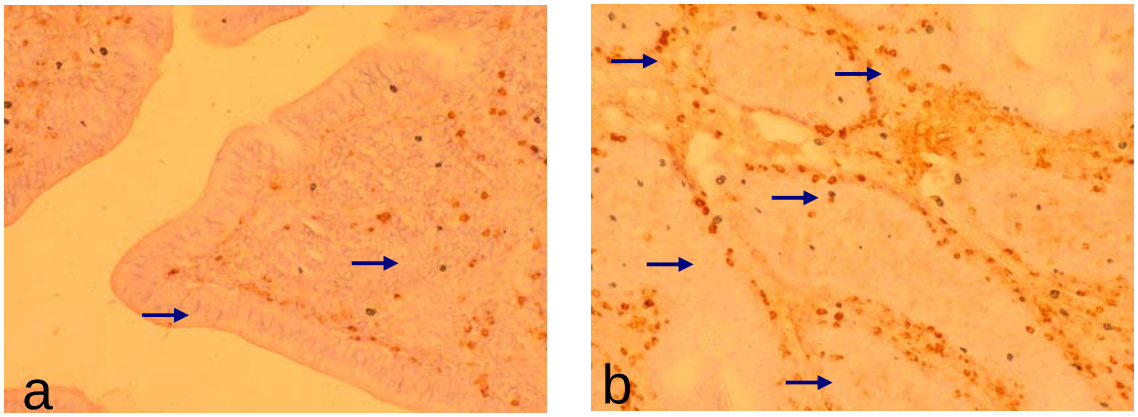

Fig. 1 (a-b). Immunostained sections from bursa of Fabricius in the chickens of 25 days old (a) and 32 days old (b) showing IgG positive cells (arrow) x200.

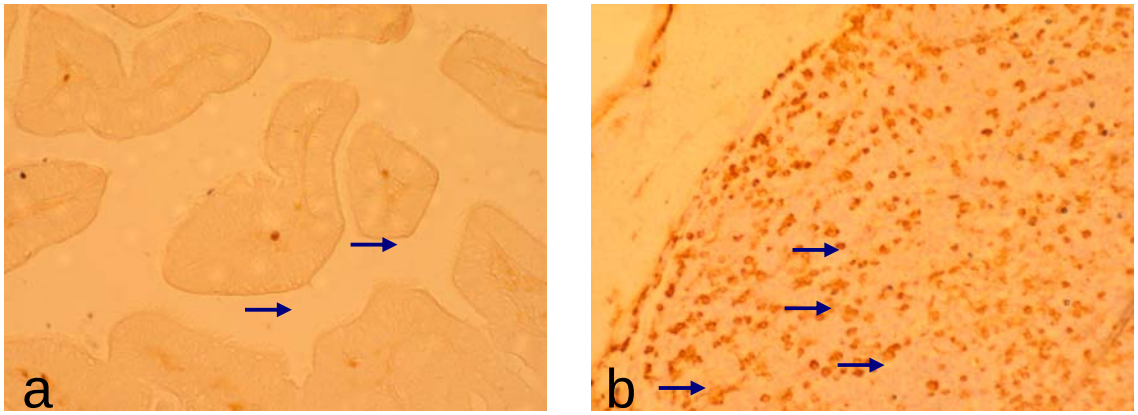

Fig. 2 (a-b). Immunostained sections from cecal tonsils in the chickens of 3 days old (a) and 32 days old (b) showing IgM positive cells (arrow) x200.

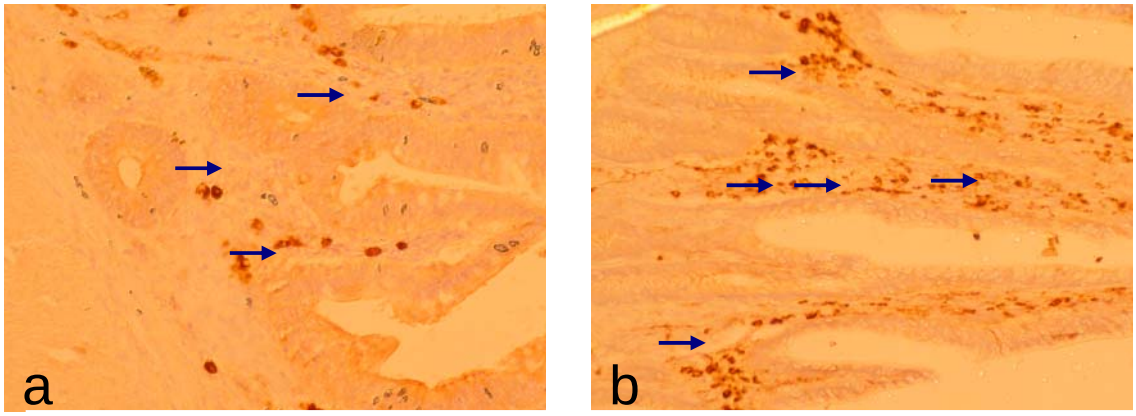

Fig. 3 (a-b). Immunostained sections from ileum in the chickens of 11 days old (a) and 32 days old (b) showing IgA positive cells (arrow) x200.

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