
RESEARCH NOTE**MORPHOLOGICAL AND HISTOLOGICAL FEATURES OF THE BURSA OF
FABRICIUS FROM NON-VACCINATED ONE MONTH OLD FEMALE
GAMEFOWL CHICKS FROM HYPERIMMUNIZED BREEDERS**

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ABSTRACT

The bursa of *Fabricius* of chickens plays an important role in early immunity, providing B lymphocytes that produce antibodies to neutralize pathogenic organisms, such as viruses and bacteria to protect the body from detrimental effects. This study aimed to determine the normal morphological and histological parameters of the bursa of *Fabricius* in a month old female gamefowl chicks coming from hyperimmunized breeders. The study utilized 25 gamefowl chicks from five different gamefowl farms. The results of the study across farms, show normal and uniformity of values for both morphology and histology; threshold values were obtained for each parameter and a strong positive linear relationship was observed between the weight of the chicken and the bursa of *Fabricius*. Immune and protective competency of the bursa started at four weeks in gamefowls. The results suggest that vaccination can be postponed up to four weeks old in case of gamefowls coming from hyperimmunized breeders.

Keywords: bursa of *Fabricius*, gamefowl, histology, immunization, morphology

INTRODUCTION

The bursa of *Fabricius* is a rudimentary organ that plays an immunological role during the early stages of life in poultry species (Riddell, 1996). It is a globular pear-shaped lymphoepithelial organ peculiar to birds, found in the dorsal wall of the terminal part of the cloaca; normal bursas are creamy white in color. The bursa is known to be mainly responsible for the development of humoral immunity, but few studies have been done on this organ in disease conditions (Tizard, 2009).

The mechanisms by which gamefowl chickens mount an immune response during the early stages of their life have been a common problem in the industry. The bursa of *Fabricius* was found to be the same across species used in the poultry industry. Broiler, layers, and gamefowls share similar characteristics, functions and structure of a normal bursa. In a study made by Kumar *et al.* (2014) the bursa of ducks were also found to be similar to the bursa of fowls. The bursa of *Fabricius* is an organ responsible for early immunity (humoral) in avian species (Tizard, 2009). These data lead to the hypothesis that there are no significant differences between the bursae of gamefowls, broilers and layers.

One important tool to assess and determine the flock health status is through bursal scoring; it is divided into two morphological and histological aspects. Bursal scoring is a reflection of the vaccination protocols done in a farm; efficient monitoring and evaluation of the morphological and histological aspects of the bursa of *Fabricius* can lead

to the improved health status of the flock, different vaccination protocols, assessment and management procedures. Most importantly, the intervention determines the extent of disease conditions caused by different pathogens.

This study, conducted on non-vaccinated female gamefowl chicks coming from hyperimmunized breeders, aimed to determine the normal histological and morphological features of the bursa of *Fabricius* at 4 weeks old. By examining and analyzing these parameters, a veterinarian can formulate and establish an effective, fast, reliable and inexpensive tool to diagnose a disease, a process that compares it with the normal values obtained from the study.

MATERIALS AND METHODS

Gamefowl chicks coming from five different gamefowl farms located in Rosario, Batangas; San Pablo, Laguna; and Lucban, Quezon were utilized in the study. A total of 25 samples were collected. Only gamefowl chicks at 4 weeks of age coming from hyperimmunized parent stock were used in the study. The following vaccination protocols were done on the breeders: live ND + HB (Hipraviar® S/H120 live vaccine, Newcastle disease La Sota strain, and infectious bronchitis H120 strain in oral freeze – dried tablet) and live IBD (Hipragumboro® CH80 Attenuated live intermediate Gumboro disease virus in oral freeze – dried tablet) were used as priming doses 1 week before administration of killed ND + IB + IBD (Bronipra® Inactivated infectious bronchitis virus, H52 strain: 2.4-16 SN; Inactivated Newcastle disease virus, La Sota strain: 1/16- 1/1024 HAI; Inactivated Gumboro disease virus, W2512 strain: 357-13500 ELISA. Oil based adjuvant). Live ND + IB vaccination were given intranasally at a dose of 0.03 ml per bird while live IBD vaccine was given orally also at a dose of 0.03 ml per bird. Killed ND + IB + IBD vaccine was given via deep intramuscular injection at a dose of 0.5 ml per bird.

The gamefowl chicks were not given any form of vaccination. They were weighed and physically examined for possible signs of disease, only healthy gamefowl chicks were used in the study. The chickens were sacrificed via atlanto-occipital dislocation and necropsy was conducted to check for any lesions. The bursa was then exteriorized at the dorsal aspect of cloaca, washed and measured. The length, width, bursa meter score, bursal index and weight were obtained. A bursa meter was used to determine the bursal score, a scale of 1 to 8, with 1 being the smallest and 8 being the largest; the smallest hole in which the bursa of *Fabricius* easily pass through indicates the bursal score. The bursal index was computed as:

$$(\text{Weight of the bursa} / \text{weight of the chicken}) \times 100 = \%$$

The collected samples were transferred to leak proof containers which contained 10% neutral buffered formalin solution and properly labelled. The samples were then sent to the Philippine Kidney Dialysis Foundation at Quezon City for histological processing and examination. Hematoxylin and eosin-stained samples were observed under the microscope and the different histological parameters were measured using the built in measuring application on the microscope. Pearson correlation, regression analysis, Wilk Shapiro test, Bartlett's test, Analysis of variance, and Kruskal Wallis test were used to analyze the relationships between the different morphological and histological values obtained.

RESULTS AND DISCUSSION

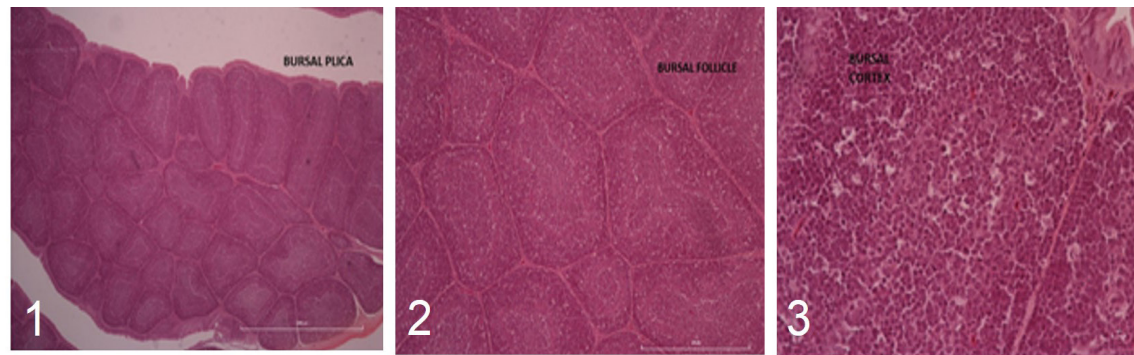
The morphological and histological structures present in the bursa of *Fabricius* of a month old non-vaccinated female gamefowl chickens showed no evidence of any pathological lesions or abnormality caused by any disease or other factors affecting the health status and the bursa of the sample population. The normal structures including the basic functional unit which is the bursal follicle of the bursa of *Fabricius* were also observed. All values obtained from both morphological and histological aspects showed normality and homogeneity across farms involved in the study

The bursa of *Fabricius* was globular in shape, and located on the dorsal aspect of the cloaca and opened into the proctodeum at its dorsal surface. The average measurements across farms were: bursal plica – 1.22 mm (range 1.10-1.33 mm); bursal follicle – 50 µm (range 45.53-54.48 µm); cortex – 8.89 µm (range 7.49-10.30 µm); and medulla – 20.51 µm (range 16.25-24.77 µm) at 95% confidence interval. The average bursal plica present in the study was 12, which is the normal number present at 4 weeks of age.

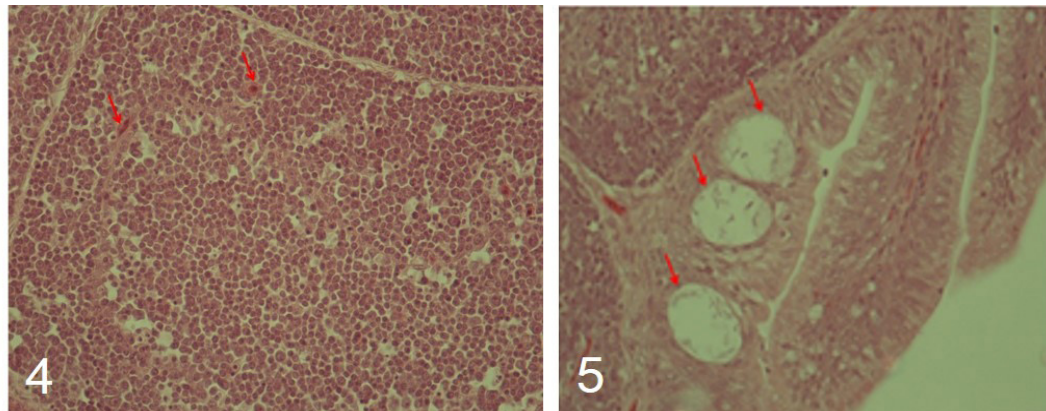
Morphologically, the color of the bursa was creamy white to pink. No lesions were observed in any of the samples. The average bursal index, which is the weight of the cloacal bursa in percent relative to the weight of the chicken obtained from the study was 0.42%, which is the same value obtained by Wolfe *et al.* (1962). The bursal score obtained across farms was 5, which is the average size based on the bursal meter scale. The average length of the bursa was 16.92 mm, average diameter was 12.31 mm and average weight was 1.32 g. In addition, there was a strong positive linear relationship between the weight of the chicken and the weight of the cloacal bursa at 4 weeks of age and statistical analysis showed that for every 1 g increase in the weight of the chicken there was a 0.006 gram increase in the weight of the bursa at 4 weeks of age. Statistical analysis showed that the weight of the chicken had a strong positive linear relationship with the weight of the bursa, peaking at 4 weeks of age with obtained value of 1.32 g and achieving its maximum weight of 4.25 g at 10 weeks of age (Wolfe *et al.*, 1962).

Histological examination revealed the following features. The wall of the bursa included a thin serosal layer, which is the outermost part of the organ, a muscularis layer which is formed by smooth muscle fibers and is composed of two longitudinal layers and an interposed circular layer. It also included the main branches of the blood vessels that supply the cloacal bursa, a mucosa which is divided into two components: connective tissue framework and the follicles. The mucosal layer was formed by 12 thick, vertical folds or plicae (Figure 1). Each plica consisted mainly of large numbers of polyhedral follicles closely packed together and separated by small amounts of connective tissue. The follicle (Figure 2), which is the basic functional unit of the cloacal bursa, was divided into two parts; namely, the cortex (Figure 3) and medulla separated by a thin basal membrane which was composed of well-defined capillary network.

The cortex which stained darker compared to the medulla was composed of closely packed small lymphocytes, lymphoblast and many mitotic figures. However, macrophages were not seen due to the abundance of lymphocytes and also these cells are only activated in an inflammatory process (Riddell, 1996). The blood supply was not well developed. The medulla, on the other hand, which stained lighter, was composed of lymphoblast found in greater concentrations in the periphery with medium and small lymphocytes. They were also seen and appeared to be almost devoid of blood capillaries (Figure 4). The 4th layer was the surface epithelium that lined the inner surfaces of the plicae. It was composed of tall, columnar, pseudo-stratified type of cells. There were three



Figures 1-3. 1) Bursal plica; 2) bursal follicle; and 3) bursal cortex H&E stain.



Figures 4-5. 4) Small blood vessels located in the cortex (arrows), the medulla is avascular; 5) well-defined type 3 goblet cells (arrows) on the surface epithelium (H&E stain).

definitive types of cells found in the surface epithelium: a) type 1 cell, an oval cell which contains clear cytoplasm and less common compared to the other cell types; b) type 2 cell, the most abundant which appears as columnar cells with oval nucleus; and c) type 3 cell, a goblet cell containing hyperchromatic nucleus (Figure 5) and is mainly responsible for the mucus secretion on the surface epithelium of the bursal plica. A well-defined characteristic of a young cloacal bursa is an epithelial tuft which is located between the medulla and the lumen and composed of undifferentiated epithelial cells.

Analysis and comparison of the morphological and histological structures in this study with a previous report involving diseased bursa (Matias, 1993) revealed that the values and parameters in this study do not indicate any pathogenic lesions and abnormalities. This is because the sample population in the study, even though they were not vaccinated with core vaccines, possessed maternally derived antibodies from hyperimmunized breeders

which are still protective against diseases, such as infectious bursal disease, Newcastle disease and Marek's disease. Mullins (2014) stated that maternally derived antibodies at 4 weeks of age are still above the threshold of protective immunity.

In the study done by Hussan *et al.* (2013), it was observed that the population of bursal lymphocytes and the follicular size were found to increase in vaccinated broiler chickens (IBDV) compared to control non-vaccinated chickens of the same age. Therefore, vaccination could lead to hypertrophy of the bursa of *Fabricius* that can affect the normal measurements of the bursa when assessing the flock health status and establishing normal range values, but can be very important in determining indirectly the protective capacity of the vaccine given to the flock. In addition, the number of bursal follicles located within the bursal plica is correlated with increasing maturity and development of the cloacal bursa. Therefore, as the animal grows, humoral immunity increases in relation to the production of B lymphocytes within the organ. These findings suggest that the present vaccination protocol for broiler and layers can also be adapted to gamefowls. In a study done by Kumar *et al.* (2014) it was found out that the most predominant age of functional competency of the bursa of *Fabricius* is at 4 weeks of age in case of ducks. However, the same findings histologically and morphologically, were noted to be similar with the values and structures in this study, suggesting that the bursa of *Fabricius* of gamefowls at 4 weeks of age is also functionally competent.

Comparison of the structures and function of the bursa of *Fabricius* of gamefowls to published literature of broilers and layers revealed that there are no observed significant difference between the structures, characteristics and function of the bursa of *Fabricius*. Also, this study supports a strong positive linear relationship between the weight of the chicken and the weight of the cloacal bursa during the early stages of development. As the animal grows and develops, the protective immunity increases due to an increase in bursal follicles within the bursal plica, which in turn produces B lymphocytes important in humoral immunity.

The above results suggest that the bursa of *Fabricius* of 4 week old gamefowl chicks coming from hyperimmunized breeders have immunity and are protected against diseases that cause detrimental effects on the health status of the chicken, since the bursa was found to be free from any pathological lesions and abnormality. Vaccinations, as early as 4 weeks of age, can be postponed until needed if gamefowls come from hyperimmunized breeders. In addition, vaccination protocols done on broilers and layers can also be adapted. The morphological and histological features obtained in this study can be used to assist in formulating a diagnostic protocol to assess flock health status, vaccination protocols, management procedures or a disease process for a specific farm.

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