Histopathological Changes of Bursa Fabricius of Imported Broilers and Local Chicks Vaccinated With Two Types of Infectious Bursal Disease Vaccines*

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Abstract

This study was designated to investigate the Histopathological changes of bursa of Fabricius (BF) in broilers and local chicks after vaccination with two types of Infectious Bursal Disease (IBD) vaccines. Eighty Hubbard broilers and one hundred local, one-day-old chicks were reared on litter floor for 35 days. They were divided into six groups, A, B and C for broilers, and D, E and F for local chicks. Chicks of group A, and D were vaccinated with an intermediate type vaccine (Bursine ® -2) at 14th day, whereas group B and E were vaccinated with an intermediate-plus vaccine (Bursine ® plus) at 14th day also (Fort Dodge Animal Health, Fort Dodge, Iowa, USA). Chicks of group C and F were acted as control. For histopathological examination of the bursa, five chicks were sacrificed from A, B, D and E groups at 21st, 28th and 35th day and at 1st, 7th, 14th, 28th and 35th day from C and F control groups. Histopathological lesions in this investigation were scored from 0 to 6 based on the presence of degenerative alterations, necrosis, follicular atrophy in the bursa of Fabricius and the percentage of altered lymphoid follicles. The present study revealed that the more pathogenic intermediate vaccine (intermediate- plus) caused severe bursa of Fabricius injury in vaccinated local chicks. This could be explained by the lower degree of attenuation of this kind of vaccine and may be also related to

* part of thesis of the first author
the lack of maternally-derived antibody in these chicks. It seems that there are no changes in the bursae of control groups.

Introduction

Infectious bursal disease is an acute, highly contagious disease of young chickens caused by a Birnavirus (1), and it is responsible for the major economic losses in the poultry industry (2). The bursa of Fabricius is a unique, primary lymphoid organ in avian species (3). Histologically, the wall of the BF is composed of tunica mucosa, tunica muscularis and tunica serosa (4). Tunica mucosa forms plicae towards the bursal lumen (5). The bursal follicles are the major sites of B-cell maturation; they develop from endothelial buds, and are made up of a cortex and medulla. The cortex contains lymphoblasts, lymphocytes, macrophages and plasma cells; the medulla also contains reticular cells (6).

Lymphocyte loss and alterations in the structure of BF may be observed when the chicken is infected with Infectious bursal disease virus (IBDV); nevertheless, it is very important to evaluate the stage and the extension of the injury. Some IBDV isolates cause intense loss of the bursal stroma and of the follicular microenvironment that sustains B cell differentiation, which explains the consequent severe immunodepression (7). Histopathological examination of the BF identifies the occurrence of IBD because very typical microscopic alterations are present after infection (8).

Lymphocyte necrosis is the most common histopathological lesion and is accompanied by edema, hyperemia, accumulation of heterophils and cystic cavities replaces lymphocytes in follicles and later there is some regeneration of lymphocytes (9). The intensity of microscopic alterations in the BF may also be quantified to evaluate the level of immune protection (10,11) or immune modulation of the infection (12). The aim of this study was to determine the effect of immune response on bursal histopathology after the vaccination with intermediate and intermediate plus IBD vaccines at 14 days of age in broiler and local chicks.

Materials and Methods

Eighty Hubbard broilers and one hundred local, one-day-old chicks were raised under control conditions in separated portions. The birds were supplied with feed and water ad libitum. The broiler chicks were divided into A, B and C groups, whereas the local birds were placed into group D, E and F. The chicks of group A, B, D and E were vaccinated with IBD vaccines as shown in Table 1 at 14th day, whereas chicks of group C and F were used as control. All groups were reared for thirty five days of age.

Two commercial vaccines available in the local market were used. They were administered according to the manufacturer's recommendations. The IBD vaccines used were: an intermediate vaccine (Bursine®-2) and an intermediate-plus (Bursine® Plus) of Fort Dodge Animal Health, Fort Dodge, Iowa, USA. Each vial of vaccines
contained 1,000 doses. Each bird received one dose of IBD vaccine in 0.5 ml distilled water, given intracrop using a syringe and blunted needle to ensure that all birds received the vaccine.
### Table 1: Experimental Design

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of birds</th>
<th>Breed &amp; Age</th>
<th>IBD Vaccine</th>
<th>Date of vac.</th>
<th>Histopathology of Bursa</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>25</td>
<td>Broiler</td>
<td>Intermediate</td>
<td>14th day</td>
<td>21st, 28th and 35th days</td>
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<tr>
<td></td>
<td></td>
<td>1 day old</td>
<td>(Bursine - 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>Broiler</td>
<td>Intermediate plus</td>
<td>14th day</td>
<td>21st, 28th and 35th days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 day old</td>
<td>(Bursine-plus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>Broiler</td>
<td>Control</td>
<td>-</td>
<td>1st, 7th, 14th, 21st, 28th &amp; 35th days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 day old</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>25</td>
<td>Local</td>
<td>Intermediate</td>
<td>14th day</td>
<td>21st, 28th and 35th days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 day old</td>
<td>(Bursine - 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>45</td>
<td>Local</td>
<td>Intermediate plus</td>
<td>14th day</td>
<td>21st, 28th and 35th days</td>
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<td></td>
<td></td>
<td>1 day old</td>
<td>(Bursine-plus)</td>
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<tr>
<td>F</td>
<td>30</td>
<td>Local</td>
<td>Control</td>
<td>-</td>
<td>1st, 7th, 14th, 21st, 28th &amp; 35th days</td>
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<tr>
<td></td>
<td></td>
<td>1 day old</td>
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For histopathology, five bursae were collected at 21\textsuperscript{st}, 28\textsuperscript{th}, and 35\textsuperscript{th} from each group of A, B, D and E. Five chicks were also taken from each control group C and F at 1\textsuperscript{st}, 7\textsuperscript{th}, 14\textsuperscript{th}, 21\textsuperscript{st}, 28\textsuperscript{th}, and 35\textsuperscript{th} day old for the same purpose. The bursal specimens were fixed in 10\% buffered formalin. Each piece of tissue was trimmed at 5 microns (\(\mu\)) in thickness, fixed and dehydrated in a series of increasing alcohol concentrations, and embedded in paraffin wax using rotary tissue processor. The sections were stained with Mayer's Hematoxylin and eosin (13), and studied under low and high power objective lenses of light microscope for histological changes (14). The histopathological changes were scored from 0 to 6 based on lesion characteristics in the lymphoid follicle, interstitial connective tissue and bursa of Fabricius follicles as described by (15), (16), (12) and (7).

Therefore, scores for the tissue alterations were 0 = no alteration; 1 = cells with pyknotic nuclei in the medullary region of the lymphoid follicles and discrete dissociation of the interstitial connective tissue, or lymphoid rarefaction in the medullary zone and/or roughening of the corticomedullary cord and/or central epithelization foci on the medullary region; 2 = hypotrophy of medullary and cortical regions of the lymphoid follicle due to the degeneration of lymphoepithelial cells, and/or lymphocytes and/or macrophage- or plasmocyte-type cell afflux, associated to a discrete dissociation and infiltration of inflammatory cells in the interstitial connective tissue. During the recovery phase, roughed epithelium and the presence of mitosis or sparse lymphoid cells in the medullary zone of the lymphoid follicles may be noticed; 3 = lymphoid depletion in the cortical and medullary zones and/or intense degeneration of lymphoepithelial cells in the lymphoid follicle, associated to a discrete edema and interstitial infiltration of inflammatory cells, besides roughening and/or discrete epithelial invagination and perivascular lymphoid hyperplasia at the septum of the conjunctive tissue; 4 = degeneration of cells from the medullary and cortical regions, with moderate infiltration of inflammatory cells in the lymphoid follicle, intersticium and epithelial layer, or lymphoid follicles depleted from lymphoid cells, with sparse lymphoepithelial cells, interstitial fibroplasia and many foci of epithelial invagination; 5 = medullary necrosis with cyst formation, intense granulocyte infiltration in the parenchyma and/or necrosis with hemorrhage in the intersticium or lymphoid follicles and/or lymphoid follicles with epithelial invagination or fibrosed and intense interstitial fibroplasia; 6 = absence of lymphoid follicles due to hemorrhage, necrosis, atrophy or fibrosis.

**Results and Discussion**

The histopathological examination of the bursa of Fabricius identifies the occurrence of IBD because very typical microscopic alterations are present after infection (Riddell, 1987). The intensity of microscopic alterations in the bursa of Fabricius may also be quantified to evaluate the level of immune protection (Abdel-
Alim and Saif, 2001) or immune modulation of the infection (Poonia and Charan, 2000). In the present study, the histological lesions in the bursa of Fabricius were scored from 0 to 5 based on the presence of degenerative alterations, necrosis, follicular atrophy and other types of alterations in the lymphoid follicles, these changes were presented in (Table 2) below:

**Table 2: Histological lesions score of Bursa of Fabricius in all experimental groups at different ages.**

<table>
<thead>
<tr>
<th>Age (day)</th>
<th>Experimental groups</th>
<th>No. of birds</th>
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<tbody>
<tr>
<td></td>
<td>Broiler</td>
<td>Local</td>
</tr>
<tr>
<td></td>
<td>A B C</td>
<td>D E F</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
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<tr>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>3 3 0</td>
<td>3 3 0</td>
</tr>
<tr>
<td>28</td>
<td>4 3 0</td>
<td>5 5 0</td>
</tr>
<tr>
<td>35</td>
<td>5 5 0</td>
<td>5 5 0</td>
</tr>
</tbody>
</table>

Histopathological scores showed that the intermediate (Bursine 2) and intermediate – plus (Bursine- plus) vaccinated broiler and local chicks (A, B, D and E respectively), exhibited similar degree of injuries at 21st day (Fig 1), whereas more noticeable lesions in the local chicks were noticed than in the other breed at 28th day (Fig 2). According to Cheville (1967), score 5 of bursal lesions was recorded in all vaccinated groups at 35th day, but they were more significant in the local chicks and more severe with intermediate- plus vaccine than the other (Fig 3). These findings suggest that the hot vaccines were able to cause severe bursal injuries. There were no bursa l lesions in group C and F (throughout the experiment (Fig 4).

These results were in disagreement with those of Bolis et al., (2003) who found that birds vaccinated at 14 days of age, with (intermediate-plus vaccines) didn't induce IBD-typical microscopic lesions in the bursa of Fabricius and with that of Moraes et al., (2004) who recorded that the intermediate vaccines were not different from the control (Fig 5).

The present study also revealed that intermediate- plus vaccine produced severe alterations than that of intermediate (Fig 6&7). Nishizawa et al., (2007) indicated that based on lesions distribution and scores at 17 and 21 days of age, there
was no significant differences in the BF of birds which were vaccinated with intermediate or intermediate-plus and those of non-vaccinated, whereas three hot vaccine strains which were tested by the same author were found to produce slightly atrophic bursa with moderate microscopic lesions.

Davelaar et al., (2007) reported that the lesion score after vaccination is very low and at an acceptable level for a vaccine, whereas a very high score is found in unvaccinated birds after challenge. The bursa of vaccinated birds again showed to be unaffected by the challenge virus.

On the other hand, many authors supported the findings of the present study, Alloui, (2005) found that broiler chicks vaccinated against IBD with an Intermediate-plus vaccine had lesions score more than the half (53%) of the examined organs which were characterized by atrophy and lymphocytes depletion. In the more serious cases, a follicular lymphoid necrosis and interfollicular interstitial fibrosis had been noticed in this study (Fig 8).

The results of the present study were in agreement with those of Abdul Ahad, (2002) who evaluated the degree of bursal damage produced by the local Bangladeshi isolate in the local cross breed which was characterized by severe depletion of lymphocytes leaving many small vacuoles, pyknotic nuclei, severe edema was also demonstrated in the interfollicular septa, cystic cavities developed in the medullary areas of the follicles and loss of demarcation between the follicular septa (Fig 9).

The same results were reported by Moraes et al., (2004) who mentioned that the strong vaccines induced more pronounced lesions than the other tested vaccines and the more pathogenic intermediate vaccines as well as very virulent vaccines caused severe BF injury in vaccinated birds, resulting in lymphocytes depletion of approximately 90%.

Ezeokoli et al., (1990) evaluated the histopathological modifications of BF associated with poultry vaccination against IBDV, describing severe lesions in the bursa between three and seven days after vaccination. The results of the study were also in agreement with those of Rautenschlein et al., (2005) who reported that commercial broilers vaccinated with IBDV vaccines of different virulence (intermediate) and (intermediate plus) showed variable severity in the lesions. The intermediate vaccine expressed only mild lesions (score 1) while the severity of the lesions of the intermediate-plus vaccine was of score 4.

Further more, the results of the present study were in agreement with those of Mazariegos et al., (1990) who assessed the effects of intermediate vaccine samples and reported that the lesion scores ranging between 1.4 in controls, and up to 4 in the vaccinated birds. Therefore, it was considered that the birds were protected until a histopathological score of 3. Similar findings were obtained by Hair-Bejo et al., (2004) who determined the efficacy of an intermediate strain of live attenuated IBD
vaccine in broiler chickens at 1 day old and 14 respectively, and found that bursa lesions scoring of the control group remained normal to mild throughout the experiment, while the lesions ranged from mild to intermediate from day 21 to day 28 in the vaccinated birds.

Scanavini Neto et al., (2004) reported that the histopathologic examination of BF of broiler chicks vaccinated with intermediate strain of IBD vaccine at one, seven and fourteen days, revealed extensive lymphoid follicles necrosis and heavy plasma and inflammatory cells exudation, vaccinated birds exhibited an increasing number of T- lymphocytes in the bursa interfollicular tissue and cortex.

Al-Sereah, (2007) observed that chickens vaccinated with intermediate vaccine( Cevac) at 14th day showed more severe lesions such as hyperemia, dilatation of blood vessels and secondary follicular proliferation in the bursa of Fabricius 3 days post vaccination.

In this study, the histopathological lesion scores of the vaccinated birds of different groups were mentioned. The distribution of the observed lesions is variable within the different ages postvaccination. They were characterized by atrophy and lymphocytes depletion. In the more severe lesions; follicular lymphoid necrosis, an interfollicular interstitial fibrosis and degeneration of the coating epithelium were noted. The severity of lesions were caused by intermediate-plus vaccine. Bursal lesion scores were surprisingly high in most sections but moderate one was recorded at 21st day. Depending on the virulence of the live attenuated viruses, some vaccine strains can cause bursal damage (Mazariegos et al., 1990) and lead to immunosuppression in the vaccinated birds (Edward et al., 1982; Reece et al., 1982). The highest bursal lesion scores with cyst formation (Tsukamoto et al., 1995), lymphocytic depletion with inflammation (Mazariegos et al., 1990), acute necrosis (Rautenschlein et al., 2001), follicular atrophy (Franciosini and Coletti, 2001), extensive bursal damage with follicular repopulation (Rautenschlein et al., 2001) and increased interstitial connective tissue proliferation (Franciosini and Coletti, 2001) were produced by intermediate-plus vaccine and less with intermediate type. The histopathological features and remarkably high score of bursal lesions in this study would evaluate the virus as undoubtedly pathogenic virus vaccine. However, the pathogenicity of virus vaccines were not yet determined in a separate experiment and further experiment to evaluate it can be conducted.
Fig. 1: Bursa of Fabricius of bird in group B at 21\textsuperscript{st} day (x 100) (H and E) showed lymphocytes depletion in the follicles and an increase in the thickness of septae of interfollicular tissue.

Fig. 2: Bursa of Fabricius of bird in group A at 28\textsuperscript{th} day (x 400) (H and E) showed fatty degeneration in the bursal follicle.
Fig. 3: Bursa of Fabricius of bird in group A at 35th day (x 400) (H and E) showed an edema between bursal follicles.

Fig. 4: Normal bursa of Fabricius of bird in group C at 1st day (x 40) (H and E)
Fig. 5: Bursa of Fabricius of bird in group B at 35th day (x 100) (H and E) showed necrotic and degenerative changes.

Fig. 6: Bursa of Fabricius of bird in group D at 28th day (x 100) (H and E) showed an interfollicular edematous area and lymphatic depletion
Fig. 7: Bursa of Fabricius of bird in group D at 35th day (x 400) (H and E) showed an area of necrosis in the bursal follicle.

Fig. 8: Bursa of Fabricius of bird in group E at 35th day (x 100) (H and E) showed vacuolation and cyst-like structures due to lymphoid depletion.
Fig. 9: Bursa of Fabricius of bird in group E at 35th day (x 100) (H and E) showed an increase in the thickness of capsule.

References


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دراسة التغيرات النسيجية لجراب فابريشيا في أفراخ فروج اللحم والأفراخ المحلية بعد تلقيحها بثنين من لقاحات التهاب جراب فابريشيا المعدي

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فرع الأمراض وأمراض الدواجن / كلية الطب البيطري/ جامعة البصرة / العراق

الخلاصة
صمم هذا البحث لدراسة التغيرات النسيجية المرضية لجراب فابريشيا في أفراخ فروج اللحم والدجاج المحلي بعد التلقيح بنانين من لقاحات التهاب جراب فابريشيا المعدي. استخدم لغرض أنجاز البحث ثمانون من أفراخ فروج اللحم نوع هابرد ومانية من الأفراخ المحلية بعمر يوم واحد. تم تربية الأفراخ لمدة 35 يوما. قسمت الأفراخ (F و E, D) إلى ستة مجاعيم (B, A وC) للأفراخ المحلية. لقحيت أفراخ المجموعات (D و A) للفروج اللحم و (F و E, D) للافراخ المحلية. لقحيت أفراخ المجموعات (C و B, A) للفروج اللحم و (F و D) للفروج المحلية. تم قتل 5 أفراخ من كل مجموعه للأيام (21, 28 و35) بعد التلقيح، أما مجاعيم السيطرة فقد تم قتل 5 طيور منها أبتداء من الأسبوع الأول إلى السادس وتمت إزالة جراب فابريشيا لغرض الفحص النسيجي. الفحص النسيجي المرضي لمقطع جراب فابريشيا صنف إلى ست درجات اعتمادا على وجود مناطق التنخس والتكتس والضمور والتغيرات المرضية الأخرى للجريبات الملاوية. أظهرت النتائج بأن اللقاح العالي الضراوة كان أكثر تأثيراً من اللقاح المتوسط في إحداث درجات عالية من التغيرات النسيجية وخاصة في أفراخ الدجاج المحلي في حين لم يلاحظ أي من تلك التغيرات المرضية في مجاعيم السيطرة. الآفات النسيجية الشديدة التي ظهرت ربما تعزى إلى قلة تضعيف اللقاحات عالية الضراوة أو ربما بسبب فقدان المناعة الأمية في أفراخ الدجاج المحلي.

جزء من رسالة الماجستير للباحث الأول.