Effect of live infectious bursal disease vaccines on bursa of Fabricius

Pooja Kundu, Gulshan Narang, Sushma Kajal, Pravesh Kumari and Babu Lal Jangir

Abstract
An experiment was planned to study the effect of live infectious bursal disease vaccines in broiler chickens. One hundred and fifty day old broiler chicks were reared for 45 days and divided into A, B1, B2, C1, C2 group. Chicks of group A were acted as control. Chicks of group B1, B2 were vaccinated with commercially available intermediate plus vaccine P while in chicks of group C1, C2 vaccine Q was used. Chicks were vaccinated on 17 days of age as decided by maternal antibody titre. Booster vaccination was done in group B2 and C2 with respective vaccines P and Q. Bursal index and histopathological changes were studied in Bursa of Fabricius on 1, 7, 14 days of age and 7, 14, 21, 28 days post vaccination (DPV). Histopathology showed lymphoid depletion, medullary necrosis with cyst formation, fibrous connective tissue proliferation in vaccinated groups which was initiated earlier with vaccine Q. Bursal index was comparable with control in case of birds vaccinated with vaccine P at 7 DPV only. However, it was reduced significantly in birds vaccinated with vaccine Q at 7 DPV itself. Later the bursal index was significantly lower in all the vaccinated groups vaccinated with either of the vaccine at 14, 21, 28 DPV. The present study revealed that vaccine Q seemed to be little more invasive as compared to vaccine P as it induces the immunosuppressive changes in bursa at 7 DPV itself.

Keywords: Bursal index, infectious bursal disease, vaccines, histopathology

Introduction
Infectious bursal disease (IBD) or Gumboro disease is an acute, highly contagious viral infection of poultry caused by infectious bursal disease virus (IBDV) which is classified in the Avibirnavirus genus of Birnaviridae family.[3,14] IBDV primarily targets the lymphoid tissues and actively replicates in B-lymphocytes of bursa of Fabricius. The disease causes heavy economic losses in poultry industries due to immunosuppression in subclinical cases[15] and in acute cases; it is associated with mortality, haemorrhages and also bursal damage.[5] IBDV is very stable in the environment and once infected with IBDV, chickens are capable of shedding the virus in faeces for as long as 16 days.[15] Chickens are prevented from infection by using live vaccines mainly. The emergence of variant or newer strains of the virus in the recent times has also been reported to cause vaccination failures.[10] The present study was used to check commercially available live infectious bursal disease vaccines effect on bursal histopathology in broiler chickens.

Materials and Methods
One hundred and fifty, day old broiler chicks were procured from a local hatchery. Commercially available live vaccines named P and Q belonging to intermediate plus strain of IBDV were used in this study. Optimum day of vaccination was 17th day of age on the basis of maternal antibody titre of the chicks procured. Booster vaccination was done on 24th day of age. The birds were grouped into five treatment sub-groups as shown in the Table 1. Birds in group A were used as control. Birds in group B1 and B2 were immunized with recommended doses of intermediate plus vaccine P at 17th day of age. The birds of group B2 were given booster dose of vaccine P at 24th day of age. Similarly birds of group C1 and C2 were immunized with recommended doses of intermediate plus vaccine Q at 17th day of age. The birds in group C2 were given booster dose of vaccine Q at 24th day of age. Vaccines were administered via the recommended intraocular route. Bursal index was calculated on 1, 7, 14 days of age in group A and later at 7, 14, 21 and 28 DPV (Days Post Vaccination) in all the groups. Ten birds from group A on 1, 7, 14 days of age and six birds from each group were taken on 7, 14, 21, 28 DPV. Birds were weighed before sacrifice and bursa weight was taken...
after sacrificing the bird for calculating bursal index. Bursal index \(^{(1)}\) of each bird was calculated at each sampling as follows:

\[
\text{Bursal index} = \frac{\text{weight of bursa}}{\text{body weight}} \times 1000
\]

After sacrificing the birds at each interval the representative samples of bursa were collected in 10% buffered formalin for histopathological studies. Section were cut at 4 \(\mu\) thickness and stained with haematoxylin and eosin stain \(^{(9)}\) for histopathological studies.

### Results and Discussion

Bursal index reflects the relative changes in weight of bursa of Fabricius with respect to body weight. Mean bursal index in different groups is presented in Table 2. Bursal index in day old chicks was 1.56, increased to a level of 1.95 at 7 day of age and 2.54 at 14 day of age in control group A. Thereafter it showed declining trend from 24 day of age onwards and reached up to a level 1.34 at 45 day of age. Bursal index in birds vaccinated with vaccine P (group B1, B2) was comparable with the control group A at 7 DPV. However it was reduced significantly in birds vaccinated with vaccine Q (group C1 and C2) at 7 DPV itself. Later the bursal index was significantly lower in all the vaccinated groups vaccinated with either of the vaccine at 14, 21 and 28 DPV. Decrease in weight of bursa of Fabricius without any clinical signs due to IBD vaccine was observed in another study which supports the result \(^{(12)}\). In earlier study it was reported that reduction in bursal index was more by intermediate vaccines as compared to mild vaccines \(^{(7, 8)}\).

Decrease in bursal index in IBD vaccinated birds correlated with the sequential gross and microscopic changes observed in bursa. Bursal atrophy was comparatively higher in group C1 as compared to birds of group B1 at 7 DPV (Fig. 1a). Bursa of Fabricius was smaller in size in IBD vaccinated groups as compared to control group at 28 DPV (Fig. 1b). Histopathologically mild depletion was observed at 7 DPV (Fig. 3, 4) in vaccinated groups as compare to group A (Fig. 2) followed by moderate to severe depletion and formation of cystic cavities at 14, 21 and 28 DPV. Cystic cavities were observed at 7 DPV itself in group C1 and C2 vaccinated with vaccine Q however cystic cavities were observed at 28 DPV in group B1 and B2 vaccinated with vaccine P (Fig. 5, 6, 7 and 8). These early histopathological changes correlates with lower bursal index observed in group C1 and C2 birds at 7 DPV as compared to group B1, B2 and A. Lymphoid depletion along with other bursal damages had also been supported by a study on IBD virus inoculation \(^{(2)}\) and by Kumar \(^{(1)}\) on IBD vaccination with intermediate plus vaccine. The effect of IBD vaccine and levamisole on bursal index in HPS infected chicks revealed significant reduction in the bursal index due to IBD vaccination indicating immunosuppression. Levamisole was able to significantly improve the bursal index in uninfected and IBD vaccinated birds \(^{(11)}\).

### Table 1: Experimental design

<table>
<thead>
<tr>
<th>Group</th>
<th>Immunogen</th>
<th>Sub group</th>
<th>Immunization (days of age)</th>
<th>Bursal index and histopathology (days of age/DPV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=60)</td>
<td>Vaccine P</td>
<td>A</td>
<td>Day-17</td>
<td>1, 7, 14 day of age</td>
</tr>
<tr>
<td>B (n=45)</td>
<td>Vaccine P</td>
<td>B-1 (25)</td>
<td>Day-17</td>
<td>7, 14, 21, 28 DPV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B-2 (20)</td>
<td>Day-17</td>
<td>14, 21, 28 DPV (first vaccine)</td>
</tr>
<tr>
<td>C (n=45)</td>
<td>Vaccine Q</td>
<td>C-1 (25)</td>
<td>Day-17</td>
<td>7, 14, 21, 28 DPV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-2 (20)</td>
<td>Day-17</td>
<td>14, 21, 28 DPV (first vaccine)</td>
</tr>
</tbody>
</table>

### Table 2: Mean bursal index in single and double IBD vaccinated broiler chickens vaccinated with Intermediate Plus Vaccine P and Q

<table>
<thead>
<tr>
<th>Days</th>
<th>Day Post Vaccination</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPV</td>
<td>Control</td>
<td>B1</td>
<td>B2 (booster)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>1.56±.42(^{a})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>1.95±.33(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>2.54±.41(^{c})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>7</td>
<td>2.41±.87(^{a})</td>
<td>2.78±.43(^{a})</td>
<td>2.78±.43(^{a})</td>
</tr>
<tr>
<td>31</td>
<td>14</td>
<td>1.71±.56(^{a})</td>
<td>.88±.29(^{b})</td>
<td>.69±.29(^{b})</td>
</tr>
<tr>
<td>38</td>
<td>21</td>
<td>1.31±.19(^{a})</td>
<td>.54±.13(^{b})</td>
<td>.73±.35(^{b})</td>
</tr>
<tr>
<td>45</td>
<td>28</td>
<td>1.34±.09(^{a})</td>
<td>.57±.32(^{b})</td>
<td>.67±.24(^{b})</td>
</tr>
</tbody>
</table>

- Means with different superscripts are significantly different \((p<0.05)\) within same row.
- Means of control group A at 1, 7 and 14 day age are significantly different \((p<0.05)\).

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\[^{1}\] Statistical weight. Both the vaccines P and Q. 1a). % buffered formalin for × ore invasive as compared to vaccine P.ination indicating
Histopathologically mild depletion was observed at 7 DPV as compared to control group at 28 DPV (Fig. 1b). C1 as compared to birds of group B1 at 7 DPV (Fig. 1a). Bursa of Fabricius was smaller in size in IBD vaccinated groups as compared to control group at 28 DPV (Fig. 1b). Histopathologically mild depletion was observed at 7 DPV as compared to group A at 1, 7 and 14 day age are significantly different \((p<0.05)\). These early histopathological changes correlates with lower bursal index observed in group C1 and C2 birds at 7 DPV as compared to group B1, B2 and A. Lymphoid depletion along with other bursal damages had also been supported by a study on IBD virus inoculation \(^{(2)}\) and by Kumar \(^{(1)}\) on IBD vaccination with intermediate plus vaccine. The effect of IBD vaccine and levamisole on bursal index in HPS infected chicks revealed significant reduction in the bursal index due to IBD vaccination indicating immunosuppression. Levamisole was able to significantly improve the bursal index in uninfected and IBD vaccinated birds \(^{(11)}\). Immunosuppressive effect of vaccination on immune system of broiler chickens having maternal antibody against IBD virus was also studied. Histopathological studies of lymphoid organs indicated that the vaccines induced bursal damage after vaccination \(^{(6, 13)}\). Both the vaccines P and Q gave the protective immune response. However, the vaccine Q seemed to be little more invasive as compared to vaccine P as it induces the immunosuppressive changes in bursa at 7 DPV itself.

### Statistical analysis

One-way analysis of variance (ANOVA) test, followed by Duncan post hoc test was used to determine the statistically significant differences in mean values of bursal index between the experimental groups. Alpha was set at 95%. Statistical software SPSS™ 20.0 (IBM, Corp. USA) was used.

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The Pharma Innovation Journal

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~ 92 ~
**Fig 1**: Gross lesions in bursa of Fabricius
(a)- Bursa of group B1 and C 1 at 7 DPV
(b)- Bursa of group B1 and A at 28 DPV

**Fig 2**: Normal bursa of Fabricius of bird in group A at 14 day age (x10) (H and E)

**Fig 3**: Bursa of Fabricius of bird from group C1 at 7 DPV/24 day age (x20) (H and E) showing lymphoid depletion in follicles

**Fig 4**: Bursa of Fabricius of bird from group C1 at 7 DPV/24 day age (x10) (H and E) showing medullary cyst cavities

**Fig 5**: Bursa of Fabricius of bird from group B1 at 28 DPV/45 day age (x20) (H and E) showing lymphoid depletion in follicles

**Fig 6**: Bursa of Fabricius of bird from group B1 at 28 DPV/45 day age (x10) (H and E) showing lymphoid depletion in follicles and fibrous connective tissue proliferation

**Fig 7**: Bursa of Fabricius of bird from group B1 at 28 DPV/45 day age (x20) (H and E) showing medullary cyst cavities
Fig 8: Bursa of Fabricius of bird from group C1 at 28 DPV/45 day age (x20) (H and E) showing lymphoid depletion in follicles and medullary cystic cavities

Conclusion
Bursal index was comparable with control in case of birds vaccinated with vaccine P at 7 DPV only. However, it was reduced significantly in birds vaccinated with vaccine Q at 7 DPV itself. Later the bursal index was significantly lower in all the vaccinated groups vaccinated with either of the vaccine at 14, 21, 28 DPV. Histopathology showed lymphoid depletion, medullary necrosis with cyst formation, fibrous connective tissue proliferation in vaccinated groups which was initiated earlier with vaccine Q. From the present study, it can be concluded that commercially available live infectious bursal disease vaccine Q seems to be little more invasive as compared to vaccine P because the bursal changes developed earlier and were more severe with vaccine Q. However, further studies may be conducted to strengthen the observations.

References