Immunosuppressive effects of an infectious bursal disease-immune complex vaccine in broilers

A. SADRZADEH, S. M. PEIGHAMBARI* and B. SHOJADOOST

Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, P.O.Box: 14155-6453, Iran.
Corresponding author: mpeigham@ut.ac.ir

This study was conducted to test the immunosuppressive effects of an infectious bursal disease virus (IBDV) vaccine administered in ovo or to day-old chicks. Three hundred embryonated eggs from a 30-weeks old broiler breeder flock were incubated in a hatchery. At embryonation day 18 in the hatchery, 120 embryonated eggs were injected with the IBDV-Icx vaccine through the eggshell. At day 1, 60 chicks received IBDV-Icx vaccine subcutaneously. All 300 day-old chicks were transferred to the isolation facility, divided into 5 groups of 60 birds, settled in separated rooms, and kept under identical conditions. The IBD vaccinated groups were immunized against Newcastle Disease (ND) with an entrotropic apathogenic (at day 1) or B1 (day 7) and LaSota (day 18) strains. One group received only B1 and LaSota vaccine strains and one group, as control, did not receive any vaccine. At 31 days of age, birds in all groups were challenged with a virulent ND virus strain (HERTZ33). Prior to and after challenge, birds were bled at weekly intervals for antibody detection. At 45 days of age, all birds were euthanatized and necropsied. The geometric mean of the NDV-HI titres of birds in 5 groups were not differed significantly from each other at 1 and 7 days of age. The mean titres of two in ovo-vaccinated groups were differed significantly at days 14, 21, 38 and 45 days of age. The mean titres of birds in groups vaccinated with B1 and LaSota, with and without prior vaccination with IBD-Icx vaccine, were not significantly different from each other at weekly intervals till the end of the experiment. The frequency of mortality was significantly higher in unvaccinated group. There were no significant differences in mortality rate among groups vaccinated by IBDV-Icx vaccine. Birds in group 4, however, which were immunized by NDV vaccines only, suffered the lower mortality when compared with birds in groups received IBD vaccine prior to ND vaccination, although the difference was not significant. This study demonstrated that in ovo or day-old immunization of broiler chicks against IBD with an infectious bursal disease-immune complex vaccine did not have any significant immunosuppressive effects on broilers.

Keywords: broiler; Infectious bursal disease; in ovo vaccine; immunosuppression; Newcastle disease

Introduction

Infectious bursal disease (IBD) is a highly contagious disease of young chickens (Saif et al., 2003). Chickens are most susceptible to clinical infection from 3 to 6 weeks of age. Lymphoid tissue, in particular bursa of Fabricius, is the primary target of IBD virus (IBDV). Subclinical infection may occur in chickens less than 2 weeks of age leading usually to immunosuppression (Saif, 1991; Sharma et al., 1994; Sharma et al., 2000). Because the early IBD infection decreases responses to vaccines and increases susceptibility to other infections in young chickens, vaccines able to protect the chicks as early in life as possible are desired. The intermediate plus IBD vaccines developed to control very virulent IBD virus strains. These vaccines combined with homologous antibody (IBDV-immune complex [Icx]) vaccine have been successfully used to immunize 18-day-old embryos via in ovo vaccination or day-old chicks via subcutaneous route (Kelemen et al. 2000; Corley et al., 2001). However, these high virulent vaccine strains may have immunosuppressive effects in chickens.
Information on the virulence and immunosuppressive effects of IBDV-Icx vaccines is very limited. This study was designed to evaluate the immunosuppressive effects of an IBDV-Icx vaccine in broilers based on HI serum antibody and its protective effect against challenge with a virulent Newcastle disease virus strain.

Materials and methods

**Vaccine and challenge viruses:** In this study we used an IBDV-Icx vaccine (Transmune, CEVA, France), and three live Newcastle vaccine strains including entrotropic apathogenic Phy.LMV.42 strain (Vitapest, CEVA, France), B1 and LaSota (Serum and Vaccine Razi Institute, Iran). A virulent strain of NDV, HERTZ33, was used for the challenge (Saif et al., 2003).

**Experimental design:** Three hundred embryonated eggs (Ross 308) were provided from a 30-weeks old broiler breeder flock that had high maternal antibody against IBD, and incubated in a modern hatchery. At embryonation day (ED) 18 in the hatchery, 120 embryonated eggs were injected with the IBDV-Icx vaccine through the eggshell. A commercial automated egg injection system, the INOVOJECT® (EMBREX, US), was used to inoculate the eggs. After hatch, the percent of hatchability among vaccinated and non vaccinated chicks was recorded. All 300 day-old chicks were transferred to the faculty of veterinary medicine, weighed, divided into 5 groups of 60 birds, settled in separated rooms, and kept under identical conditions. The birds were observed for any mortality and disease problems and were provided feed and water ad libitum during the study. Groups 1 and 2 were consisted of chicks which had received the in ovo vaccine at ED 18. Chicks in group 3 received IBDV-Icx vaccine at day 1 subcutaneously. Chicks in group 1 received entrotropic apathogenic NDV strain (eye drop at day 1 of age and chicks in groups 2, 3, and 4 received B1 (eye drop at day 7) and LaSota NDV strain (in drinking water at day 18 of age). Birds in group 5 did not receive any vaccines and served as controls. At 31 days of age, 50 randomly selected birds from each group were challenged with the virulent NDV HERTZ33 strain. Each bird received a dose of $10^6$ ELD$_{50}$ viruses via intramuscular (IM) route. All birds in different groups were kept under tight control for 14 days post-challenge (PC) and were monitored for the observation of the clinical signs (especially nervous signs), mortality and development of typical lesions of ND among dead birds. At 14 days PC (45 days of age), all birds were euthanatized and necropsied for gross lesion observations. At days 1, 7, 14, 21, 28 (prior to challenge), 38, and 45 of age (after challenge), 20 birds from each group were weighed and bled. Serum samples were prepared to determine the serum HI antibody titre to the NDV (Mazariegos et al., 1990).

**Statistical analysis:** Average weight gains and serum HI antibody titre among birds in 5 groups present in this study were compared using one-way ANOVA and Tukey’s test ($P \leq 0.05$) by SPSS program (Version 11.0). For differences in mortality between each two treatment groups, data were subjected to chi-square analysis.

Results and discussion

This study demonstrated that in ovo or day-old immunization of broiler chicks against IBD with an infectious bursal disease-immune complex vaccine does not have any significant immunosuppressive effects on broilers. Prior to challenge, the comparison of the mean NDV-HI titres among groups showed that in the most cases there were no significant differences among groups that received both IBD and NDV vaccines or NDV vaccine alone (Table 1). After challenge, there were increases in the mean titres of all vaccinated groups. The geometric mean of the NDV-HI titres of birds in 5 groups were not differed significantly from each other at 1 and 7 days of age. In group 1, the geometric mean of the HI titres of birds was significantly higher than those of birds in other groups at 14 days of age. The mean titres for non-vaccinated birds (group 5) were significantly lower than those of vaccinated birds in groups 1-4 at 21 and 28 days of age (there were no live birds in group 5 at 38 and 45 days of age to make such comparison). Two in ovo-vaccinated groups (1 and 2) were differed significantly at days 14, 21, 38 and 45 days of age. The mean titres of group 1, which received the entrotropic
Vaccination schedule of each apathogenic NDV strain, were significantly lower than those of groups 2 and 4. Whether this difference may be an indication of a lower efficacy of a single dose vaccination by the entrotropic apathogenic NDV vaccine strain needs to be more elucidated. The mean titres of birds in groups 2, 3 and 4 were not significantly different from each other at weekly intervals till the end of the experiment. All birds in unvaccinated group (5) died 14 days PC.

The frequency of mortality was significantly higher in unvaccinated group that is an indication of values of vaccination program against NDV challenge (Table 2). High frequency of deaths occurs in non-immunized birds after challenge with a virulent strain of NDV (Saif et al., 2003). There were no significant differences in mortality rate among groups vaccinated by IBDV-Icx vaccine. Birds in group 4, however, which were immunized by NDV vaccines only, suffered the lower mortality when compared with birds in groups received IBD vaccine prior to ND vaccination, although the difference was not significant. This finding may show slightly immunosuppressive effect of IBD vaccine strain used in this study. No significant differences were found for weight gains among surviving birds in different groups at the end of experiment.

Susceptibility to a variety of bacterial, protozoal and viral diseases of chickens may be enhanced in chicks infected with IBDV early in life. The immunosuppressive effects of IBDV infection on the antibody response to vaccines have been reported previously (Saif, 1991). Suppression of response to Newcastle disease virus vaccines has been commonly used to study the immunosuppressive effects of IBD vaccines. The strain of the IBDV vaccine virus plays an important role in its immunosuppressive ability (Giambrone and Clay, 1986; Mazariegos et al., 1990). Less virulent IBDV strains were shown not to be immunosuppressive, whereas, the more virulent strains induced immunosuppression (Giambrone and Clay, 1986). These researchers studied two intermediate vaccines and showed that they produced slightly atrophic bursae with moderate microscopic lesions but did not demonstrate immunosuppression. They concluded that the severe bursal lesions (high atrophic bursal and severe microscopic bursal lesions) are required to induce immunosuppression.

The lower level of HI titre afforded by ND vaccination after exposure to IBD vaccine as reported previously (Allan et al., 1972; Muskett et al., 1979; Mazariegos et al., 1990; Kelemen et al., 2000) was not comparable to our findings. Except in one group, this study showed no significant difference in HI titres induced by ND vaccination between groups vaccinated or not vaccinated with IBD previously. The group which received the entrotropic apathogenic NDV vaccine strain (with prior exposure to IBD vaccine) demonstrated a significantly lower HI titre compare to the group, which received B1 and LaSota NDV vaccine strains. The difference, however, may not be related to effects of IBD vaccination.

### Table 1. Mean serum HI antibody titre of birds in treatment group 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (day)</th>
<th>1</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>38</th>
<th>45</th>
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<td>1</td>
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<td></td>
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<td>4.2&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>3.9&lt;sup&gt;ab&lt;/sup&gt;</td>
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<sup>a</sup>Means with different superscripts within the same column differ significantly (P ≤ 0.05). <sup>b</sup>Vaccination schedule of each group (60) was as follows: Group 1 = IBDV-Icx (<i>in ovo</i>) + entrotropic apathogenic NDV strain, Group 2 = IBDV-Icx (<i>in ovo</i>) + B1 + LaSota, Group 3 = IBDV-Icx(SC) + B1 + LaSota, Group 4 = B1 + LaSota, Group 5 = No vaccination. Chickens (50) were challenged at 31 days of age with a virulent NDV strain.

### Table 2. Rate of bird mortalities in treatment groups 1.

<table>
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<tr>
<th>Group</th>
<th>Days post-challenge</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>14</th>
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</tr>
<tr>
<td>2</td>
<td></td>
<td>0</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
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<td></td>
<td>0</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>7&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td></td>
<td>0</td>
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</tr>
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<sup>a</sup>Means with different superscripts within the same column differ significantly (P ≤ 0.05). <sup>b</sup>Refer to Table 1.
Acknowledgement

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References


