HUMORAL AND CELLULAR IMMUNE RESPONSES FOLLOWING SIMULTANEOUS APPLICATION OF LIVE NEWCASTLE DISEASE, AVIAN METAPNEUMOVIRUS AND INFECTIOUS BRONCHITIS VACCINES

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Summary

We report the results of protection studies and humoral and cellular immune responses in specific-pathogen-free (SPF) chicks that received live Newcastle disease virus (NDV), avian metapneumovirus (aMPV) or infectious bronchitis virus (IBV) vaccines in single, dual or triple combinations. Following the vaccination of day-old chicks, blood and trachea were collected at 21 days post vaccination (dpv). The blood was used for the detection of serum antibodies against NDV, aMPV and IBV. The trachea was examined for CD4+, CD8+ and IgA-bearing B-cells by immunohistochemistry (IHC). Between the NDV-vaccinated groups, there were no significant differences in the mean titres of the NDV haemagglutination inhibition (HI) titres and they remained above the protective titre. For antibodies against aMPV, the mean titres were suppressed when aMPV vaccine was given with other live vaccines but the aMPV-vaccinated groups were fully protected when challenged with virulent aMPV. For IBV, the mean levels of enzyme-linked immunosorbent assay (ELISA) antibodies were similar in the IBV-vaccinated groups and all IBV-vaccinated groups were almost 100% protected against M41 challenge. Between the vaccinated groups, there were no significant differences in the mean numbers of CD4+, CD8+ and IgA-bearing B-cells, reflecting similar levels of tracheal cellular and IgA responses irrespective of single, dual or triple vaccine applications. Despite the aMPV humoral antibody suppression, the efficacy of none of the live vaccines was compromised when they were given simultaneously to young SPF chicks.

Introduction

Newcastle disease virus, avian metapneumovirus and infectious bronchitis virus are important and common causes of respiratory diseases in chickens. All three viruses initially replicate in the epithelium of the respiratory tract (Alexander, 2000, Cavanagh and Gelb, 2008, Gough and Jones, 2008), and their ability to induce protective immunity may therefore be reduced if it is necessary to apply all three live vaccines simultaneously. Previous \textit{in vivo} studies, demonstrated

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that IBV interfered with the replication of live NDV vaccines (Thornton and Muskett, 1975) and aMPV vaccines in chickens (Cook et al., 2001). Ganapathy et al. (2005) demonstrated that NDV vaccination delayed the replication of the live aMPV vaccine, and also reduced the humoral antibody responses. Systemic antibody responses have been shown to have a poor correlation with protection against IBV (Pensaert and Lambrechts, 1994) and aMPV infections (Cook et al., 1989). Working with live NDV + IBV and aMPV vaccine viruses (Cook et al., 2001, Ganapathy et al., 2005, Tarpey et al., 2007), the authors suggested that the protection against these viruses was likely induced by cell-mediated and local immune responses. The objective of this study was to evaluate the humoral antibody responses. The cell-mediated and local antibody responses in the trachea were also assessed. Furthermore, the protection conferred against virulent aMPV and IBV was evaluated.

Material and Methods

One-day-old SPF chicks were allocated into eight groups (Table 1). The control (C) group was sham inoculated with sterile water (SW). Each chick was inoculated by ocular (50 µl) and oral (50 µl) routes. Through the same route of application, using the same volume for inoculation, different groups of chicks were vaccinated singly, dually or triply (Table 1). Dosages received by each bird were as recommended by the manufacturers.

Blood sample were collected at 21dpv from eight chicks in each group for antibody response against NDV (HI), IBV (ELISA) and aMPV (ELISA). At 21 dpv, five chicks from each group were humanely killed and trachea samples were collected from each bird, immediately placed in aluminium foil cups containing cryo embedding compound and frozen in liquid nitrogen (-190°C). The trachea was used for detection of CD4+, CD8+ and IgA-bearing B cells by IHC as described before (Rautenschlein et al., 2011). At 21 dpv, ten birds from each group were transferred to a different isolation room and challenged with 0.1 ml virulent aMPV subtype B (10^5.5 CD50/bird) by the oculo-nasal route. An additional ten birds from each groups were challenged with 0.1 ml virulent IBV M41 (10^5 CD50/bird) via the same route. Following aMPV challenge, birds were visited once a day for 13 days post-challenge (dpc) to record the clinical signs, each bird was examined (squeezing) individually and clinical signs were recorded as described before (Jones et al., 1992a). For IBV challenge, five birds from each unchallenged and challenged groups respectively were examined for ciliary scores. Scoring of the cilia beating was recorded as described before (Cook et al., 1999).

The data of the serological responses, cell-mediated immune responses (CMI) and aMPV clinical signs were analysed statistically using analysis of variance (ANOVA), followed by Tukey’s test. Differences were considered to be significant when p < 0.05. All analyses were conducted using the GraphPad Prism software, 6.0.1.

Results

Detection of NDV, IBV and aMPV serum antibodies. Unvaccinated control chicks were free of the antibody titre against NDV (Fig.1), aMPV (Fig 2) and IBV (Fig. 3). Chicks vaccinated with
NDV vaccine either alone or in combination had significantly higher antibody titres than chicks not vaccinated with NDV vaccine (Fig. 1). There were no significant differences in the level of HI antibody titres between groups that received the NDV vaccine.

Chicks vaccinated solely with the aMPV vaccine showed significantly higher levels of antibody titre than chicks vaccinated with the aMPV vaccine combined with either NDV and/or IBV (Fig. 2).

Chicks vaccinated with H120 vaccine alone or in combination with other vaccines showed high level of antibody titre against IBV (Fig. 3). However, there were no significant differences in the level of antibody titres between the groups that received H120 vaccine.

CD4+, CD8+ and IgA-bearing B-cells in the trachea. Single, dual or triple vaccinated groups showed significantly greater expression of CD4+, CD8+ and IgA bearing B-cells in the trachea compared to the unvaccinated control groups (Fig. 4). However, there were no significant differences between vaccinated groups.

Protection against aMPV challenge. Following virulent aMPV challenge, no clinical signs were observed in the groups vaccinated with aMPV alone or co-delivered with NDV and/or IBV. In contrast, chickens not vaccinated against aMPV showed clinical signs, such as clear to turbid nasal discharges at 3 dpc and had completely recovered by 11 dpc.

Protection against IBV challenge. Virulent IBV M41 caused substantial damage to the tracheal epithelium of chicks not vaccinated with the H120 vaccine. Groups that were given the H120 vaccine alone or in combination showed 95-98 % protection against IBV M41.

Discussion

In this study, IBV and aMPV humoral antibodies were measured using commercial ELISA kits and HI was used for detection of NDV antibodies. For IBV and NDV, the results demonstrate that simultaneous dual or triple vaccinations did not affect the levels of the antibodies induced. This is particularly important for NDV, as HI antibody levels are commonly used as an indicator of protection (Goddard et al., 1988). For aMPV, the lower humoral antibody titres in groups that were given dual or triple vaccinations were at the same level as previously reported (Cook et al., 2001, Ganapathy et al., 2005, Tarpey et al., 2007).

There are currently no published data on the cell-mediated responses to dual or triple vaccinations, even though such programmes are increasingly practiced in the poultry industry worldwide. We attempted to measure the CD4+ and CD8+ responses in the trachea following single, dual or triple vaccinations. In addition, the trachea was also assessed for IgA-bearing B-cells as an indicator of the local immune response. Our findings showed significant increases in the expression of CD4+, CD8+ or IgA bearing B-cell in the trachea from vaccinated compared to unvaccinated groups. The absence of significant differences between the vaccinated groups at 21 dpi demonstrates that at the tracheal level, similar intensities of cell-mediated and local immune responses were induced by the single, dual and triple vaccinations.

For NDV, due to local regulations, no challenge was carried out; instead NDV HI titres following vaccination were used. The mean HI titres of 2-5 log2 and above were considered to provide clinical protection (Reynolds and Maraqa, 2000). In this study, it appears that simultaneous ap-
plication of live NDV vaccine with aMPV, IBV or both does not compromise the immune response or protection conferred against NDV.

Protection against virulent aMPV challenge was achieved irrespective of the humoral antibody levels. This finding supports previous reports contending that humoral antibodies do not have a major role in protection against aMPV challenge (Jones et al., 1992b). Our study confirms previous suggestions that the protection against aMPV was due to cell-mediated and local immune responses, as the levels of cell-mediated and IgA-bearing B-cells were similar in all aMPV-vaccinated groups.

Results of IBV challenge showed that irrespective of live vaccine combinations, protection against IBV was not affected, as ciliary protection of 95-98% was achieved in all IBV-vaccinated groups. Similar findings have previously been shown in other in vivo studies (Cook et al., 2001).

In conclusion, there was a suppression of humoral antibody responses to aMPV, but responses to IBV and NDV were not affected. At the tracheal level, no variations in the levels of cell-mediated or IgA-bearing B-cells were seen between vaccinated groups.

References


Table 1: Chicks were randomly divided into 8 groups and immunized against NDV, aMPV and/or IBV.

<table>
<thead>
<tr>
<th>Groups/vaccines</th>
<th>Number of birds</th>
<th>Oculo-orlal dose</th>
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<tbody>
<tr>
<td>NDV</td>
<td>40</td>
<td>5.5 log_{10} EID_{50}/chick</td>
</tr>
<tr>
<td>aMPV</td>
<td>40</td>
<td>2.3 log_{10} TCID_{50}/chick</td>
</tr>
<tr>
<td>IBV</td>
<td>40</td>
<td>5.0 log_{10} EID_{50}/chick</td>
</tr>
<tr>
<td>NDV+aMPV</td>
<td>40</td>
<td>as above</td>
</tr>
<tr>
<td>NDV+IBV</td>
<td>40</td>
<td>as above</td>
</tr>
<tr>
<td>aMPV+IBV</td>
<td>40</td>
<td>as above</td>
</tr>
<tr>
<td>NDV+aMPV+ IBV</td>
<td>40</td>
<td>as above</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>40</td>
<td>SW</td>
</tr>
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Figure 1: NDV HI antibody titres in chicks administered with live NDV vaccine alone or with other live vaccines. Absent of letters indicate no significant difference between vaccinated groups. Vertical bars represent standard error of the means (n = 8).

Figure 2: aMPV ELISA antibodies in chicks vaccinated with aMPV or with NDV and/or IBV. Different superscripts represent significant differences in the antibody response. Vertical bars represent standard error of the means (n = 8).
Figure 3: IBV ELISA antibodies in chicks vaccinated with IBV alone or with NDV and/or aMPV. Absent of letters indicate no significant difference between vaccinated chickens. Vertical bars represent standard error of the means (n = 8).

Figure 4: Immunostaining of trachea sections at 21 dpv using monoclonal antibodies (Mab) for presence of CD4+ and CD8+ T-cells and for IgA-bearing B cells. No significant differences were observed between vaccinated groups. Vertical bars represent standard error of the means (n = 5).