IMMUNE RESPONSES IN CHICKS AFTER SINGLE OR DUAL VACCINATION WITH LIVE INFECTIOUS BRONCHITIS MASSACHUSETTS AND VARIANT VACCINES: SOME PRELIMINARY FINDINGS

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SUMMARY

Infectious bronchitis (IB) is an economically important viral disease in chickens, mainly affecting the respiratory, urinary and reproductive systems. For the prevention of the disease, live and inactivated vaccines have been used for many decades but due to the ability of the virus to mutate or to form recombinants, occasionally novel variant viruses can penetrate through the protection conferred by existing vaccines. To overcome this, it has been shown that when chicks are vaccinated with two different serotypes, there is a broader protection against a range of unrelated IBV challenge viruses compare to homologous vaccines. Despite the widespread use of such vaccination regimes, the underlying immune mechanisms involved are not known. This paper reports on some preliminary findings on humoral and cell-mediated immune responses of chicks vaccinated first with live Massachusetts (H120) vaccine then followed by a live 793B (CR88) vaccine. In addition, protection conferred against virulent IBV M41, 793B, QX, Italy-02 and D1466 were assessed using an in vitro tracheal organ culture challenge model.

INTRODUCTION

Infectious bronchitis (IB) caused by a coronavirus, is arguably the most important respiratory viral disease in chickens in regions where avian influenza and Newcastle disease are absent. It mainly affects the respiratory, urinary and reproductive systems causing substantial economic losses (Cavanagh and Gelb, 2008; Dhinakar Raj and Jones, 1997).

For the prevention of the disease, live and inactivated vaccines have been used for many decades but the disease persists through out the world. The challenge posed
by IB is mainly related to the ability of the virus to mutate or to form recombinants (Cavanagh et al., 1992; Kottier et al., 1995; Wang et al., 1997), which occasionally breaks through the protection conferred by existing vaccines. With decreased host ability to clear an infection, virulent viruses tend to persist in the flocks, and occasionally variant strains may emerge. In recent years, variant genotypes such as 793B, Italy-02 and QX have been reported in Europe (Worthington et al., 2008).

For control of IB, it has been shown that when chicks are vaccinated with two different serotypes/genotypes, there is a broader protection against various unrelated IBV challenge viruses compared to use of homologous serotype of live vaccines (Cook et al., 1999; Terregino et al., 2008; Worthington et al., 2008). Despite the broad protection, the underlying immune mechanisms are not known. Thus, the objective of this study is to examine the immune responses of chicks vaccinated with Massachusetts (H120) at day old followed by a 793B (CR88) at 13 days old.

MATERIAL AND METHODS

Vaccine preparation
IBV H120 and CR88 live vaccines were provided by Merial Animal Health Limited (Europe). One vial of each vaccine was thoroughly mixed with 100 ml of SW (Sterile water).

Chicks
Day-old Rhode Island Red specific pathogen free chicks from Institute of Animal Health (Compton) were used.

Experimental design
Day-old chicks were randomly divided into 6 groups as shown in the Table 1. They were inoculated with 0.1 ml of SW or prepared vaccines according to schedule outlined in Table 1. Each chick was inoculated by ocular (0.05 ml) and nasal (0.05 ml) routes.

Clinical signs
Chicks were monitored daily for clinical signs.

Sampling
A number of samples were collected including for virus detection (RT-PCR and isolation), serology, cytokine detections, immunohistochemistry and in vitro tracheal protection. Some of the related samplings are outlined below.

Sera
Blood samples were collected at 1, 6, 13, 19 and 26 days old. Sera were used for routine ELISA and HI antibody assays. For ELISA, a commercial ELISA kit (BioChek, Gouda, Holland) was used and data were analysed as recommended by the manufacturer. For HI, serum antibody levels against M41, 793B, D1466, QX and Italy-02 were determined using 4HA units of IB antigens. The IBV HI antigens were kindly provided by Ruth Manvell (Veterinary Laboratory Agency, Weybridge, UK).
**Tissues:** At 6, 13, 19 and 26 days old, 5 chicks from vaccinated and 3 chicks from unvaccinated groups were sacrificed. Samples of trachea, lung, kidneys, caecal tonsil and spleen were removed and immediately placed in RNA later solution. These were stored at –20 °C until further use. Tissues were processed and various cytokines were determined using quantitative real-time RT-PCR (Powell et al., 2009). This was carried out at the Institute of Animal Health, Compton.

**Tracheal Organ Culture (TOC) and in-vitro challenge**
At 26 days old, tracheas were harvested from five vaccinated and 3 unvaccinated chicks. After cleaning off excess fat, TOC were prepared as described before (Cook et al., 1976). After overnight incubation at 37 °C in a rotating rack, rings with 100% ciliary movement were used for in vitro protection studies. After separation into groups, the rings were inoculated with 0.1 ml of virulent M41, 793B, D1466, It-02 or QX IB viruses. The rings were read daily and scored for ciliostasis. Number of days taken for 50% of the rings to become non-viable (ndTOCNV$_{50\%}$) was obtained after plotting graphs.

**RESULTS**

Following the first vaccination at day-old, mild respiratory signs were observed only in the group given H120 and CR88 simultaneously. However, the signs disappeared before 13 days old. No clinical signs were observed in any other groups.

Humoral antibody titres varied depending on the assay used. With IBV-specific ELISA, highest antibody titres were detected in the group that received heterologous vaccines apart, H120 at day old and CR88 at 13 days old. Interestingly, second highest levels of antibodies were detected in the group that received H120 vaccine at day-old and again at 13 days old. The group of chicks that had been given CR88 vaccine at 13 days old showed moderate levels of antibodies.

In contrast, HI antibodies against M41 and 793B of all the vaccinated groups showed high and 'clustered' levels. However, the levels were markedly higher against M41 compare to the 793B antigen. The levels of HI against It-02, QX and D1466 were variable, ranging from high against It-02 but only trace levels against QX and D1466.

For cell-mediated immune responses, the levels of mRNA in various tissues were detected in samples of trachea, kidney and spleen. The results of tracheal analysis showed a storm of IFN-γ, IL-1 and IL-6 after primary but not after the second vaccination. For IL-13, cytokine storm was only found in the group given H120 and CR88 simultaneously. No IL-10 was detected. Results of other tissues are currently being analysed.

*In vitro* TOC challenge showed that the ndTOCNV$_{50\%}$ was the highest among the homologous challenges, particularly against M41 and 793B. For heterologous challenges, the ndTOCNV$_{50\%}$ differed depending on the vaccination group and challenge virus used. Broadly, ndTOCNV$_{50\%}$ for the group given H120 at day-old followed by CR88 at day 13 was the highest.
DISCUSSION

In this study, for the first time, it was attempted to evaluate the underlying immunological mechanisms for broader protection conferred by chicks vaccinated with heterologous compare to homologous IBV vaccines. The heterologous vaccines (H120 and CR88) were administered apart (H120 at day old and CR88 at 13 days old) or simultaneously at day old, and other groups were included as vaccinated and unvaccinated controls for comparison. Based on clinical signs observed, it appears that it is unwise to administer the H120 and CR88 vaccines simultaneously at day old, as post-vaccination reactions were seen until 13 days old. If such clinical signs can develop in SPF chicks kept under experimental condition, it is likely that much more severe respiratory disease may develop under the field condition.

Humoral antibodies were assayed for ELISA and HI antibodies. In both assays, the level of antibodies in the chicks that received H120 at day old and CR88 at 13 days old were the highest. This may have contributed to enhanced protection seen in this group. Humoral antibodies, even though not directly correlated with respiratory protection, play an important role in limiting the spread of the virus to visceral tissues (Yachida et al., 1985).

Cellular immune responses were monitored by assaying for various cytokines in the spleen, trachea, lungs and caecal tonsil. To date, IFN, IL-1B, IL-6, IL-10 and IL-13 were determined in the trachea. It appears that following the first vaccination, there were cytokine storms at day 6 and 13 days old. For IL-13, cytokine storms were not seen. For IL-10, only trace amount were detected in few of the chicks. Further work is in progress to establish the significance of these findings.

This study highlights kinetics of underlying humoral and cell-mediated immune responses following the heterologous live IBV vaccination. Broadly, it further strengthens the wider use of H120 followed by 793B vaccine.

Table 1: Experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of chicks</th>
<th>Vaccination (days)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td>H120</td>
</tr>
<tr>
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</tr>
<tr>
<td>3</td>
<td>35</td>
<td>H120</td>
</tr>
<tr>
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<td>H120</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>H120&amp;CR88</td>
</tr>
<tr>
<td>6</td>
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REFERENCES


