DYNAMICS OF MAREK'S DISEASE VIRUS AND HERPESVIRUS OF TURKEY
SHEDDING IN FEATHER DANDER OF BROILER CHICKENS

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Summary

In an attempt to measure the excretion dynamics of HVT and MDV via feather dander, broiler chickens were vaccinated with HVT or sham-vaccinated at day old and than challenged with MDV at days 2, 4 and 7 post-vaccination. The effect of the interval between vaccination and challenge on the protection of vaccine was determined. MDV and HVT were quantified in the dust samples collected at various days post-placement. There was an effect of the interval between vaccination and challenge on protection, higher the interval, lower the protection. Shedding of HVT was recorded from day 9 to 58 post-vaccination with a peak at around day 20. MDV was recorded in the dust from day 7 to 56 post-challenge with a peak at around 30-35 days post-infection. MDV shedding was significantly reduced by HVT vaccination, higher the protection less the shedding. Identification of HVT and MDV in the same dust sample can be a useful tool for monitoring vaccinal presence and MDV status of a chicken flock by analysing a single dust sample.

I. INTRODUCTION

Marek's disease (MD) is a lymphoproliferative disease of chickens caused by a cell-associated herpesvirus called MD virus (MDV). Chickens are infected with MDV via the respiratory route. Following uptake in the lungs the virus infects lymphocytes where initial viral replication occurs. Virus is then transmitted to the feather follicle epithelium (FFE) where fully productive viral production occurs and large amounts of virus are excreted to the environment in feather dander (Calnek et al., 1979). MDV in host cells such as lymphocytes, is infective but infectivity is lost if the integrity of the host cell is lost. However virus excreted in dander remains infective in the environment for a long time and is the main means of MDV transmission (Gilka and Spencer, 1993). MDV shedding in feather dander is thought to commence two weeks after infection (Carrozza et al., 1973).

A naturally occurring turkey herpesvirus (HVT) is antigenically related to MDV, does not produce disease in turkeys or chickens, but maintains horizontal transmission within turkey populations. HVT used as a vaccine against MD and is not thought to transmit horizontally between chickens unless they are infected at around 8 weeks of age, in which case limited horizontal transmission has been reported (Cho, 1976; Cho and Kenzy, 1975). HVT replication in the FFE has been reported for a short period during weeks 2 and 3 post-infection (Zygraich and Huygelen, 1972). With the advent of fully quantitative methods for measuring MDV in poultry dust samples (Walkden-Brown et al., 2004) it is of interest to determine whether this replication in the FFE is manifest as HVT in dander.

As for other live vaccines, the interval between vaccination and challenge is an important determinant of vaccine efficacy. If so, varying the interval between HVT-vaccination and MDV-challenge may produce variable protection. MDV shedding rate may also vary with the protection status of chickens. In this paper we report the excretion dynamics of MDV and HVT in feather dander in broiler chickens in an experiment in which

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the intervals between HVT-vaccination and MDV-challenge varied, giving rise to chicken populations of different protection status against MD.

II. MATERIALS AND METHODS

One hundred and fifty HVT-vaccinated (8,000pfu s/c at hatch) female Cobb broiler chickens were placed in 6 positive pressure isolators, 25 chickens in each isolator. Fifty more sham-vaccinated chickens were placed into two other isolators. Chickens of two sham- and two HVT-vaccinated isolators were challenged with 100pfu of MDV (strain MPF57) at 2 day post-vaccination (dpv) intra-peritoneally. HVT-vaccinated chickens in two isolators were challenged with MDV at 4 dpv and those in the remaining isolators were challenged at 7 dpv.

Dust samples were collected from each isolator between days 9 and 58 after placement. Chickens dying during the experiment were examined post-mortem for gross MD lesions. At day 56 post-challenge, all survivors were euthanased and examined post-mortem for MD lesions. The protective index of each challenge group was calculated as previously described (Islam et al., 2001).

DNA was extracted from 5mg of dust using DNeasy kits (Qiagen Pty Ltd, VIC Australia) and quantified by spectrophotometry (BioRad SmartSpec, TM3000). MDV and HVT were quantified using a real-time PCR described previously (Islam et al., 2004). MDV quantity was expressed as viral copy number (VCN) per mg of dust while HVT quantity was expressed as calculated concentration of HVT in arbitrary units, in the absence of absolute quantification for this assay.

III. RESULTS

There was an increase in protection with increasing interval between vaccination and MDV challenge. Protective index and percent MD to day 56 for the various vaccination-challenge treatments is presented in Table 1.

Table 1. Incidence of gross MD lesions (%MD) to day 56 post-challenge and protective index (PI) of sham-vaccinated or HVT-vaccinated broiler chickens challenged with 100pfu of MDV intra-peritoneally at various intervals after vaccination.

<table>
<thead>
<tr>
<th>Isolator</th>
<th>Vaccine</th>
<th>Vaccination-challenge interval (day)</th>
<th>MD positive</th>
<th>MD Negative</th>
<th>Total</th>
<th>% MD</th>
<th>Mean %MD</th>
<th>PI</th>
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<tbody>
<tr>
<td>18</td>
<td>Sham</td>
<td>2</td>
<td>16</td>
<td>2</td>
<td>18</td>
<td>88.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Sham</td>
<td>2</td>
<td>13</td>
<td>8</td>
<td>21</td>
<td>61.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>HVT</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td>14</td>
<td>42.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>HVT</td>
<td>2</td>
<td>6</td>
<td>11</td>
<td>17</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>HVT</td>
<td>4</td>
<td>6</td>
<td>17</td>
<td>23</td>
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<tr>
<td>19</td>
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<td>4</td>
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<tr>
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<tr>
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<td>15</td>
<td>17</td>
<td>11.76</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Total chickens present at day 31 post-challenge when the first mortality with MD lesions was observed.

Excretion of HVT in dander started from 9 dpv, the first dust sample collection day. The rate of excretion increased sharply at around 20 dpv and then decreased slowly up to 58 dpv.
dpv, the last dust sample collection day. No HVT was detected in the dust samples collected from unvaccinated chickens. The calculated concentration of HVT is presented in Figure 1.

Marek's disease virus was first detected in the dust collected at day 7 post-challenge in both sham and HVT-vaccinated chickens. Viral copy number was significantly higher overall in sham-vaccinated than HVT-vaccinated chickens (P=0.005). In sham-vaccinated chickens MDV shedding increased rapidly from day 14 post-challenge, peaked at around days 35-40 and then declined to day 56 post-challenge. In HVT-vaccinated chickens MDV increased slowly from days 14 to 21 and then tended to plateau at a lower level than in sham-vaccinated chickens for the remainder of the experiment (Figure 2A).

![Figure 1](image1.png)

Figure 1. Excretion of herpesvirus of turkey (HVT) by broiler chickens vaccinated at day old with 8,000pfu of HVT vaccine. Each point represents one isolator.

![Figure 2](image2.png)

Figure 2. MDV copy number per mg of dust sample at various days post challenge in vaccinated and unvaccinated chickens (left panel) and in chickens with various levels of protection (right panel).

The rate of MDV shedding was also influenced by the timing of challenge post-vaccination and thus the vaccinal protection status of the treatment (Figure 2B). Sham-vaccinated chickens shed MDV at the highest rate. An intermediate level of shedding was observed in the day 2 challenge group (PI 48) and low levels of shedding were observed in the day 4 and day 7 challenge groups (PI 69 and 78, respectively). These two groups shed significantly less MDV than unvaccinated chickens (P=0.01). The PI 48 group also shed less MDV than unvaccinated chickens between days 14 and 56 (P=0.02).

IV. DISCUSSION

This study has demonstrated for the first time the shedding of HVT in dust by chickens following vaccination with HVT. The study also demonstrated that MDV shedding may start as early as from day 7 following infection with MDV, and that HVT-vaccination significantly reduces MDV shedding into the environment.
The finding of significant HVT shedding in dust supports earlier reports of HVT replication in FFE (Zygraich and Huygelen 1972) and PCR detection of HVT in feather follicle extracts between days 14 and 42 (Handberg et al., 2001). This raises the obvious issue of the efficiency of lateral HVT transmission in broiler chickens. There is little evidence of efficient spread of HVT between young chickens to date, but the issue requires resolution with the improved methods for measuring virus now at our disposal.

Significant shedding of MDV may start much earlier than the 2 weeks previously reported (Carrozza et al., 1973). We observed MDV load of 600-7000 and 200-400 copies per mg dust at day 10 in unvaccinated and HVT-vaccinated chickens respectively; and very limited shedding (VCN 21-36/mg dust) was found as early as day 7. However, infectivity of this early MDV in dust was not confirmed. Using conventional PCR, MDV was identified in feather tip at day 14-16 post-challenge (Davidson and Borenshtain 2003; Handberg et al., 2001). MDV detection in dust at day 7 in our study might be due to higher sensitivity of our method, or dust samples may contain more virus than feather tip in the early stages of infection. Our finding of early shedding is supported by the finding of Cho et al. (1996), who detected aggregates of lymphocytes in the perifollicular dermis in MDV-infected chickens as early as day 7.

Vaccination with HVT overall reducing peak MDV shedding by approximately four fold but the extent of reduction was dependent on the PI. A PI of 78% reduced peak viral shedding by more than five fold. It is unclear at this point whether this reduction reflects a uniform reduction across the group of birds, or simply a greater proportion of individual birds in which shedding is dramatically reduced.

Identification of MDV and HVT in the same dust sample is now feasible and may be a new turning point in the routine monitoring of MDV status of chickens using dust samples.

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REFERENCES


Davidson, I., and Borenshtain, R. (2003). *FEMS Immunological and Medical Microbiology*, 15, 199-203


