SITE OF VACCINE DEPOSITION DURING *IN OVO* VACCINATION OF BROILER CHICKENS AGAINST MAREK'S DISEASE INFLUENCES THE TIMING OF POST-VACCINAL VIRAEMIA.

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**Summary**

*In ovo* vaccination of broiler chickens against Marek’s disease is a relatively recent industry practice. This study was designed to determine whether the timing of post-vaccinal viraemia following vaccination with cell-associated herpesvirus of turkeys (caHVT) is influenced by the site of vaccine deposition or dose of vaccine. Extra-embryonic vaccine deposition resulted in significantly later development of viraemia (~4 weeks) relative to both intra-embryonic vaccination (~2 weeks) and vaccination at hatch (~3 weeks). There were no effects of vaccine dose (4,000 or 8,000 pfu) on the timing of post-vaccinal viraemia, irrespective of site of deposition. There were no treatment effects on egg hatchability, mortality, feed intake or growth, but feed conversion ratio was shown to improve with increased dose of vaccine.

I. INTRODUCTION

*In ovo* vaccination of chickens is an important emerging technology (Ricks *et al.*, 1999) and it is now widely used to vaccinate broiler chickens against Marek’s disease (MD). Vaccination by this method deposits vaccine either extra-embryonically (EE) into the tissues surrounding the embryo or intra-embryonically (IE) into the body of the embryo. We have previously demonstrated that the deposition site of vaccine in the embryo by the automated INOVOJECT® method varied considerably with embryo age and egg size (Islam *et al.*, 1998). The present study formed part of our ongoing investigations into the role of site of vaccine deposition on the efficacy of vaccination and was designed to test the following hypotheses:

1. that intra-embryonic (IE) injection with cell associated HVT (caHVT) will result in earlier post-vaccinal HVT viraemia than extra-embryonic injection.
2. that vaccine dose (4,000 or 8,000 pfu) will influence the timing of post-vaccinal viraemia when vaccine is deposited EE but not if deposited IE.

II. MATERIALS AND METHODS

Before the main experiment a manual *in ovo* vaccination method was validated by the injection of 0.2ml vegetable food dye into embryos at day 18 of incubation and subsequent determination of the injection site. For IE injection, a sharp 21Gx1½” hypodermic needle was inserted through the pre-made hole in the air cell end of the egg until the embryo was encountered and the vaccine was then inoculated into it. For EE injection, a blunted 21G needle was inserted carefully through a hole and the air cell, into the extra embryonic space to a depth of 2.5-3cm, carefully avoiding the embryo proper before vaccine was deposited. The injection technique was found to be reproducible with an accuracy rate of >95%.

The design of the main experiment was a 2x3 factorial with two injection sites (IE and EE) and three doses of vaccine (0, 4000 and 8000pfu). An external control treatment was also

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included comprising the industry standard of subcutaneous injection with 4000pfu caHVT at hatch. For each of the seven treatment combinations five replicates of seven chickens were used.

The vaccine used was caHVT, strain FC 126 (The Mareks Company, North Ringwood, Victoria, Australia). All vaccine doses were administered in 0.1ml diluent. In ovo vaccination was performed manually at day 18 of incubation by a single operator using the previously validated method and aseptic technique.

The birds used in this experiment were Cobb broilers. The parent flock had been vaccinated with a MD virus serotype 1 (MDV1) vaccine (Rispens CVI 988) so the embryos and chickens used would have had maternal antibody directed against MDV1 but not HVT. Immediately after the hatch was taken off and the chicks vaccinated for infectious bronchitis (VicS strain) and MD (external control treatment), they were transferred to enclosed brooding facilities at UNE to limit exposure to natural challenge. Commercial feed and water were provided ad libitum throughout the experiment. At three weeks of age the birds were transferred from multi-deck electric brooders to Californian slide cages.

Individual bird weights and group (replicate) feed intakes were recorded weekly throughout the experiment. At weeks one and two of age, two chickens were removed from each replicate (n=10/treatment), stunned, sampled for blood and then euthanased. At weeks three, four and five of age, blood samples were collected from the wing veins of two birds in each replicate. Peripheral blood lymphocytes (PBLs) were separated using Ficoll-paque medium. HVT isolation was performed by inoculating PBLs into a secondary culture of chicken embryo fibroblast cells derived from specific pathogen free chicken embryos (SPAFASS Australia Pty Ltd, Woodend, Victoria, Australia) at day 10-12 of incubation. Cell monolayers were read for cytopathic effects (plaque formation) at 4-day intervals up to 20 days.

Associations between continuous variables were analysed using linear regression. Non-continuous data were analysed using logistic transformation and the generalised linear model method of S-plus 4.5 (Mathsoft Inc. Cambridge, MA, USA).

III. RESULTS

Overall hatchability of eggs containing embryos (determined by candling at day 17 of incubation) was 93%. Treatments had no effect on hatchability.

There were no effects of site of vaccine deposition or dose of vaccine on bird weight, feed intake or mortality. Final means (±SEM) for these variables at the end of week five were 1545±27g/bird, 2471±14g/bird, and 3.75% respectively. However, within the in ovo vaccinated treatments there was a significant negative association between vaccine dose and FCR (feed/gain, R² = 0.15, P<0.05) with mean FCR (±SEM) for the 0, 4000 and 8000pfu vaccine doses being 1.71±0.09, 1.66±0.05 and 1.52±0.02 g feed/g gain respectively.

The timing of post-vaccinal viraemia was significantly influenced by the treatments applied (Figure 1). Amongst in ovo vaccinated groups vaccinated with 4,000 or 8,000pfu of HVT there was a significant (P<0.05) effect of site of vaccine deposition with more IE birds being viraemic at weeks two and three (7/13 and 18/19 respectively) than EE birds (2/17 and 3/18 respectively). Vaccine dose (4,000 vs 8,000 pfu) had no effect. Subcutaneous vaccination at hatch produced viraemia in 10/10 birds at three weeks, which was significantly earlier than that in EE but not IE groups. No data were available from week one.
IV. DISCUSSION

Hypothesis 1, but not 2, was supported by the data. The major finding of this experiment was that EE deposition of caHVT at day 18 of incubation delayed post-vaccinal HVT viraemia relative to both, IE deposition at day 18, or subcutaneous injection after hatch. Vaccine dose did not affect this. To our knowledge this is the first published report on differences in the timing of post-vaccinal HVT viraemia following in ovo vaccination due to differences in the site of vaccine deposition.

![Graph showing percentage of chickens showing post-vaccinal HVT viraemia in each treatment.](image)

Figure 1. Percentage of chickens showing post-vaccinal HVT viraemia in each treatment during the experiment. Columns within each week not having a common letter are different (P<0.05).

We have already demonstrated that under Australian commercial hatchery conditions there is considerable variation in the ratio of EE to IE vaccine deposition due to egg size and more importantly, the day of incubation when vaccination takes place (Islam et al., 1998). In that work, we found that the proportion of IE vaccination sites when birds were vaccinated in ovo at day 18 of incubation was only 15%. Together with those data the present study suggests that a high proportion of chicks vaccinated extra-embryonically in ovo with caHVT may have significantly delayed post-vaccinal viraemia relative to those vaccinated IE or subcutaneously at hatch. The extent to which these differences impact on protective immunity to virulent MD virus (MDV) is currently being investigated.

The first report of in ovo vaccination against MD stated that vaccine deposition, both IE and EE, resulted in post-vaccinal viraemia within one week (Sharma and Burmester, 1982). However this work did not test the effect of site of deposition and furthermore it was performed on SPF White Leghorn birds, quite different from the industry situation.

The reasons why IE injection should produce a much earlier detectable vaccinal viraemia than EE injection are not known and were not examined in this experiment. Reasons may include one or more of the following: differences in the mode of entry into the embryo; the presence of antiviral activity in the extra-embryonic tissues; or differences in the level of interference from maternal antibody. Sharma et al. (1984) claimed that EE vaccine deposition results in infection of the embryo via the respiratory tract with initial replication in the lymphoid tissues associated with the lung, whereas IE deposition probably results in initial viral replication in the spleen and other lymphoid tissues. It is possible that the latter leads to a more rapid spread of infected lymphocytes into the blood. Anti-viral activity has been reported for mammalian amniotic fluid and a similar phenomenon may occur in avian species (discussed by Sharma and Graham, 1982). However, were this so, one could expect the
negative effects to be partially compensated for with an increased dose of vaccine and there was no suggestion of this in the present experiment. The birds used in our experiment came from a serotype 1 vaccinated parent flock (the normal industry situation) and would thus have passed on maternal antibody to MDV to the chicks. This may have contributed to both the delay in viraemia (often detected in less than a week in SPF birds) and possibly the observed difference between IE and EE vaccination. Cell-associated HVT vaccines are far less susceptible to the effects of maternal antibody than cell free HVT vaccines (Sharma and Graham, 1982) but are unlikely to be completely free of effects. In avian species maternal antibody is transferred via the yolk and so the yolk sac would be expected to contain a high concentration of maternal antibody. In an earlier study we found that 20% of EE injection sites involved the yolk sac, possibly contributing to the delay in post-vaccinal viraemia associated with EE vaccine deposition. The isolation of HVT from a small number of control birds was unexpected and may be due to lateral spread of virus although this is generally not considered to occur.

The effects of vaccine dose on FCR are difficult to explain in the context of an experiment where challenge with wild virus was not controlled, but assumed to be low. The improved FCR associated with higher vaccine doses suggests either that challenge with wild MDV occurred during the experiment or that vaccination with caHVT triggered a non-specific immune response that enhanced bird performance in the face of challenge from the normal range of pathogens.

V. CONCLUSION

This experiment has clearly demonstrated that the site of vaccine deposition is an important determinant of the timing of post-vaccinal viraemia following in ovo vaccination. Should delay in post-vaccinal viraemia be shown to compromise protective immunity to MD these findings will have important ramifications for the poultry industry. We are currently addressing these questions.

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REFERENCES