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Abstract: The Rispens (CVI988) attenuated Serotype 1 Marek¿s Disease virus vaccine is highly effective and used worldwide. It has been shown that this virus is present in feather tips of vaccinated chickens but the extent to which current commercial Rispens vaccines transmit effectively between chickens is unknown. To determine this, we measured the shedding rate, lateral transmission and changes in viral load over time, of the three commercial Rispens vaccines available in Australia. In each of three cli ...

VIRAL LOAD, SHEDDING RATE AND LATERAL TRANSMISSION OF MAREK'S DISEASE VACCINAL VIRUS (RISPENS/CVI988) IN SPF CHICKENS

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Summary

The Rispens (CVI988) attenuated Serotype 1 Marek's Disease virus vaccine is highly effective and used worldwide. It has been shown that this virus is present in feather tips of vaccinated chickens but the extent to which current commercial Rispens vaccines transmit effectively between chickens is unknown. To determine this, we measured the shedding rate, lateral transmission and changes in viral load over time, of the three commercial Rispens vaccines available in Australia. In each of three climate-controlled rooms, 10 SPF (specific pathogen free) chickens were vaccinated with a commercial Rispens vaccine at day old and left in contact with 10 unvaccinated chickens. A separate room contained unvaccinated control birds. As determined by quantitative real-time polymerase chain reaction (PCR) of room dust and peripheral blood lymphocytes (PBL), vaccine virus was shed from the vaccinated chickens in dander from day 7 and transmitted effectively from vaccinated to in-contact chickens with a lag period of 2-3 weeks.

I. INTRODUCTION

The most effective current Marek's disease vaccine worldwide is the Rispens CVI988 vaccine which is an attenuated serotype 1 Marek's disease virus (MDV1) (Witter et al. 1995; Baigent et al. 2005). As part of a wider investigation into interaction between vaccinal and wild-type MDV, this study was designed to determine the kinetics of Rispens replication at tissue & cell level at different times post vaccination and the spread of Rispens to in-contact chickens following vaccination. Rispens et. al. (1972) showed that CVI988 virus spreads laterally from vaccinated to in-contact chickens at low passage level (35), but working with a high passage clone (65), Witter et al. (1987) found that there was only limited transmission (0/8 and 4/10 for virus isolation and sero-conversion respectively). It is not known whether vaccination with the current Rispens vaccines will result in bird to bird effective transmission or not. In Australia, three different Rispens vaccines are commercially available. We used these vaccines in an experiment designed to test the following hypotheses:

1. Vaccine virus will be shed in dander and this will start from 7 day post-vaccination;
2. Vaccine virus will transmit laterally to in-contact chickens;
3. Viral kinetics will not vary significantly between the three commercial vaccines; and
4. Replication rate and shedding will be lower than published values for pathogenic MDV.

II. MATERIALS AND METHODS

Vaccines: The experiment utilised 3 vaccines (Nobilis®Rismavac, Intervet Australia; Poulvac®CVI Vaccine, Fort Dodge/Pfizer; Vaxsafe®RIS, Bioproperties.

Chickens: SPF white leghorn chickens were used, 20 in each of three vaccine study treatments and 10 in the negative control treatment.

Vaccination Protocol: Ten chickens in each vaccine group were vaccinated subcutaneously at hatch (day 0) with the manufacturer's recommended dose of vaccine {$\geq$1000 $TCID_{50}$ (tissue culture infective dose), $\geq$1000 and $\geq$4000 pfu (plaque forming unit) for the 3 vaccines above

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respectively} in 0.2ml of the supplied diluents, and 10 chickens for each group remained unvaccinated but were injected with the respective diluent alone (0.2ml).
The chickens were reared on deep litter in four climate control rooms of the University of New England Animal House. Vaccinated and unvaccinated in-contact chickens were individually identified using wing tags and housed together. Negative control chickens were injected with a mixture of the three vaccine diluents (0.2ml).

Blood samples were collected from the vaccinated and control birds weekly from 7 days of age and from in-contact birds weekly from 21 days of age up to day 56. Blood samples were collected into sodium citrate anticoagulant prior to separation of PBL using ficoll paque^{TM} PREMIUM. For the day 56 sampling, serum was also collected for Enzyme-linked immunosorbent assay (ELISA) to measure antibody levels directed against MDV. Dust collection from an open tray in each room started at day 7 and continued weekly up to day 56.

DNA was extracted from PBL using the X-tractor Gene DNA extraction robot (Corbett, ROBOTICS, Australia). From dust, DNA was extracted using the DNeasy® tissue kit (Qiagen, Clifton Hill, Australia). All DNA was quantified using a NanoDrop® ND-1000 UV-Vis spectrophotometer (NanoDrop® Technologies Wilmington, USA). Taqman real-time qPCR assay for MDV1 was performed using a Rotor Gene 3000 real-time PCR machine (Corbett Research, Australia) using the method of Islam et al., (2006). Indirect ELISA to detect MDV-specific antibody was adapted from the method of Zelnik et al., (2004). Samples were randomized prior to testing.

The rate of which birds become infected was determined by detection of MDV in PBL and treatments effects were investigated using survival analysis (Kaplan-Meier product-limit method). MDV1 load and MDV titer values were log transformed [$\log_{10}$ (x+1)] prior to analysis using ANOVA, fitting the effects of Vaccine (randomly coded A, B, C), Type of infection (vaccinated, in-contact), Day (when required) and their interactions. For repeated measures (PBL, dust) a mixed REML model was used with animal or room fitted as a random effect. Analyses were performed with JMP8 (SAS Institute, NC, USA). Least squares means and standard errors of means are presented for continuous variables. A statistical significance level of $P<0.05$ was used throughout.

III. RESULTS

Control chickens remained negative for MDV in PBL and dust (qPCR), and serum (ELISA) at day 56. Most (93%) vaccinated birds were MDV1-positive in PBL at 7 days of age and 100% by 28 days of age. For the in-contact birds, 38% were positive by the first measurement at 21 days of age, reaching 96% by day 56 (Figure 1). Infection of in-contact birds lagged behind vaccinated birds by 2-3 weeks (Figure 1).

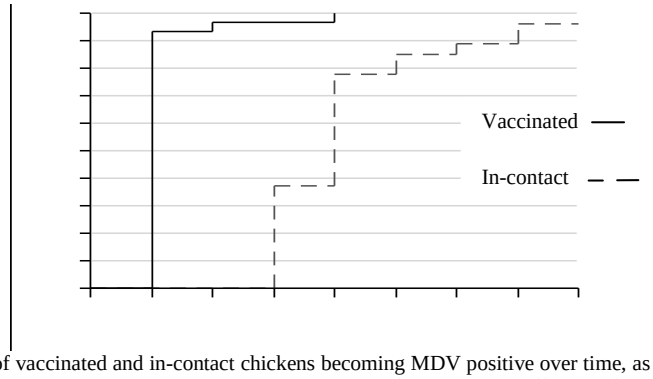

Figure 1 Cumulative proportion of vaccinated and in-contact chickens becoming MDV positive over time, as determined by qPCR of PBL for MDV1 commencing at days 7 (Vaccinated) and 21 (In-contact) respectively (failure curves differ significantly, $P < 0.001$)

There was a significant effect of Type of Infection (P = 0.007, Figure 2) and Vaccine (P<0.001) on $\log_{10}$ MDV copy number per 10^6 PBL with no significant interaction between these effects (P=0.696). In vaccinated birds mean viral load was maximal at day 7 {$10^{3.5}$ VCN (viral copy number)/10^6 PBL} decreasing to $10^{2.5}$ VCN/10^6 PBL at day 21 then plateauing until day 42 before decreasing further to $10^{1.2}$ VCN/10^6 PBL at 56 days of age (Figure 2). In the in-contact birds, mean MDV load in PBL was low ($10^{1.5}$ VCN/10^6 PBL) at 21 days of age (first sampling day for this group) increasing to $10^{3.2}$ VCN/10^6 PBL on day 28 before plateauing at this level until day 42 days before decreasing to 10^2 VCN/10^6 PBL at day 56 (Figure 2).

There was a significant effect of Vaccine (P < 0.045) and Day (P < 0.001) on MDV load in dust with vaccines B and C inducing overall values 0.38 and 0.52 logs higher than vaccine A. Dust from all three vaccine treatments was MDV positive from day 7 onwards with the viral load in dust increasing to day 21 then more slowly and erratically thereafter (Figure 3).

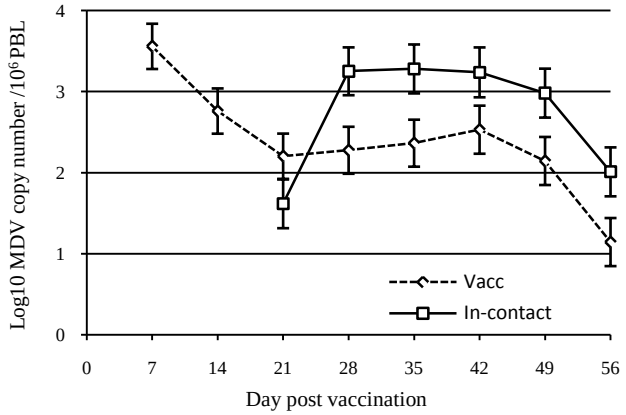

Figure 2 Mean (LSM±SEM) MDV load in PBL in vaccinated and in-contact chickens over time.

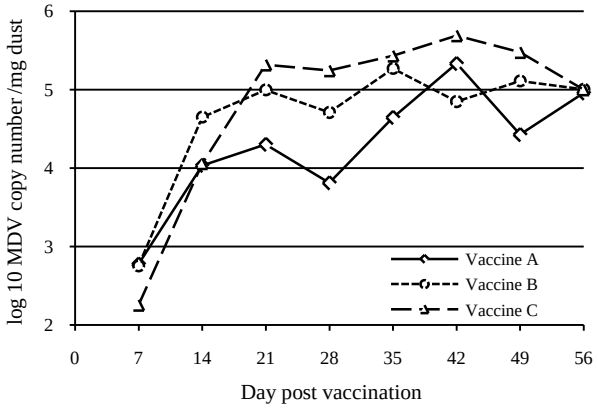

Figure 3 Mean MDV load in dust by vaccine type over time (no SE as only one sample per vaccine/week)

There was no significant effect of Type of Infection (P=0.358) and Vaccine (P=0.189) on MDV antibody titre at day 56, with no interaction between these effects (P=0.714).

IV. DISCUSSION

The study demonstrated that all three serotype 1 MDV vaccines available in Australia induce shedding of vaccinal virus in feather dander as early as day 7 post-vaccination. The experiment also revealed that the vaccine virus transmits very effectively to in-contact chickens, producing higher viral loads in these birds than observed in the vaccinated chickens. There was lag time of 2-3 weeks in infection between vaccinated and in-contact chickens.

Baigent *et. al.* (2005) detected CVI988 in feather tips at 6 days of age but did not extend their findings to measuring shed virus in feather dander or dust. The present study has demonstrated that the current vaccinal strains of CVI988, used in Australia according to the manufacturers' directions, all readily shed from the vaccinated chickens into the environment in large amounts (up to $10^{5.7}$ VCN/mg dust), with shed virus readily detected in dust samples from 7 days of age. Thus the first hypothesis of this experiment is accepted.

When CVI988 was first detected and tested for its properties, Rispens et al., (1972) reported that it spread directly to in-contact chickens using virus isolation and detection of antibody levels from in-contact chickens. However, the Clone C vaccinal strain of CVI988 spread poorly to in-contact chickens, probably due to subsequent passage and attenuation (Witter et al. 1987). Since those two studies, there has been no quantitative data reported on

the transmission of commercial CVI988 vaccine strains. The effective spread of the vaccine virus in the present study is consistent with the earlier results of Rispens et. al. (1972) and thus the second hypothesis of this experiment is accepted.

Regarding the viral load in PBL over time, Baigent et. al. (2005) first detected the CVI 988 genome in PBL at 4 days post vaccination (dpv). Viral load then increased to a peak of 4.7 x 10^3 VCN/10^6 PBL at 14 dpv and steadily decreased to a level of 3.91 x 10^2VCN/10^6 PBL on the last day of experiment at 28 dpv. In the present experiment, very similar viral loads and patterns were observed but the peak in vaccinated birds was earlier, that is at 7 days post vaccination. From day 28 onwards, MDV load in PBL was consistently higher in in-contact birds than in the vaccinated chickens, indicating very effective natural transmission and subsequent replication. Baigent et. al. (2005) reported data on the kinetics of Rispens vaccine manufactured by Ford Dodge UK. Until this present experiment, no other published studies have compared the kinetics or spread of Rispens vaccines from different manufacturers. While differences between vaccine products was not a significant focus of our experiment, it did show that there were significant differences between the three Rispens vaccines in the viral load induced following vaccination, with some trends also evident for rate of spread. However MDV titre at the end of the experiment did not differ significantly between the 3 vaccines. Thus, based on the PBL and dust data, hypothesis 3 is rejected.

Our fourth hypothesis, that replication rate and shedding of CVI988 will be lower than published values for pathogenic MDV is accepted. Mean vaccine virus in PBL never exceeded 10^4 VCN/10^6 PBL while values of 10^5-10^6 VCN/10^6 PBL have been reported for pathogenic MDV up to 35 dpi (day post infection) using the same methods (Islam et al., 2006). Furthermore MDV load in PBL continues to increase up to day 35 with virulent MDV (Islam et al., 2006), whereas with CVI988 vaccine, values peak early (days 7-14) then decline (present study; Baigent et al., 2005). Regarding virus shedding, vaccinal MDV values in dander in the present study were approximately 1-1.5 logs lower than reported values of 10^6-10^7 VCN/mg of dander for virulent wild type virus (Islam and Walkden-Brown 2007).

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