Quantitative profiling of the shedding rate of the three Marek's disease virus (MDV) serotypes reveals that challenge with virulent MDV markedly increases shedding of vaccinal viruses

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INTRODUCTION

Marek’s disease viruses (MDVs) are cell-associated viruses belonging to the family Herpesviridae, the subfamily Alpha-herpesviridae and the genus Mardivirus (Fauquet et al., 2005). Antigenically, they are closely related and their classification into three serotypes, namely serotype 1 (MDV1), 2 (MDV2) and 3 (MDV3 or herpesvirus of turkeys, HVT) is widely used. MDV1 is oncogenic and is the aetiological agent of Marek’s disease (MD), a lymphoproliferative and neoplastic disease of poultry (Churchill & Biggs, 1967; Nazerian et al., 1968) with an estimated worldwide economic impact of US$1–2 billion (Morrow & Fehler, 2004). MDV1 isolates vary widely in their pathogenic and oncogenic potential (Witter, 1997). The other two serotypes (MDV2 and HVT) are non-oncogenic and are used in live vaccines against MD, either alone, in combination or in combination with attenuated MDV1 strains (Witter & Schat, 2003). Attenuated strains of MDV1 and naturally occurring vaccine strains of MDV2 or HVT produce non-sterile immunity against MD tumours and persist together with virulent MDV1 in the host (Witter et al., 1971; Witter & Schat, 2003).

Whilst the primary target cell for MDV pathology is the lymphocyte, MDV1 is contagious and is readily transmitted by the airborne route (Biggs, 1985). This is due to replication of fully infectious virus in the epithelial cells of the keratinizing layer of the feather follicle epithelium, which then slough off and are shed as highly infective dander (Calnek et al., 1970). MDV2 is also contagious (Witter, 1987), but HVT does not spread readily between chickens infected early in life (Cho & Kenzy, 1975). Understanding the rate of shedding of the different MDVs, and factors influencing this, is a prerequisite to a detailed understanding of the epidemiology of MDV, but our current understanding of this is limited. Earlier reports suggested that shedding of MDV1 commences from 2 to 4 weeks after infection, well before the appearance of clinical signs, and may continue throughout the life of the chicken (Witter et al., 1971; Carrozza et al., 1973). Since then, studies using more sensitive molecular methods have...
found that MDV1 is shed as early as 7 days post-infection (Baigent et al., 2005; Islam et al., 2005b). In recent studies, it has been shown that all three serotypes of MDV are prevalent in dander collected from commercial broiler farms across Australia (Islam, 2006; Renz et al., 2006). These studies and others (e.g. Walkden-Brown et al., 2004, 2005) have also demonstrated that poultry dander is a suitable material for monitoring of MDV1, MDV2 and HVT on commercial poultry farms. However, the pattern of shedding of these viruses over time in chickens infected singly or co-infected with different serotypes of MDV under controlled conditions has yet to be reported.

In order to understand in more detail the dynamics of MDV shedding from commercial meat chickens, the current experiment was designed to: (i) measure the amount of MDV1, MDV2 and HVT present in dander shed from broiler chickens infected with one or more of these viruses; (ii) estimate the amount of dander shed by broiler chickens during their growth cycle; and (iii) calculate the profiles of total virus shed for each MDV serotype during the broiler chicken growth cycle.

**METHODS**

**Experimental design.** This paper reports results from a single experiment using a complete 4 × 3 factorial design with two replicates using 24 isolators (4 × 3 × 2 = 24). The two experimental factors and their levels were challenge virus (mock, MPF57, 02LAR and FT158) and vaccine virus (mock, HVT and bivalent HVT/MDV2). Vaccination was administered on the day of hatch (day 0) and challenge was on day 5 post-vaccination (p.v.). Mock-treated chickens received diluent only.

In this experiment, the individual isolator was the experimental unit. Each treatment combination was replicated in two separate purpose-built positive-pressure chicken isolators in a physical contamination level 2 animal house. The isolators had approximately 20 air changes per hour. The University of New England Animal Ethics Committee approved the experiment protocol, and chickens were maintained in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NH & MRC, 2004). The experiment started on 28 February 2005 and terminated on 5 May 2005. All surviving chickens were euthanized at day 56 post-challenge (p.c.).

**Experimental chickens.** The experiment commenced with 648 newly hatched chickens providing 27 per isolator and 54 per treatment combination initially. The chickens were commercial female Cobb broilers from parent stock vaccinated with the Rispens CVI988 strain of attenuated MDV1. They were hatched at a commercial hatchery and were unvaccinated.

Approximately five chickens per isolator were removed on each of days 7 and 14 p.c. to evaluate immunosuppression (data not presented), reducing the effective maximum number per 17 to 17 per isolator.

**Vaccination.** This was performed manually by subcutaneous injection of the recommended dose at hatch (day 0) on arrival of the chickens at the isolator laboratory at the University of New England (UNE).

Both the cell-associated (ca) HVT vaccine (caHVT strain FC-126, batch number HO2308) and the bivalent vaccine (caHVT strain FC-126 and caMDV2 strain SB1, batch number SBH 4101) were supplied by Bioproperties Pty Ltd. The dose rate used for both vaccines was 8000 p.f.u. per bird in 0.2 ml diluent supplied by the manufacturer. The dose for the bivalent vaccine was for both viruses combined, with an HVT:MDV2 ratio of 63:37.

**MDV1 challenge.** Chickens were challenged with three different Australian isolates of MDV1 (MPF57, 02LAR and FT158) on day 5 p.v. For each isolate, a dose of 500 p.f.u. per chicken in 0.2 ml diluent was inoculated via the intra-abdominal route. Challenge virus was kindly provided by Professor Greg Tannock (RMIT, Melbourne, Australia) and virus titrations were performed on chick kidney cells in his laboratory. MPF57 is the standard Australian challenge MDV1 and was included as a reference strain. It was isolated in 1994 from a bivalent (HVT/MDV2)-vaccinated layer flock (De Laney et al., 1998). The isolate 02LAR was isolated from an unvaccinated broiler flock in 2002, whilst FT158 was isolated from a meat breeder flock vaccinated with Rispens CVI988 in 2002.

**Collection of dander for MDV analysis.** For determination of MDV load in dander, samples were collected weekly from the dust deposits at the 90° bend in the exhaust air outlet duct of each isolator. For mock-challenged isolators (n=6), this commenced at 7 days p.v., and for those challenged with MDV1 (n=18), dust collection commenced at day 7 p.c. (=12 days p.v.). Using a disposable wooden spatula, approximately 1 g of dust was scraped into an Eppendorf tube following brief closure of the exhaust air outlet valve. After each collection, the outlet duct was thoroughly cleaned, so that the next collection represented the past 7 days of dust accumulation.

**Estimation of daily dander production per chicken.** Six isolators were selected randomly to measure dander production, and dander production was measured weekly throughout the experiment from these isolators. Dander production was measured by capturing the total dander output in the exit air of each isolator in commercial vacuum cleaner filter bags over a period of 48 h for weeks 1–4 and over 24 h for weeks 5–8. Daily dander production per chicken was then calculated from the total dander collected, the duration of the collection period and the number of chickens in the isolator.

**DNA extraction from dander.** DNA was extracted from 5 mg dander using a DNeasy tissue kit according to the manufacturer’s instructions (Qiagen) and stored at –20 °C. Prior to use in the quantitative real-time PCR (qPCR) assay, extracted DNA was quantified by spectrophotometric analysis (SmartSpec TM 3000; Bio-Rad) for DNA yield and purity, and then diluted to a concentration of 5 ng μl⁻¹ for use in the qPCR assay.

**Assay of MDV genome copy number by qPCR.** The primers and probes used were specific for the MDV1 mgi gene, MDV2 dnapel gene and HVT sorf1 gene as described previously by Islam et al. (2004). MDV1, MDV2 and HVT genome copy numbers were determined in the extracted DNA from dander samples according to the absolute quantification method described by Islam et al. (2006a, 2007) and Renz et al. (2006). Briefly, each reaction contained 0.3 μM each primer and 0.2 μM corresponding probe, 12.5 μl Platinum Quantitative PCR System-UDG (Invitrogen) and 5 μl DNA template (25 ng) in a total reaction volume of 25 μl. The cycling parameters consisted of 50 °C for 2 min and 95 °C for 2 min, followed by 40–45 cycles of denaturation at 94 °C for 15 s and annealing/extinction at 60 °C for 45 s. Amplification and data acquisition were carried out using a Rotor Gene 3000 real-time PCR machine (Corbett Research). A standard curve for each primer set was generated in each assay and used to derive the copy number of target sequences in unknown samples. For each assay run, individual standard curves were generated using four to five 10-fold dilutions of MDV1, MDV2 and HVT standards of known concentration of virus. Samples that did not amplify or that had a threshold cycle (Ct) value below the lowest
standard were coded as zero/negative. Viral loads in dander were expressed as viral copy number (VCN) (mg dander)⁻¹, taking into account the total amount of DNA extracted from each sample. The lower limits of detection (mean ± SEM) based on the mean calculated VCN of the lowest standard in each of the assays were 3.34 ± 0.22, 11.73 ± 5.02 and 60.80 ± 30.91 VCN per reaction for the MDV1, MDV2 and HVT assays, respectively.

All samples were randomized across assays to minimize individual assay effects, and amplified in duplicate. Mean intra-assay coefficients of variation for all qPCR runs were 0.68 ± 0.11 % for MDV1, 1.67 ± 1.09 % for MDV2 and 1.07 ± 0.29 % for HVT based on Ct values. The co-efficients of variation based on VCN were 14.0 ± 2.7 % for the MDV1, 30.3 ± 18.5 % for the MDV2 and 23.5 ± 4.7 % for the HVT assays, respectively.

**Statistical analysis.** Data were analysed using JMP version 5.1 statistical software (SAS Institute). MDV1, MDV2 and HVT viral load data obtained from qPCR assays of isolator dander were square-root-transformed to meet the assumptions of analysis of variance (Petrie & Watson, 2006). The transformed data were then analysed using a restricted maximum likelihood mixed model with each isolator fitted as a random effect and the relevant treatment effects and their significant interactions fitted as fixed effects. Differences between individual values were determined using Tukey’s HSD test or specific contrasts within the model. Viral load data are presented in the text as back-transformed least-squares means with 95 % confidence intervals (CI). A significance level of P≤0.05 was used throughout.

**RESULTS**

All treatment combinations were applied successfully and there was no cross-infection between isolators. No MD lesions were recorded in the two control isolators, and qPCR analysis of dander confirmed the absence of MDV1 in control and mock-challenged isolators. MD lesions were first detected at day 27 p.c. and the challenge viruses induced a high level of visible gross MD lesions in at-risk unvaccinated birds (86 %), which was considerably reduced by vaccination with either HVT (27 % MD lesions) or the bivalent vaccine (28 % MD lesions). Detailed data on the level of immunosuppression and MD lesions induced and the vaccinal protection will be reported separately.

**MDV1 load in dander from chickens challenged with MDV1**

MDV1 load varied significantly due to the effect of vaccination (P=0.002) and the number of days p.c. (P<0.0001), with significant interaction between these effects (P<0.0001, Fig. 1). However, the effect of challenge with three isolates of MDV1 (P=0.91) and its interaction with the number of days p.c. (P=0.083) were not significant (Fig. 2). The interaction between challenge virus and vaccination was also not significant (P=0.257).

Dander from mock-vaccinated chickens had a significantly higher MDV1 load than that of HVT- and bivalent-vaccinated chickens (Table 1), with this effect being most marked between days 14 and 28 (Fig. 1). MDV1 appeared in dander from day 7 p.c., increased markedly between days 14 and 28 and then plateaued until day 56.

**HVT load in dander from chickens vaccinated with HVT and bivalent vaccine**

The effect of challenge with MDV1 was highly significant (P<0.0001), with mock-challenged chickens having HVT loads in dander nearly 2 logs lower than that found in chickens challenged with MDV1 (Fig. 3, Table 1). There were no significant differences due to the different challenge

![](http://vir.sgmjournals.org)
viruses used. HVT was present in dander from day 7 p.v. onwards. In the groups challenged with MDV1, HVT load increased sharply between days 12 and 26 p.v. and then decreased between days 26 and 40 p.v. before stabilizing. The pattern in unchallenged chickens was broadly similar but with a greatly reduced load (Fig. 3). The effect of vaccine type was significant \((P=0.031)\), with slightly higher overall values in chickens vaccinated with HVT than in those vaccinated with the bivalent vaccine \([160\, 221\, \text{vs}\, 110\, 789\, \text{copies (mg dander)}^{-1}]\), respectively.

**Table 1.** Summary of overall treatment effects on the mean load of MDV1, HVT and MDV2 in relevant groups of chickens during the experiment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect</th>
<th>(P) value</th>
<th>Level</th>
<th>Back-trf. LSM*</th>
<th>95 % CI</th>
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<tbody>
<tr>
<td>MDV1 load in dander ((\times 10^6 \text{copies mg}^{-1})) in chickens of different vaccination status</td>
<td>Vaccination treatment</td>
<td>0.002</td>
<td>Mock, HVT</td>
<td>6.086&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.752–7.585</td>
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<td></td>
<td></td>
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<td>Bivalent</td>
<td>3.492&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.508–4.639</td>
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<td></td>
<td></td>
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<td></td>
<td>2.331&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.542–3.283</td>
</tr>
<tr>
<td>HVT load in dander ((\times 10^3 \text{copies mg}^{-1})) in chickens vaccinated with HVT or bivalent vaccine and given different challenge treatments</td>
<td>Challenge treatment</td>
<td>&lt;0.0001</td>
<td>Mock, 02LAR, MPF57, FT158</td>
<td>2.981&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.034–13.329</td>
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<td></td>
<td></td>
<td></td>
<td>164.168&lt;sup&gt;b&lt;/sup&gt;</td>
<td>116.865–219.490</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>238.013&lt;sup&gt;b&lt;/sup&gt;</td>
<td>181.020–302.794</td>
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<td>268.961&lt;sup&gt;b&lt;/sup&gt;</td>
<td>208.130–337.579</td>
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<tr>
<td>MDV2 load in dander ((\times 10^6 \text{copies mg}^{-1})) in chickens vaccinated with bivalent vaccine and given different challenge treatments</td>
<td>Challenge treatment</td>
<td>&lt;0.0001</td>
<td>Mock, 02LAR, MPF57, FT158</td>
<td>0.330&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.081–0.746</td>
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<td>8.735&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.877–10.814</td>
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<td>15.514&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>13.480&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.347–15.802</td>
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*Back-transformed least-squares means. Means not sharing a common superscript letter in differ significantly using Tukey’s HSD \((P<0.05)\). This is a more conservative test than Student’s \(t\)-test used to calculate the 95 % CI.

**Fig. 3.** Effect of challenge treatment on HVT load in dander. The results show mean HVT load \((\pm \text{SEM, log scale})\) in isolator exhaust dander over time from isolators containing chickens vaccinated with either HVT or bivalent vaccine at hatch, and challenged with three isolates of MDV1 (02LAR, FT158 and MPF57) or mock challenged at 5 days of age. Each data point represents the mean of four isolators (two vaccinated with HVT and two with bivalent vaccine). Sampling days for MDV1 and mock-challenged chickens differed as shown.

**Fig. 4.** Effect of challenge treatment on MDV2 load in dander. The results show mean MDV2 load \((\pm \text{SEM, log scale})\) in isolator exhaust dander over time from isolators containing chickens vaccinated with bivalent vaccine (HVT/MDV2) at hatch and challenged with three isolates of MDV1 (02LAR, FT158 and MPF57) or mock challenged at 5 days of age. Each data point represents the mean of two isolators. Sampling days for MDV1 and mock-challenged chickens differed as shown.
MDV2 load in dander from chickens vaccinated with bivalent vaccine

The effect of challenge treatment on MDV2 load was highly significant \((P<0.0001)\), as were the effect of the number of days p.v. and its interaction with challenge treatment \((P<0.001, \text{ Fig. 4, Table 1})\). MDV2 was not present in dander sampled at day 7 p.v., but was present from day 12 onwards. In MDV1-challenged groups, MDV2 load in dander increased sharply between days 12 and 19 p.v. and then slightly between 19 and 26 days p.v., after which little change occurred. There were no significant differences due to the different challenge viruses used. In mock-challenged chickens, a similar pattern was observed, but with greatly reduced levels. In mock-challenged groups, MDV2 loads in dander also increased sharply between days 12 and 19 p.v. and then slightly between days 19 and 26 p.v., before stabilizing.

MDV1, MDV2 and HVT load in dander in chickens vaccinated with bivalent vaccine and challenged with MDV1

All three MDV serotypes were only present in dander from chickens vaccinated with bivalent vaccine and challenged with MDV1 isolates. In these samples, the level of the different serotypes of MDV varied significantly \((P<0.001)\), and they also varied significantly over time, with significant interaction between these two effects \((P<0.0001)\). Overall, the viral load of MDV2 was significantly higher than that of MDV1, which in turn was significantly higher than that of HVT \([12.628 \times 10^6, 2.331 \times 10^6 \text{ and } 0.210 \times 10^6 \text{ VCN (mg dander)}^{-1}\), respectively]. The interaction between days p.c. and MDV is shown in Fig. 5, with clear differences in the pattern of viral load over time for the different MDV serotypes.

Daily dander production per chicken

Daily dander production per chicken increased significantly between days 14 and 49 with non-significant increases between days 7 and 14 and days 49 and 56 (Fig. 6). There was no effect of isolator. Overall, dander production increased from \(8.9 \pm 0.9 \text{ mg}\) at day 7 to \(232.4 \pm 22.5 \text{ mg}\) at day 56.

Daily shedding rate of MDV1, MDV2 and HVT per chicken

By combining MDV levels in dander with estimated daily dander production, the daily shedding rates of the three serotypes of MDV in dander from chickens in the different treatment combinations could be estimated and these are presented in Fig. 7. Shedding of MDV1 increased sharply between days 7 and 28 p.c., with minor changes thereafter, for both vaccinated and unvaccinated groups (Fig. 7a). From day 35 onwards, both groups were shedding approximately \(10^8\) copies per chicken per day. Shedding of MDV2 and HVT also increased rapidly between days 12 and 28 p.v. and stabilized thereafter. However, shedding of the vaccinal viruses was much greater in MDV1-challenged than in mock-challenged chickens for both MDV2 and HVT (Fig. 7b). In challenged chickens vaccinated with the bivalent vaccine, shedding of MDV2 was always higher than that of MDV1, which in turn was always higher than that of HVT.

DISCUSSION

This paper describes the shedding profile of three virulent isolates of MDV1 (MPF57, 02LAR and FT158) and two
strains of vaccine virus, HVT strain FC-126 and MDV2 strain SB1, in single and mixed infections. For the first time, viral load in dander and total dander production by broiler chickens have been estimated, providing good estimates of the rates of shedding into the environment of the different MDV serotypes. MDV1 and HVT were detectable in isolator exhaust dander from day 7 post-infection, whilst MDV2 was detected on day 12 p.v. All viruses continued to be shed in dander throughout the experimental period (up to 61 days of age). Both HVT and bivalent vaccines significantly reduced MDV1 shedding in dander between days 14 and 28 p.c., although the effect of vaccination was not large, and vaccinated challenged chickens continued to shed approximately 10^9 copies of MDV1 daily. A major unexpected finding was that MDV1 challenge increased the total shedding rate of the vaccinal viruses (HVT and MDV2) by 38- and 75-fold, respectively (based on back-transformed least-squares means). This is the first report quantifying the shedding rate of all three serotypes of MDV over the lifetime of broiler chickens, particularly in chickens infected with all three serotypes.

There were no significant differences in MDV1 load in dander from birds challenged with three different virulent MDV1 isolates, but there were significant differences among different serotypes of MDV. Nazarian & Witter (1970), using feather follicle epithelium, found positive correlations between virulence, contagiousness and replication of virulent and attenuated MDV1 and HVT viruses. They found that virulent strains of MDV1 were more readily detected in the feather follicle epithelium and more easily transmitted to contact chickens than avirulent strains including attenuated MDV1 and HVT. However, in a recent study using qPCR, Baigent et al. (2005) reported that an avirulent vaccine strain of MDV1 (CVI988 Rispens) was readily detectable in the feather tips after only 7 days, and subsequently rose to levels in this tissue that were almost 10^3-fold greater than that in the lymphoid tissues (spleen, bursa of Fabricius and thymus). In the current study, shedding of avirulent HVT in dander was certainly lower than that of virulent MDV1, but shedding of the avirulent MDV2 was greater than that of MDV1, suggesting that there is not an overarching relationship between virulence and the rate of viral replication and shedding from the feather follicle epithelium.

Both HVT and a bivalent vaccine combining HVT and MDV2 significantly suppressed MDV1 shedding in dander between days 14 and 28 p.c., but did not prevent MDV1 shedding. Indeed, the effect of vaccination was modest, and older vaccinated chickens continued to shed large amounts of MDV1 (~10^9 copies day^-1). The effects of HVT vaccination on MDV1 appear to be somewhat different in lymphoid tissues. In both splenic (Islam et al., 2005a) and circulating lymphocytes (Islam et al., 2006b), MDV1 load was unaffected by vaccination until days 21 and 28 p.c., respectively, with vaccinated chickens having significantly lower MDV1 loads thereafter. This differs from the pattern seen in dander in the present experiment in which the effects of HVT vaccination were greatest at days 14–28 and reduced thereafter, thus demonstrating early rather than late suppression of MDV1 replication in the feather follicle epithelium.

Shedding of HVT and MDV2 in dander was significantly higher in MDV1-challenged chickens than in mock-challenged chickens. This was an unexpected observation and clearly demonstrates that concurrent challenge with MDV1 enhanced both HVT and MDV2 replication and subsequent shedding in dander. The exact mechanism of such enhancement is unknown. It complicates the use of this technology for monitoring of vaccine efficiency, as higher vaccine virus loads in dander may not relate to vaccine quality or vaccination efficacy, but rather to co-infection with MDV1.

HVT was shed in dander throughout the experimental period, as has been reported previously (Islam et al., 2005b). It has also been shown that HVT is present in many dust samples from commercial broiler farms (Islam, 2006). Although HVT exhibits limited lateral transmission

Fig. 7. Estimated daily shedding of MDV per chicken. (a) Shedding of MDV1 in chickens vaccinated with HVT or HVT/MDV2 bivalent vaccine compared with mock-vaccinated chickens. (b) Shedding of all three serotypes of MDV (MDV1, MDV2 and HVT) in chickens either challenged or not challenged with three isolates of MDV1 at day 5 p.v. with bivalent (HVT/MDV2) vaccine.
in older chickens (Cho & Kenzy, 1975), transmission between broiler-aged chickens infected early in life is not thought to occur (Cho & Kenzy, 1975). This was confirmed in a recent study using molecular methods (Tink et al., 2005). This indicates that the HVT shed by chickens is largely non-infective for other chickens. With the CVI988 strain of MDV1, the original uncloned isolate spread readily by contact (Rispens et al., 1972), but the clone CVI988/C showed limited transmission between in-contact birds (Witter et al., 1987). The ability to spread is affected by serial passage and attenuation of CVI988 and this characteristic may vary among different subclones of the virus (Baigent et al., 2005).

This study provides the first estimates of dander production by individual broiler chickens (based on capturing dander in isolator exhausts) during their life span. The pattern of dander production was a typical sigmoidal growth curve and appeared to be closely associated with body weight, which exhibited a similar-shaped curve over this period. The method of calculation of dander production assumed that all dander shed by chickens was expelled in the isolator exhausts. This assumption clearly underestimated dander production, as dander accumulated over time in the isolators, although it was clear that most of it was expelled. For this reason, the estimates provided are likely to have underestimated total dander production by a small factor.

The combination of quantitative evaluation of viral load in dust and quantitative estimation of dust production by individual chickens enabled the first estimates to be made of actual shedding rates of the different MDV serotypes in commercial chickens under typical vaccination conditions. The combination of rapid increases in viral load in dander over the first 4 weeks of life, and a concomitant increase in dander production by individual birds led to a very rapid rise in shedding of all of the MDV serotypes over this period, followed by a plateau of viral shedding rates. A key finding was that the MDV2 strain examined had very high shedding rates, several-fold higher than that of three MDV1 isolates, whilst shedding of HVT was between 10- and 100-fold lower than that of the other two MDV serotypes. More importantly, there was a major interaction between the viruses in their effects on shedding rate. Vaccination with HVT or MDV2 provided significant, but not complete, protection against Marek’s disease, but had minor effects on viral shedding of MDV1 when birds were given a high challenge dose (500 p.f.u.) at day 5 of age. Indeed, vaccinated chickens continued to shed approximately 10^9 copies of MDV1 per day, approximately half the amount shed by unvaccinated chickens. On the other hand, infection with MDV1 caused marked increases (approx. 1–2 logs) in the shedding rate of HVT and MDV2.

The implications of these findings are numerous. Firstly, they provide data on which to base further studies into, or models of, the epidemiology of Marek’s disease and the spread of both vaccinal and virulent viruses in chicken populations. From a diagnostic sense, the findings are mixed. The ability to quantify either vaccinal or MDV1 viral load in poultry dust has considerable diagnostic and monitoring potential for MD in industry (Walkden-Brown et al., 2004, 2005; Islam, 2006) and indeed currently is being used for this purpose in Australia. However, the potential application of qPCR for monitoring vaccinal virus to confirm efficacy of vaccination is limited by the finding in this experiment that high values for vaccinal virus may be less a reflection of successful vaccination than an indication of post-vaccinal challenge with MDV1. It is likely, therefore, that routine monitoring of MDV using shed dust samples would be best based on analysis of MDV1 load alone. The other area where the findings of the present experiment find application is that of evolutionary biology. Marek’s disease is almost unique in that the combination of mass vaccination against this disease worldwide using vaccines that limit oncogenicity but do not preclude co-infection by virulent MDV1 appears to have resulted in a steady evolution in virulence of MDV1 such that MD vaccines fail every decade or so in the USA (Witter, 1998). Gandon et al. (2001) have proposed a theoretical framework by which imperfect vaccines such as the MD vaccines may contribute to the evolution of virulence. The present study provides objective and quantitative data to show that shedding of MDV1 in vaccinated chickens remains at high levels and thus may contribute to evolution in virulence of MDV1 to overcome the effects of vaccination.

In summary, this study has shown that: (i) all three serotypes of MDV are detectable in feather dander, even when all three are co-existing in the same population; (ii) there was no significant difference in viral shedding rates among three isolates of MDV1 of broadly similar virulence; (iii) vaccination with HVT alone or in combination with MDV2 had only limited effects on shedding of MDV1 by infected chickens; (iv) shedding of the vaccinal viruses MDV2 and HVT increases significantly with co-infection with virulent MDV1; (v) a commercial broiler chicken challenged with 500 p.f.u. MDV1 on day 5 of age sheds approximately 10^9 copies of the MDV1 genome per day from day 28 onwards, irrespective of vaccination status; and (vi) qPCR assays can play an important role in the monitoring of MD status in poultry flock by assaying MD load in feather dander.

ACKNOWLEDGEMENTS

A.I. received an Australian Postgraduate Award (Industry) from Australian Research Council project LP 0211607, which also directly supported the research, in association with Baiada Poultry Pty Ltd, Bioproperties Pty Ltd and the Queensland Department of Primary Industries and Fisheries. The pathotyping experiment on which this additional research was conducted was funded by the Australian Poultry CRC (Project 03-17) established and supported under the Australia Government’s Cooperative Research Centres Program. We are grateful for all of these sources of support. We also thank Ms Susan Burgess and Mr Paul Reynolds of the Centre for Animal Health and Welfare at UNE for their technical assistance, and Professor Greg
Tannock and Ms Julie Cooke of RMIT, Melbourne, Australia, for supplying the challenge viruses.

REFERENCES


