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Replication kinetics and shedding of very virulent Marek's disease virus and vaccinal Rispens/CVI988 virus during single and mixed infections varying in order and interval between infections

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ABSTRACT

Vaccination is thought to contribute to an evolution in virulence of the Marek's disease virus (MDV) as vaccines prevent disease but not infection. We investigated the effects of co-infections at various intervals between Rispens/CVI988 vaccine virus (Rispens) and very virulent MDV (vvMDV) on the replication and shedding of each virus. The experiment used 600 ISA Brown layer chickens in 24 isolators with all treatments replicated in two isolators. Chickens were vaccinated with Rispens and/or challenged with the vvMDV isolate 02LAR on days 0, 5, or 10 post hatching providing vaccination to challenge intervals (VCI) of −10, −5, 0, 5 or 10 days with the negative values indicating challenge prior to vaccination. Peripheral blood lymphocytes (PBL), feathers and isolator exhaust dust were sampled between 7 and 56 days post infection (dpi) and subjected to quantitative real-time polymerase chain reaction (qPCR) to differentiate the two viruses. Overall Rispens significantly reduced the viral load of vvMDV in PBL and feather cells and shedding in dust. Similarly vvMDV significantly reduced the viral load of Rispens in PBL and feather cells but not in dust. VCI significantly influenced these relationships having strong positive and negative associations with load of vvMDV and Rispens respectively. Differences between the two viruses and their effects on each other were greatest in PBL and feathers, and least in dust. This study expands our understanding of the interaction between pathogenic and vaccinal viruses following vaccination with imperfect vaccines and has implications for selection of appropriate samples to test for vaccination success.

1. Introduction

Marek's disease (MD) is an economically significant and rapidly progressive lymphoproliferative disease of chickens caused by a highly cell-associated infectious oncogenic alphaherpesvirus known as Marek's disease virus (MDV). There are three species namely gallid herpesvirus 2 (GaHV-2), gallid herpesvirus type 3 (GaHV-3) and meleagrid herpesvirus type 1, (MeHV-1) which are commonly referred to as MDV serotype 1 (MDV-1), MDV serotype 2 (MDV-2) and turkey herpesvirus (HVT) respectively (Osterrieder and Vautherot, 2004). Of the serotypes only MDV-1 causes clinical disease in chickens, the other two species are non-pathogenic and widely used as vaccines to

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protect against MD (Afonso et al., 2001; Schat and Calnek, 1978; Witter, 1982). The pathogenic MDV-1 viruses may be further classified on the basis of virulence using a range of methods. One widely used classification system subdivides MDV-1 into mild (m) MDV, virulent (v) MDV, very virulent (vv) MDV, and very virulent plus (vv+) MDV strains based on standardized tests in vaccinated and unvaccinated chickens (Witter, 1997; Witter et al., 2005).

Vaccination is the only known method to prevent susceptible chickens from developing lesions when infected with MDV. In 1970, the first vaccine was introduced to the industry to control MD (Churchill et al., 1969). This vaccine was based on the oncogenic HPRS-16 strain of serotype 1 MDV that had been attenuated by serial passages in chicken kidney cell culture (Churchill et al., 1969). This vaccine was replaced by the naturally avirulent HVT vaccine (FC126 strain), which was shown to be highly effective in preventing MD (Burmester et al., 1972; Ianconescu et al., 1971; Okazaki et al., 1970; Purchase and Okazaki, 1971). MDV isolates of increasing virulence have been isolated since the 1950s and, after the introduction of vaccines; this became associated with vaccine failure (Witter, 1998). When failure of protection with HVT occurred in the USA, a bivalent (HVT + MDV-2) vaccine was introduced in 1983 giving better protection against very virulent MDV (vvMDV) (Calnek et al., 1983; Witter et al., 1984). Again in the early 1990s major outbreaks occurred due to bivalent vaccine failure associated with the development of a new pathotype of MDV namely very virulent plus MDV (vv + MDV) (Witter, 1996, 1997). The Rispens/CVI988 vaccine virus, a mild and attenuated serotype 1 MDV provided superior protection against vv + MDV (Witter et al., 1995). In earlier studies, both HVT and Rispens provided excellent protection against MD (Bulow et al., 1976; Maas et al., 1982; Vielitz and Landgraf, 1971) but in later studies the Rispens vaccine provided better protection against highly virulent challenge strains of MDV (Witter et al., 1995). In Australia, Rispens was introduced in 1997 to control a major MD outbreak against which neither HVT nor MDV-2 were protective (Jackson, 2000). Vaccines of all three serotypes continue to be widely used worldwide alone or in combination, with the highly effective Rispens vaccine often used to provide protection in long-lived layer and breeder chickens.

While these vaccines prevent clinical MD they are "imperfect" vaccines as they not prevent co-infection with pathogenic MDV and long term shedding of virus of both vaccinal and wild-type virus (Islam and Walkden-Brown, 2007). Co-infection of imperfect vaccine organisms with the target organism can drive evolution towards greater virulence in the target organism (Gandon et al., 2001) and this is thought to contribute to the ongoing evolution of MDV towards higher virulence (Davison and Nair, 2005). Recent modeling of experimental data appears to confirm this (Atkins et al., 2012).

To properly understand how imperfect vaccine viruses affect the fitness of the target organisms requires studies into the basic replication and shedding of the viruses so that estimates of reproductive success can be made. Good progress has been made in studying the kinetics of infection with mixed infections of MDV with HVT and MDV-2 vaccines (Islam and Walkden-Brown, 2007; Islam et al., 2006b). For single infections with the Rispens virus the kinetics of viral replication in the early post infection stage in feather tips and lymphocytes has been reported (Baigent et al., 2005b) as have the longer term kinetics in lymphocytes, feather tips and dust, and the efficacy of transmission to unvaccinated chickens (Islam et al., 2013a). With the development of real time quantitative PCR (qPCR) assays which can differentiate between Rispens and pathogenic serotype 1 MDV the possibility of examining the effects kinetics of mixed infections of Rispens and pathogenic MDV became possible. An initial study examining kinetics of both viruses up to 21 dpc in *in ovo* vaccinated chickens challenged at 5 days of age found that vaccination with Rispens reduced MDV load in feathers but challenge with MDV did not affect the load of Rispens virus (Haq et al., 2012).

The kinetics of viral replication and shedding differ somewhat between vaccinal and wild-type MDV. In peripheral blood lymphocytes (PBL) Rispens virus tends to show an early peak in viral load in PBL at day 7 (Islam et al., 2013a) or 14 (Baigent et al., 2005a) after vaccination before declining whereas wild-type MDV in PBL continues to increase, at least to day 35 post infection (Islam et al., 2006a). Baigent et al. (2005a) also reported a similar early peak in Rispens viral load in feather tips at 14 days post vaccination (dpv) before gradually decreasing until the last day of their experiment at 28 dpv. On the other hand the viral load of Rispens in dust shows no such early peak and remains at high levels up to 56 dpv (Islam et al., 2013a), albeit at much lower levels than reported for pathogenic MDV. Lower load of Rispens than pathogenic MDV has also been reported in feather tips (Haq et al., 2012).

Co-infection of chickens with vaccinal and pathogenic MDV can also influence viral kinetics and shedding. Vaccination has been shown to reduce pathogenic MDV load in lymphoid cells (Baigent et al., 2011; Islam et al., 2006a, 2008; Walkden-Brown et al., 2013), feathers (Baigent et al., 2011; Haq et al., 2012) and dust (Atkins et al., 2012; Renz, 2008). On the other hand, challenge with MDV at 5 dpv was shown to greatly increase the shedding of HVT and MDV-2 vaccinal virus in dander relative to vaccinated but unchallenged chickens (Islam and Walkden-Brown, 2007). However Haq et al. (2012) found that challenge with vvMDV did not change the viral load of Rispens in feather tips. The effects of vaccination on viral load of pathogenic MDV may be dependent on the interval between vaccination and challenge. Islam et al. (2008) reported that the reduction in MDV-1 load in spleen cells caused by vaccination with HVT increased as vaccination to challenge interval was extended up to 7 days.

Recently Renz et al. (2013) reported a qPCR method to differentiate between the Rispens virus and Australian isolates of pathogenic MDV-1. Based on the above discussion, we designed an experiment using this method to determine the effects of widely divergent vaccination to challenge intervals, including challenge prior to vaccination, on the level of vaccinal protection provided by the Rispens vaccine and the replication kinetics of Rispens and vvMDV in PBL, feather tips and dust samples.