Spotlight on avian pathology: Marek’s disease

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ABSTRACT
Marek’s disease (MD), characterised by rapid-onset lymphoid tumours, immunosuppression and paralysis, is one of the most widespread economically important diseases of poultry world-wide. Caused by the highly contagious Marek’s disease virus, control of MD is mainly achieved through vaccination with live attenuated vaccines, although improvements in genetic resistance have also been an important component of disease control. Despite the overall success of the vaccination policy in controlling MD in the last 40 years, continuous evolution of virulence and emergence of hypervirulent pathotypes remains the major challenge for sustainable control of this disease.

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Background
Marek’s disease (MD) is an important viral disease of poultry associated with rapid-onset lymphoid tumours, paralysis and immunosuppression. Caused by the highly contagious Marek’s disease herpesvirus (MDV), MD is initiated by the inhalation of infected poultry house dust and dander where the virus can remain infectious for long periods (Schat & Nair, 2013). Affected birds remain infectious for life continuously shedding the virus into the environment contaminating the poultry house environment. MD has been reported in all poultry-rearing countries (Dunn & Gimeno, 2013; Mete et al., 2016) and a 2004 estimate suggested that MD causes annual losses of up to US$2 billion to the poultry industry (Morrow & Fehler, 2004).

Pathogenesis
Pathogenesis of MD has many unique distinguishing features. During the early cytolytic phase, lasting for approximately one week after infection, MDV causes massive destruction of lymphoid cells and macrophages inducing severe immunosuppression (Schat & Nair, 2013). After this period, MDV enters a lifelong latent phase in the CD4+ and CD8+ T cells where most of the viral gene expression is shut off. Some of the latently infected CD4+ T cells are neoplastically transformed leading to the development of multiple lymphomas in visceral organs resulting in mortality from around 3–4 weeks post infection. Paralysis, due to the lymphoid infiltration of the peripheral nerves, also occurs in some of these birds. In these different cell types, MDV remains cell-associated and is spread by cell-to-cell transmission. However, from around 10 days after infection, the infection is transferred to the feather follicle epithelial cells, the unique cell type from where the cell-free infectious virus is shed into the poultry house environment for long periods of time, acting as a source of infection to naïve newly introduced birds (Jarosinski, 2017). Thus, MDV shows infection of different types of cells with distinct virus–host interactions and outcomes.

Disease control
Vaccination with live attenuated vaccines, introduced since the early 1970s, is the single most important method of control (Biggs & Nair, 2012), although biosecurity measures and selection of birds for genetic resistance can also contribute towards the control of the disease (Bumstead & Kaufman, 2004). Vaccines used against MD include attenuated strains of MDV-1 (e.g. CVI988), antigenically related MDV-2 (e.g. SB-1) or Herpesvirus of turkeys (HVT Fc126), all of which are effective either as a single vaccine or in combination as multivalent vaccines. As most of these vaccine viruses are strictly cell-associated, they have to be injected as live infected cells, and hence must be stored and transported in liquid nitrogen (Schat, 2016).

MD vaccines are very effective in protecting the birds against the disease, preventing clinical development of tumours, immunosuppression and paralysis (Boodhoo et al., 2016) and have played a major role in the sustainable growth of the poultry industry for the last five decades (Biggs & Nair, 2012). However, the current MD vaccines have a limited effect on viral infection and transmission. Hence, vaccinated birds
continue to get infected and transmit the virus to the environment encouraging the evolution of MDV towards increased virulence (Gimeno, 2008). As a consequence, several countries with high-intensity poultry production have witnessed the emergence of the viruses classified into mild (mMDV), virulent (vMDV), very virulent (vvMDV) and very virulent plus (vv + MDV) pathotypes (Dunn et al., 2014). Our recent study demonstrated the direct effect of HVT vaccine in facilitating the spread of the vv + MDV strain 675 by preventing mortality. The study also showed the importance of the vaccinated population for sustaining vv + MDV strains, which otherwise would have been eliminated with the death of the infected birds from the population (Read et al., 2015).

**Diagnosis**

As a ubiquitous pathogen present in many poultry farms, detection of virus, viral antigens or nucleic acids in the absence of disease does not confirm the occurrence of MD. Clinical disease with signs of paralysis of legs and wings, higher than normal levels of mortality with lesions of tumours in multiple organs, and enlarged peripheral nerves is often sufficient to make a positive diagnosis. Detection of viral- or tumour-specific antigens in tumours by immunohistochemistry is valuable for further confirmation of MD (Gimeno, 2015; Gimeno & Wakenell, 2016). PCR-based molecular diagnostic tests are increasingly used for the detection and quantitation of virus in clinical/ farm materials. Availability of the nucleotide sequences of a number of pathogenic and vaccine strains of MDV has enabled the development of sensitive PCR methods for precise detection and quantitation of pathogenic and vaccine strains (Baigent et al., 2016; Kennedy et al., 2017).

**Opportunities**

Recent advances in molecular biology and sequencing technologies are providing great insights into the diversity of viruses as well as the dynamic changes in the host and viral gene expression, for a better understanding of the molecular pathogenesis of the disease to help in developing further intervention strategies. As large DNA viruses, MD vaccine strains also have demonstrated their potential as viral vectors giving opportunities for developing novel recombinant vaccines against multiple avian diseases (Reddy et al., 2017). Among the herpesvirus vectors, HVT is the most widely used vaccine vector against avian diseases (Gimeno et al., 2016; Baron et al., 2018). Recent success in the commercial development of the recombinant HVT vector for simultaneous protection against infectious bursal disease and Newcastle disease demonstrates the increasing interest for multivalent vaccines for hatchery-level vaccination. Application of the recent advances in CRISPR/Cas9 gene editing technologies for developing recombinant herpesvirus vector vaccines (Baron et al., 2018) will help in speeding up the development of such multivalent vaccines that will potentially induce protection against multiple avian viruses.

**Research challenges**

Some of the major research challenges in the area of MD pathogenesis are to understand the virus–host interactions during latency and virus reactivation, molecular events that trigger neoplastic transformation of clonal populations of the latently-infected CD4+ T cells that eventually go on to become lymphomas. Recent advances in the ability to track marker viruses in different cell types of infected chickens, together with tools to carry out analysis of viral/host gene expression, will help in getting a detailed understanding of the virus–host interactions to help in addressing some of the scientific challenges including those outlined below:

- Technological developments in simpler production methods of cell-free vaccines against MD will be a significant step forward for producing cheaper effective vaccines, but is a significant challenge.
- Evolution of MDV virulence remains the major challenge for the future control of the disease. Efforts to gain a better understanding of vaccine-induced protection and research towards the development of vaccines that can prevent/reduce transmission of the virus are critical for a sustainable vaccination-based control strategy against MD.
- Development of molecular markers for typing MDV pathotypes remains a top priority, as the current pathotyping assays are cumbersome involving the use of a large number of experimental birds.

**Disclosure statement**

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