Recent developments in Marek's disease vaccination

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Marek's disease (MD) was first described as a polyneuritis by Jozsef Marek in 1907 in Hungary. Later, it was found that the disease included lymphoid tumors and was likely infectious.

The disease became more important with high levels of mortality (up to 30%) when the poultry industry started to grow at an industrial scale in the 1950s and 1960s.

The pathogen was isolated and identified as a herpesvirus (MDV) in 1968; its complete genome sequence was obtained in the 1990s. The main phases of MDV infection were identified in the 1980s and later:

- Chickens are infected via the respiratory tract by infected danders.
- The virus is likely taken up and moved to lymphoid organs by macrophages.
- The virus induces an early lytic infection mainly in B-lymphocytes, that induces an inflammatory response and T-lymphocyte activation.
- Activated-Tcells are then rapidly (7-8 days post-infection) latently infected.
- Late cytolytic infection occurs in tissues of epithelial origin, especially in the feather follicle epithelium where cell-free viruses are produced and shed as infected danders.
- Neoplastic transformation takes place in latently-infected T-cells.

The influence of poultry genetics on MD susceptibility has been known since the 1970s and is taken into account by breeder companies.

Three types of vaccine

Three different MDV serotypes belonging to three different virus species have been described: serotype 1 (MDV1) that includes all the oncogenic MDV and their attenuated form (Gallid herpesvirus 2), serotype 2 (MDV2) that contains the non-oncogenic MDV (Gallid herpesvirus 3), and serotype 3 (MDV3) which is represented by herpesvirus of turkeys (HVT or Meleagrid herpesvirus 1). They all belong to the taxonomic genus Mardivirus, in the alphaherpesvirinae sub-family.

Marek's disease vaccine development started in 1969 and was rapidly successful. They were the first vaccines developed to control a tumorigenic disease. Most MD vaccines are produced in primary chicken embryo fibroblasts (CEFs) grown in roller bottles and are stored as live infected CEFs in glass ampoules frozen in liquid nitrogen.

HVT is a non-pathogenic virus of turkeys that is antigenically related to MDV. It is the only MDV serotype that can be freeze-dried. HVT-based vaccines were developed in the early 1970s either as cell-associated or as cell-free viruses. The latter was shown to be more sensitive to maternally-derived antibody (MDA) interference.

Non-oncogenic MDV2 vaccines were developed in the early 1980s. The SB-1 strain is the most widely used but the protection levels induced by MDV2 vaccines as stand-alone vaccines were poor. Interestingly, a true synergistic effect on protection was obtained when SB-1 was co-administered with HVT.

MDV1 of low oncogenic potential were further attenuated by serial passages in cell culture. The most effective MDV1 vaccine strain is CVI988, which was isolated in the Netherlands in the early 1970s and characterised by Bart Rispens. This Rispens strain is still considered today the most efficacious licensed MD vaccine despite four decades of research aiming at finding a better alternative.

Bivalent MD vaccines have also been developed. The most frequently used combinations are HVT+SB-1 and HVT+CVI988. The synergistic protective effect of HVT+SB-1 has been well documented. It is rarely detected with HVT+CVI988 in test conditions, but it may have some advantages in field conditions. A trivalent HVT+SB-1+CVI988 combination is also used in some countries. Chicken genetics also has an impact on the efficacy of the different types of MD vaccines.

The immune mechanisms of MD vaccine-induced protection are poorly understood. MD vaccines protect against clinical signs but they do not protect against wild type MDV infection, shedding or transmission.

Marek's disease virus virulence has increased from the 1950s to the 1990s and the introduction of vaccines may have been partially responsible. Indeed, in the US, very virulent MDV (vvMDV) and vv+MDV emerged in the 1980s and late 1990s after the use of HVT and bivalent HVT+SB-1 vaccines in the early 1970s and 1980s, respectively. The introduction of CVI988 vaccine in the US in the late 1990s was apparently not followed by a further increase of MDV virulence.

MD vaccine administration

MD vaccines are administered at the hatchery either by the in ovo route, three days before hatch, or by the subcutaneous (SC) route at hatch using specifically designed vaccination equipment.

The correct in ovo site of administration is the amniotic sac or the SC route. A double vaccine dose and/or re-vaccination with the same or a different MD vaccine are sometimes used in the field (especially in Europe, the Middle-East and Africa) to increase efficacy. Re-vaccination-improved efficacy was demonstrated in an experimental early (two days) vv+MDV challenge model for the combination HVT or HVT+SB1 in ovo followed by CVI988 at hatch; the in ovo administration of HVT may hasten the maturation of the immune system.

Vaccine take can be followed up by specific PCR test on spleen or feather follicle samples. However, the viral load is highly variable and may not correlate with protection. In contrast, the genome load of the challenge virus detected by a specific PCR is a good indicator of whether or not the bird will be protected.

Outbreaks of MD sometimes occur in MD-vaccinated flocks. Causes of vaccine failures may be multiple. Storage, shipping and handling of MD vaccines with no break in the cold chain (liquid nitrogen) is absolutely necessary. An MD vaccine ampoule that has been thawed accidentally should never be put back in liquid nitrogen because the quick freezing will kill most of the vaccine-infected cells.

Proper procedures for vaccine thawing and dilution in MD vaccine diluent are essential to conserve cell viability. The vaccine should not be diluted in titer; using a full dose is critical to get the quick onset of immunity needed for MD protection.

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Only a few antibiotics are compatible with MD vaccine; both the antibiotic active ingredient and excipient can have a negative impact on MD vaccines and it is important to use the antibiotic formulation from a specific provider proven to be compatible with MD vaccines.

The administration of MD vaccines in ovo or SC should be carefully performed with well controlled equipment and trained technicians. Some MD vaccines (CVI988, SB-1 but not HVT) are transmitted horizontally but this horizontal transmission will occur too late to provide field protection of non-vaccinated chickens and that is the reason why care should be taken to vaccinate all birds at the hatchery.

Good management practices (such as 'all in/all out', cleaning and disinfection, biosecurity) and control of early immunosuppression (such as those induced by chicken anaemia virus and mycotoxins) are essential to get optimal MD vaccine protection.

New generation of vaccine

Attempts to generate new attenuated vaccines by serial passages of virulent isolates have not given the required safety in SPF birds or efficacy in commercial birds. The genetic basis of attenuation during passages is poorly known. The development of biotechnology has allowed generation of new types of MD candidates.

Fowlpox vector expressing the gB gene was shown to protect SPF chickens against MD; however the efficacy dramatically decreased in commercial birds with MDA. Chimeric HVT, in which the HVT unique short region (US) of the genome was replaced by MDV1 US, was developed but never launched, possibly because this chimera did not bring advantages over existing vaccines. Insertion of cytokine genes into the HVT vector or co-administration of MD vaccines with immunostimulants (cytokines or pattern recognition receptor agonists) has sometimes increased MD protection.

Cosmid clones containing overlapping MDV genomic fragments or BACmid containing the whole MDV genome are now used to generate genetically modified MDV. A vvMDV Md5 virus deleted for the Meq gene, the well known MDV oncogenic gene, was shown to be better protective than the CVI988 vaccine. However, the deleted virus induced lymphoid organ atrophy. This could be suppressed by 20 additional in vitro passages, but the virus lost its protective advantage in commercial birds.

Another strategy to improve MD vaccines is the insertion of retrovirus long terminal repeat (LTR) in the MDV genome. The RM1 virus was obtained by co-infection of the vMDV JM/102W with reticuloendotheliosis virus (REV). It contains the REV LTR in the repeat region flanking the US. The RM1 virus was shown to be non-oncogenic and highly protective against vv+MDV.

However, it induced severe and persistent thymus atrophy. Lupiani et al. (2013) generated a CVI988-based chimeric virus that contains the same RM1 LTR insertion. This virus was shown to be safe and to induce a better protection than CVI988. None of these potentially new Marek's vaccines has been licensed so far.

Vectors for other diseases

The HVT MD vaccine has been developed as a vector for vaccines against other poultry diseases, including IBD, ND, ILT and AI. The major advantages of this vector are

- Excellent safety.
- Long duration of protection due to the persistence of HVT infection.
- Hatchery administration (SC or in ovo).

The HVT-IBD vaccine has been shown to be better protective than IBD modified-live vaccines (MLV). HVT-ND and HVT-ILT induce a slower onset of efficacy than the corresponding MLV and do not protect as well against local replication in the respiratory tract. Using both ND MLV and HVT-ND improved the onset and/or duration of ND immunity. The use of HVT-based vector vaccines in the hatchery has allowed a decrease in field vaccination with MLV.

The HVT based vector vaccines can be combined with SB-1 or CVI988 to improve the MD protection but most of them cannot be combined together or with parental HVT due to interference.

Conclusion and perspectives

Marek's disease vaccines have efficiently controlled MD since the early 1970s. Despite 40 years of research, the CVI988 vaccine remains the best licensed MD vaccine. Biotechnology has allowed the generation of new vaccines, some of which are more efficacious than CVI988 in experimental trials, but none have reached the market so far. The recent development of HVT as a vector for other poultry diseases has been very successful and has contributed to the move of vaccination practices from the field to the hatchery. A better understanding of the mechanisms of protection could lead to the generation of new MD vaccines and formulations that could potentially block transmission of the challenge virus and therefore, decrease the risk of increasing its virulence.

The new design of MD vector vaccines against different diseases that are fully compatible at the hatchery is a big challenge for future poultry vaccination.

References are available on request