Optimising Marek's disease control with in ovo vaccination

One of the greatest concerns facing the poultry industry is the evolutionary trend of Marek's disease virus (MDV) toward greater virulence. Although Marek's disease (MD) has successfully been controlled by vaccination since 1970, the regular emergence of new and more virulent serotypes has required control strategies to evolve as well.

by Tarsicio Villalobos, Technical Services Director, Global Biodevices and Automation, Zoetis Inc.
www.zoetis.com

Caused by an oncogenic herpesvirus, MD is associated with the development of tumors in adult birds, but some of its most damaging effects result from chronic immune suppression starting much earlier in the bird's life.

Recent research suggests that in ovo vaccination may not only provide superior MD protection, but when a turkey herpesvirus (HVT) vaccine is used, it may also have a positive impact on the overall development of the embryonic chicken's immune system.

Shift to greater virulence

MD is one of the most significant viral diseases of poultry globally, costing an estimated $1 billion to $2 billion in losses per year. The highly contagious disease has a wide range of manifestations, including immunosuppression, paralysis and other neurological problems, cutaneous lesions, visceral tumours and blindness. With morbidity of 10% to 50%, the disease also has extremely high mortality, reaching up to 100% in severe cases.

The virus that causes MD sheds through the feather-follicle epithelium and is transmitted through the respiratory route by airborne dander and contaminated dust.

Ubiquitous in poultry environments worldwide, MD wild virus exposure is virtually impossible to prevent and all flocks are considered infected. However, effective disease control can be achieved with vaccination, especially when combined with good biosecurity, hygiene, ventilation and general flock management.

According to the US National Agricultural Statistics Service, broiler condemnation losses due to MD decreased more than 99% since vaccines were introduced, dropping from 1.5% in 1970 to 0.003% in 2006.

While vaccination provides the best defence against the potentially devastating effects of MD, the emergence of new and more virulent MD wild virus strains has forced the continuous development of new vaccines and vaccination strategies.

As shown in Fig. 1, in the past HVT and bivalent vaccines (a combination of serotype-3 HVT and serotype-2 SB-1) provided adequate protection; now they are both still necessary but not always sufficient, depending on disease epidemiology.

Currently, the serotype-1 MDV strain CVI988 offers the greatest protection against virulent MD, especially when used in combination with other vaccines.

In a 2015 study, vaccine protocols that included CVI988 were found to provide better protection against the highly virulent MDV strain 648A than protocols that used only vaccines of serotypes 2 (SB-1) and 3 (rHVT) (Fig. 2) .

The inclusion of CVI988 in MD vaccination programs is therefore one important way to ensure adequate protection against virulent MD. However, given the tendency of MDV to evolve toward greater virulence, there is an urgent need to continue developing and optimising vaccination strategies for maximum disease control.

In ovo vaccination

One strategy that has proven highly effective over a prolonged period of time is in ovo vaccination, whereby a vaccine is delivered inside the egg during late-stage development to a specific site capable of stimulating immune

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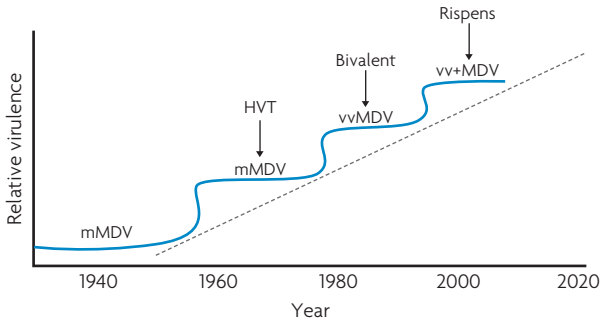


HVT: Turkey herpes virus; m: Moderate; v: Virulent; vv: Very virulent; vv+: Very virulent +

Fig. 1. Vaccines and MDV evolution. Vaccines were introduced at the time shown by the arrows. HVT, bivalent vaccine (HVT + serotype 2 SB-1) and Rispens (CVI988 strain) vaccine (from Witter, 1996 with permission from Avian Diseases).

Fig. 2. Protocols with CVI988 and/or in ovo provide the best protection against early challenge. Different letters indicate statistical difference, numbers represent protection index (Pi).

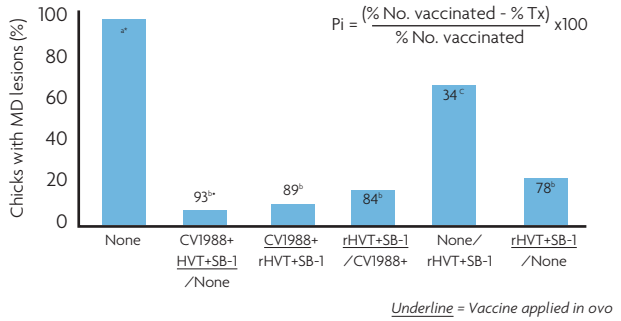
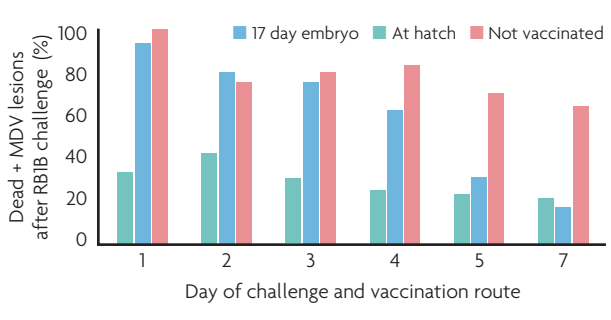


Fig. 3. In ovo vaccination provides better protection.



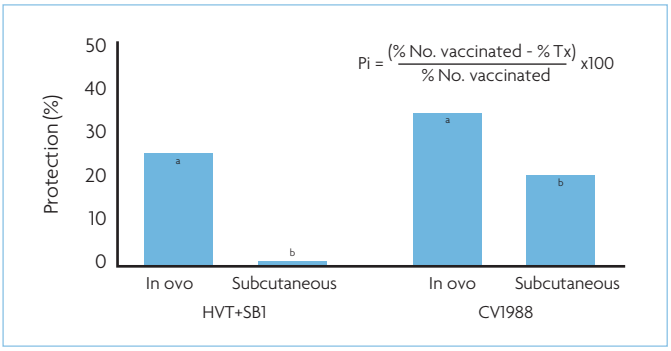


Fig. 4. In ovo vaccination at ED 18 vs. subcutaneous vaccination at hatch, with MDV challenge at day two. Different letters above each column indicate statistical difference.

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response. Studies show that all commercially available MD vaccines offer better protection when administered in ovo compared to subcutaneous vaccination at hatch.

In a 2001 study, a CVI988 vaccine was tested in specific-pathogen-free chickens to determine if it induces early post-hatch protection against MD. In one group, the vaccine was injected in ovo on embryonation day (ED) 17, and in a second group, it was administered subcutaneously at hatch.

At 1, 2, 3, 4, 5 and 7 days of age, chickens from each group were exposed intra-abdominally to MDV RB1B, a virulent serotype-1 MDV variant, and sampled to determine MDV lesions.

Results indicate that in the first four days after hatch, the in ovo vaccinated chickens were significantly better protected against virulent MDV than those vaccinated at hatch ($P<0.001$) – an important benefit in view of the very young age at which chicks are first exposed to the virus (Fig. 3).

Another study on the optimisation of double vaccination protocols produced similar findings in 2012. In this study, chicks were given the same CVI988 and bivalent (HVT+SB1) vaccine combinations either in ovo at ED 18 or subcutaneously at hatch, and then challenged at two days of age with highly virulent MDV 648A. Once again, in ovo vaccine application was found to induce significantly higher levels of MD protection than subcutaneous injection ($P<0.05$) (Fig. 4).

Txt	$CD45^+$ $MHCI^-$	$MHCI^+$	$CD45^+$	$CD3^+$ $MCHII^+$	$MHCII^+$	$CD3^+$	$CD4^+$ $CD8^+$	$CD4^+$ $CD8^-$	$CD8^+$	$CD4^+$
Sham 1d	9^a	18^a	10^a	0.7^a	6^a	2^a	0.8^a	4^a	2^a	5^a
HVT 1d	28^b	71^b	29^b	2^b	18^b	10^b	6^b	8^b	12^b	13^b
7d	18^c	79^b	18^b	5^c	12^c	12^b	5^b	6^b	11^b	12^b
14d	20^c	85^b	20^b	4^c	11^c	12^b	7^b	7^b	13^b	13^b

Table 1. Immunophenotypes in spleen. Different letters within each column indicate statistical difference.

Maturation of chicken embryo immune responses

While CVI988 offers the greatest protection against virulent MD, HVT vaccines remain important tools for MD management.

For more than 40 years, they have been used successfully to control MD, both alone and in combination with other vaccines, but recent research shows that they may also contribute to the young chicken's immune development.

A study was conducted to determine the effect of in ovo administration of a commercially available HVT vaccine on the maturation of the embryonic immune system.

Following vaccination of specific-pathogen-free chickens on ED 18, researchers evaluated splenic cell phenotypes at one day of age and the ability of the day-old chicks to respond to various antigens compared with older birds.

Results demonstrate that day-old chicks that had received HVT in ovo had higher percentages of several lymphocyte lineages in the spleen than day-old sham-inoculated chicks (one-day sham) (Table. 1).

Furthermore, in ovo administration of HVT rendered chicks at hatch more responsive to unrelated antigens, such as concavalin A and keyhole limpet hemocyanin. These findings suggest that it is possible to accelerate maturation of the embryonic immune system by administering HVT in ovo.

Summary

Early and effective protection is critical to safeguarding flocks against the potentially devastating effects of MD. Multiple studies demonstrate that in ovo vaccination with any of the commercially available MD vaccines has a positive impact on vaccine performance, with protocols including CVI988 vaccines offering the greatest MD protection.

Furthermore, in ovo administration of HVT vaccines has been shown to have a positive impact on the immune development of embryonic chicks, an important benefit in view of the well-documented immunosuppressive effects of MD.

These findings suggest that in ovo vaccination programs that include vaccines of all MDV serotypes (CVI988, SB1 and HVT) may offer optimal protection against MDV as the virus continues evolving toward greater virulence. ■

Ensuring successful in ovo vaccination

Successful in ovo vaccination depends on the consistent delivery of vaccine to the correct site inside the egg. To ensure this, in ovo injectors must optimally perform five key functions:

1. **Adaptable egg location for proper needle trajectory**
   Once eggs are in the incubation tray, the embryo orientation is subject to change due to egg size and incubation turning and, as a result, the eggs may lean off-centre in the tray. To compensate for these changes, a system that moves the injectors both vertically and horizontally to allow the needle to aim at the centre of the egg's interior is critical to ensure the correct trajectory.
2. **Consistent shell penetration**
   A system with dual-needle injection that applies a separate needle and force for shell penetration to limit the risk of embryo exposure to outer-shell contaminants while helping prevent egg cracking.
3. **Gentle vaccine delivery**
   Appropriate pumping pressure with minimal turbulence in vaccine flow pathway from bag to needle ensures no damage to cell associated MD vaccines during delivery.
4. **Accurate site of injection**
   Proper needle trajectory and injection depth ensure vaccine is deposited in the amnion or embryo, inducing an active immune response and earlier protection. Conversely, administration of MD vaccine to the air cell or allantois sac provides poor protection.
5. **Effective needle-punch sanitation**
   The usage of a dual-needle system allows for more efficient sanitation while greatly reducing the risk of egg to egg contamination during the injection process.