

Review

A novel combined approach to Marek's and Gumboro disease control

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Marek's disease virus (MDV) and infectious bursal disease virus (IBDV) are the causative agents of Marek's disease (MD) and infectious bursal disease (IBD), also called Gumboro disease, two of the most serious diseases of chickens which are of major economic importance to the poultry industry worldwide. MD is a common lymphoproliferative tumourigenic disease of chickens, usually characterised by mononuclear cellular infiltrates in peripheral nerves and various other organs and tissues. The disease is caused by a herpesvirus, is transmissible, and can be distinguished etiologically from other lymphoid neoplasms of birds. Prior to use of vaccines MD caused high mortality in pullets and laying hens as well as high condemnations and sometimes significant mortality in meat type chickens. IBD is a highly contagious disease of young chickens, especially between three and six weeks of age, which can develop into an acute syndrome clinically characterised by depression, ruffled feathers and high mortality rate. Gross lesions can be represented by swelling, oedema and haemorrhages of the bursa of Fabricius (the main target of IBDV), haemorrhages in the muscles and kidney changes in the

advanced stages of the disease. IBDV is a small, non-enveloped double-stranded RNA virus, belonging to the Birnaviridae family. There are two serotypes of IBDV, designated 1 and 2 but only serotype 1 viruses are pathogenic. VP2 and VP3 are the major structural proteins, forming the outer and inner capsid of the virus, respectively. The antigenic site responsible for the induction of neutralising antibodies is within a minimal region, called the variable domain of VP2, and is highly conformation dependent. This site is also responsible for serotype specificity. Conversely, VP3 is a group-specific antigen that is recognised by non-neutralising antibodies, which may cross-react with both serotypes. The impact of IBDV depends upon the IBDV strain, the type of chickens (commercial pullets are more susceptible than meat type chickens), their immune status as well as management factors. At the end of the 1980s, very virulent (vv) IBDV strains in Europe (antigenically similar to the 'classical strains') emerged in vaccinated flocks and rapidly spread all over the world. Infection with classical virulent strains results in high morbidity and usually low mortality, whereas vvIBDV strains can cause up to

90% of mortality in layer type birds. Direct economic losses usually occur in case of clinical forms of MD and IBD, but even in case of subclinical infections the damage caused to the immune system results in lowered resistance to other infectious agents and in a poor immune response to commonly used vaccines. The ultimate consequence of such condition is a lower overall performance of the flocks affected and an increase in production costs. No treatment exists for either disease, and chickens can only be protected by strict hygienic measures and vaccination.

Traditional vaccination

Since MDV and IBDV are widespread on industrial chicken farms and in field conditions can retain their infectivity for a long time, the sanitary measures commonly applied are not sufficient to control these infections and vaccination has become an essential tool for the protection of chickens. Traditionally vaccinations against MD and IBD are carried out separately, at different stages of the production cycle and using different tools with varying reliability. Vaccination against MD is generally carried out at the hatchery by professionals, injecting mainly cell-associated vaccines in one day- old chicks or in ovo, with the aim of protecting birds from an early exposure to the field virus. However, not everyone is aware of the economic impact of MD and even today many broiler flocks remain unvaccinated and unprotected, therefore even in the absence of

clinical signs the production performance. A strategy for control of IBD in chicks is to hyper-immunise dams with IBD inactivated vaccines so that they can transmit high levels of maternally derived antibodies (MDA) to the progeny. Although MDA provides protection during the first few weeks of life, protection against IBDV must be maintained by administering modified live vaccines (MLV) before MDA reaches sub-protective levels. Different modified live vaccines (MLV) have been developed and classified as 'mild', 'intermediate', 'intermediate plus' IBD vaccines, depending upon their ability to break through MDA. MLV sometimes are not completely efficacious against vvIBDV, particularly when they are applied in the presence of significant MDA titres. Experimental studies have shown that IBD vaccine viruses may even be completely neutralised by MDA, leading to a significant delay or even prevention of induction of humoral immunity. MLV can induce moderate to severe bursal lesions and immunosuppression that can impair a chicken's response to other vaccinations. Recently, field studies in broilers vaccinated with a live intermediate vaccine demonstrated that the induction of humoral immunity clearly correlated with the induction of bursal lesions and IBDV replication; if birds were vaccinated at the optimal time, all vaccinated flocks developed IBDV antibodies as well as bursal lesions up to 14 days post vaccination. IBD vaccination on farm by drinking water requires accurate application to maximise the

percentage of birds receiving a protective dose.

Innovative single vaccine

A new vaccine combining excellent safety and efficacy in the presence of high MDA was required. Since the turkey herpesvirus (HVT) has been widely used since the early seventies as a MLV against MD, and is well known to be safe and poorly sensitive to interference from MDA, it has been proposed as a vector for IBD and other diseases. vHVT13 is a vector vaccine in which the HVT is used as the vector expressing the protective IBDV VP2 gene inserted into its genome, to achieve protection against MDV and IBDV. Like conventional HVT vaccines, it is a cell associated vaccine that can be administered at a single dose regimen by the in ovo route three days before hatching or by the subcutaneous (s/c) route to one day old chicks. Due to the mechanism of action of vHVT13, the immune response and the consequent protection against IBDV is triggered only by the IBDV-VP2 protein produced by the vector during the replication. Consequently the chickens vaccinated synthesise only anti-VP2 antibodies that are protective against all types of IBDV challenges (classic, variant and very virulent strains). Furthermore, vHVT13 stimulates a level of protection against Marek's disease equivalent to the HVT parental vaccine strain.

Safety

Safety of vHVT13 was evaluated in one day old SPF chickens by scoring microscopical lesions of the bursa between three and 35 days after s/c vaccination using the scoring system (from 0 to 4) described by Muskett et al. [1]. The vHVT13 vaccine did not induce visible gross pathological changes or significant microscopical lesions (mean score ≤ 0.6) of the bursa, whereas an intermediate MLV strain tested in the same conditions induced moderate to severe bursal lesions (mean score of 2-3) from days three to 14 postvaccination.

Immunogenicity

The immunogenicity of vHVT13 was tested by challenge against a French vvIBDV strain (91-168) two and eight weeks after s/c vaccination of SPF chickens and full protection was achieved (data not shown). In order to evaluate the effect of MDA specific for IBD and HVT, the efficacy of vHVT13 administered by the s/c application was tested in broilers vaccinated and raised in experimental conditions. Results from a challenge performed with avvIBDV (91-168) at three and six weeks post-vaccination, showed that vHVT13 induced full protection despite the presence of very high concentrations ($>4 \log_{10}$ SN antibody titres) of anti-IBDV MDA at the time of vaccination. The cell-associated nature of vHVT13, the lack of expression of VP2 on the surface of infected cells or of the HVT vector virus, and the mode of replication of the HVT vector, probably all contribute to

the ability of this vaccine to overcome MDA. Protection against MD challenge (RB1B or GA22 strain) and compatibility with the Rispens MD vaccine were also assessed after s/c or in ovo vaccination. The protection induced against MD was similar to that induced by HVT-based MD vaccines, and combination of vHVT13 with the Rispens MD vaccine did not decrease the vHVT13 induced IBD antibody level (data not shown).

IBD serology

Vaccination with vHVT13 induces seroneutralising anti-VP2 antibodies which are likely protective as described previously and as confirmed by vaccination/challenge experiments in both broilers and pullets. IBD antibody titres can be evaluated using two types of ELISA kits, a 'standard' one such as ProFLOK[®] IBD Ab test, Synbiotics, USA, and an 'improved' kit, ProFLOK Plus IBD Ab test, Synbiotics, USA. Both types of kits are indirect ELISAs and the principle of both tests is similar but the difference between them lies in the nature of the IBDV antigen coated on the plates. The ProFLOK IBD Ab test is thought to detect mainly the antibodies produced against the VP3 protein of IBDV after a natural infection or vaccination with an MLV. ProFLOK Plus IBD Ab test allows a better detection of anti-VP2 antibodies; data suggest that when these antibodies are detectable at significant levels, birds are shown

to be protected. The combined use of the classic and IBD Plus ELISA kits makes it possible to differentiate birds vaccinated with vHVT13 from birds infected or vaccinated with MLVs. An early active immune response can be induced in commercial chickens after in ovo or day-old vaccination with vHVT13 even in the presence of high MDA, preventing an almost complete decline of MDA as normally observed when using IBD MLV.

Conclusion

This innovative vectored vaccine, with a single dose applied at the hatchery, stimulates an early and lifelong protection. It removes the doubts about the right timing for vaccination and the compromise of considering safety versus efficacy for IBD vaccination that poultry veterinarians currently face with the use of classical IBD MLV. By using two types of commercial IBD ELISA kits it is possible to monitor antibodies elicited by vHVT13 and differentiate them from those induced by an IBD field infection or by vaccination with MLV. Furthermore, vHVT13 induces a protection against MD equivalent to that achieved with conventional HVT-based vaccines.

References

- [1] Muskett J.C., Hopkins I.G., Edwards K.R. & Thornton D.H., 1979, Comparison of two infectious bursal disease vaccine strains: efficacy and potential hazards in susceptible and maternally immune birds, *Vet. Rec.*, 104, 332-334.

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