EFFICACY OF A COMBINATION VACCINE CONTAINING MDV CVI 988 STRAIN AND HVT AGAINST CHALLENGE WITH VERY VIRULENT MDV

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Summary. – With the emergence of very virulent Marek's disease virus (MDV) strains, vaccines based on herpesvirus of turkeys (HVT) appear to be not powerful enough to confer full protection, whereas in chicken flocks vaccinated with MDV CVI 988 strain protective immunity sometimes is generated not early enough for full protection. For this reason combination vaccines containing HVT as well as CVI 988 have been developed. In this paper the beneficial effect of combining both types of virus strains in one vaccine for early protection is shown in a vaccination challenge experiment, in which one-day-old chickens were vaccinated with suboptimal dosages of the monovalent vaccines and the same dosages in a combination vaccine. After 5 days the chickens were challenged with a very virulent MDV strain and subsequently observed for a period of approx. 50 days. It appeared that the combination vaccine provided better early protection than the monovalent vaccines. In addition, the combination vaccine was tested as vaccine administered *in ovo*. It appeared that after *in ovo* vaccination the vaccine conferred adequate protection against challenge with a very virulent MDV strain, 5 days after hatch, and that protection after *in ovo* vaccination was similar to that obtained after subcutaneous vaccination with the same combination vaccine.

Key words: Marek's disease; vaccine; subcutaneous vaccination; in ovo vaccination; protection; very virulent MDV strain

Introduction

MDV is a common poultry herpesvirus associated with the development of tumors and other disease symptoms. Marek's disease (MD) can cause significant losses in poultry industry. Prior to the emergence of very virulent MDV (vvMDV) strains in field, vaccination of one-day-old chickens with monovalent HVT as well as monovalent vaccines based on CVI 988 strain provided an effective means for controlling MD in susceptible flocks (Okazaki *et al.*, 1970; Rispens *et al.*, 1972). However, with the emergence of those vvMDV strains vaccines based on HVT appear to be not powerfull enough to confer full protection (Witter 1997), whereas in chicken flocks vaccinated with CVI 988 strain protective immunity sometimes is generated not early enough for full protection.

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For this reason combination vaccines containing HVT as well as CVI 988 have been developed. In the present study the beneficial effect of combining both types of virus strains in one vaccine for early protection is shown in a vaccination challenge experiment, in which the vaccines were injected subcutaneously. In addition, results are shown of a study in which the vaccine was administered *in ovo* in 18-day embryonated eggs.

Materials and Methods

Animals. Eggs were obtained from flocks of specific pathogen free (SPF) White Leghorn chickens at Hyvac, Dallas Center, IA, USA. After hatch, the birds were housed in negative pressure Horsfal-type plexiglass isolators, and fed and watered *ad libitum* for the duration of the study.

Vaccine. The experimental vaccine contained CVI 988 strain, passage level 5 from the Fort Dodge working seed virus and a commercial batch of HVT strain FC126 in a commercial batch Poulvac Marek Diluent®. The experimental vaccine was prepared

by taking ampoules CVI 988 and HVT from liquid nitrogen, thawing them and diluting the contents of the ampoules in Poulvac Marek Diluent®. The vaccine was used directly after preparation.

Challenge virus. Ampoules containing the vvMDV strain RB1B were taken from liquid nitrogen, thawed and diluted in tryptose phosphate broth at approximately 4°C, to a concentration of 3750 PFU/ml. The diluted virus was used directly after preparation.

Efficacy of CVI 988, HVT and combination vaccine after subcutaneous vaccination. Four groups of 31 to 35 one day-old chickens were formed. Three groups were vaccinated subcutaneously with 500 PFU of CVI 988 per dose, 750 PFU HVT per dose and a combination containing both 500 PFU of CVI 988 and 750 PFU HVT per dose, respectively. The injection volume was 0.2 ml. The fourth group was not vaccinated. Five days after vaccination the chickens were challenged by intraperitoneal injection of 0.2 ml of challenge virus. The challenged birds were observed for a period of 50 days. Any birds that died during the observation period were examined for gross lesions attributable to MD. At the end of the observation period all surviving birds were euthanized and examined for gross MD lesions.

Efficacy of CVI 988 + HVT combination vaccine after in ovo vaccination. At embryonation day 18, one group was injected *in ovo* with a CVI + HVT combination vaccine containing per dose 500 PFU of CVI 988 and 500 PFU of HVT and another group with 100 PFU of CVI 988 and 500 PFU of HVT per dose. The injection volume per egg was 0.1 ml. At the day of hatching 2 groups of 35 chicks were vaccinated subcutaneously with the combination of 500 PFU of CVI 988 and 500 PFU of HVT per dose, and the combination of 100 PFU of CVI 988 and 500 PFU of HVT per dose, respectively. Another group of 35 chickens was vaccinated subcutaneously with 500 PFU of HVT per dose. Furthermore, a group of 34 chickens was not vaccinated. Five days after subcutaneous vaccination all chickens were challenged as described above. Chickens were monitored as described above until the age of 49 days.

Evaluation of results. The ability to protect is reported as percentage protected and as a protective index (PI) calculated by the following formula:

$$\text{Percentage protected} = \frac{(\text{Number of birds per group}) - (\text{Number of MDV-positive birds})}{(\text{Number of birds per group})} \times 100$$

$$\text{PI} = \frac{(\%\text{ of MDV positives in nonvaccinated group}) - (\%\text{ of MDV positives in vaccineed group})}{(\%\text{ of MDV positives in nonvaccinated group})} \times 100$$

Results

The results of the study on efficacy of CVI 988, HVT and combination vaccine after subcutaneous vaccination are summarized in Table 1. They show that at the end of the observation period 31 of the 35 challenged birds had MD lesions, which indicates that the challenge was effective. Of the 35 birds vaccinated with HVT 26 were protected, whereas for CVI 988 26 of 31 birds were protected. All birds vaccinated with the combination CVI + HVT were protected.

Table 1. Assessment of day of age administration of experimental vaccines to prevent the development of MD lesions following intraperitoneal challenge by vvMDV strain RB1B 5 days after vaccination

Group	Birds per group	MDV-positive birds	Percentage protected	PI
Nonvacc. chall.[a]	35	31	11.4	NA[b]
Vacc.[c] HVT	35	9	74.3	71.0
Vacc. CVI	31	5	83.9	81.8
Vacc. HVT + CVI	35	0	100.0	100.0

[a]Nonvaccinated challenged birds.
[b]Not applicable.
[c]Vaccinated and after 5 days challenged.
CVI = CVI988 strain of MDV.

Table 2 shows the results of protection after *in ovo* vaccination. In the group vaccinated with the combination at least 32 of the 35 birds were protected. Protection after *in ovo* vaccination was similar with that after subcutaneous vaccination, and there was no difference between results with 100 PFU of CVI 988 per dose and 500 PFU of CVI 988 per dose. In the group vaccinated subcutaneously with HVT 26 of the 35 birds were protected.

Discussion

The use of combination vaccines containing vaccine strains of different MD serotypes, especially combinations of HVT strains and serotype 2 strains, has been described earlier. It was demonstrated that combination vaccines confer better protection against several virulent and very virulent MDV strains than monovalent vaccines (Witter *et al.*, 1984).

An explanation might be the concept of protective synergism among vaccine viruses (Witter, 1982). Another explanation could be the difference in response against the MD vaccine strains by different chicken breeds and strains (Bacon and Witter, 1994). Vaccines containing more than one vaccine strain would be able to generate protective immunity in a higher variety of chicken breeds or strains than monovalent vaccines.

The results found in the study in which chickens were vaccinated subcutaneously with HVT, CVI 988 and the combination and subsequently challenged are in agreement with what has been discussed above. In addition, the combination, product also conferred adequate protection after *in ovo* vac-

Table 2. Assessment of *in ovo* and day of age administration of experimental vaccines to prevent the development of MD lesions following intraperitoneal challenge with vvMDV RB1B strain 5 days after vaccination

Group	Birds per group	MDV-positive birds	Percentage protected	PI
Nonvacc. chall.[a]	34	32	5.9	NA[b]
Sc vacc.[c]CVI 500 PFU + HVT 500 PFU	35	1	97.1	96.9
Sc vacc.[c] CVI 100 PFU + HVT 500 PFU	35	0	100.0	100.0
In ovo vacc. CVI 500 PFU + HVT 500 PFU	35	1	97.1	96.9
In ovo vacc. CVI 100 PFU + HVT 500 PFU	35	3	91.4	90.8
Sc vacc.[c] HVT 500 PFU	35	9	74.3	72.6

[a]Nonvaccinated challenged birds.
[b]Not applicable.
[c]Subcutaneously vaccinated birds.
CVI = CVI988 strain of MDV.

cination in 18-day embryonated eggs. Because of technical problems no comparison was possible with *in ovo* vaccination with only HVT or only CVI 988. There was no significant difference between subcutaneous vaccination and *in ovo* vaccination. It already had been shown that HVT can be highly efficacious as *in ovo* vaccine and the protective effect after *in ovo* vaccination is already adequate at hatch (Sharma and Graham, 1982; Sharma and Burmester, 1982). The same will hold for the combination product, and taking to consideration that the efficacy of combination vaccines is better than that of monovalent vaccines, it can be assumed that vaccination with a combination vaccine *in ovo,* will result in protective immunity in the very early days of a chicken's life against a wide variety of very virulent field viruses.

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