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Effect of Vaccination with Different Types and Dosages against a Very Virulent Marek's Disease Virus Strain

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Abstract

In this study, Marek's disease(MD) vaccines including herpes virus of turkeys (HVT) vaccine, attenuated serotype 1 Marek's disease virus (MDV) CVI988/Rispens vaccine and nonpathogenic serotype 2 MDV (SB1 strain) culture were used individually or jointly to immunize 1-day-old specific pathogen free (SPF) chickens at different doses, the immunized chickens were intradermally infected with 2000 plague forming unit (PFU) of duck embryo fibroblast (DEF) passaged SD2012-1, a very virulent Marek's disease virus. All chickens of the experiments and control chickens were observed daily throughout the entire experimental period to assess the vaccines efficacy and immunization dose against MDV SD2012-1 Strain. CVI988, CVI988+HVT and CVI988+SB1+HVT could partially protect immunized chickens against SD2012-1. CVI988+HVT and CVI988+SB1+HVT could provide more efficient protection than CVI988 at normal and high immunization dose. Excessive dilution of these vaccines could result in obvious decrease on protection against SD2012-1. In this study, 450 feather pulp samples collected from the dead or living chickens on Day 150 were detected to be MDV positive by polymerase chain reaction (PCR), and the positive rate of MDV was 100%.

Keywords: Marek's disease virus; Challenge; Virulence; Vaccine

Introduction

Marek's disease virus serotype-1(MDV-1), also known as Gallid herpes virus 2 (GaHV-2), was an oncogenic poultry herpes virus, causing lymph proliferative and demyelinating disorder in infected chickens. Since the 1960s, vaccination has been used to control MDV [1,2], but the widespread use of vaccines against Marek's Disease (MD) was suggested to have led to the evolution of field viruses with greater virulence. At present, a number of pathotypes classified as vMDV, vvMDV, and vv+MDV have been isolated [3,4], and more virulent strains could overwhelm the protection conferred by currently available vaccines [5].

There are three kinds of MD vaccine strains including nonpathogenic herpes virus of turkeys (HVT) [6,7], known as Meleagrid herpes virus 1, nonpathogenic serotype 2 MDV (SB1) [8], known as Gallid herpes virus 3, and attenuated serotype 1 MDV (CVI988/Rispens) [9]. These vaccine strains could be used as monovalent vaccines or mixed with each other as multivalent vaccines [10]. Studies had shown that higher vaccine dosages which had more plaque forming unit (PFU) per dose offered higher efficacy of vaccination [11,12].

In recent years, MDV isolates in China have been reported in breeder or layer flocks which had been vaccinated with HVT or CVI988/Rispens [13-16]. The virulent Marek's Disease virus strain (MDVs) circulating in China seemed to constitute a separate genotype different from exotic MDV reference strains [15,16]. To find the possible vaccination strategy against the very virulent isolates in China, in this study, chickens vaccinated with different types and doses of MD vaccines were challenged with the very virulent MDV isolate SD2012-1 [16], to assess the efficacy of different kinds of MD immunization method against this isolate.

Methods

The following experimental researches on animals were performed with the approval of Experimental Animal Administrative Center of Shandong Province.

Chickens

510 specific pathogen free (SPF) chickens from Shandong Healthtec Laboratory Animal Breeding Limited Company (Jinan, China) were used in this study. The chickens were free of antibodies against MD, exogenous avian leukosis virus (ALV), reticuloendotheliosis virus (REV) and other common poultry pathogens.

Vaccine

HVT vaccine used in this study was a frozen dried preparation of strain FC-126, Live Vaccine (2000 PFU/dose).

CVI988 vaccine used in this study was a -196°C stored preparation of strain CVI988/Rispens, Live Vaccine (3000 PFU/dose).

SB1 vaccine used in this study was fresh chick embryo fibroblast (CEF) cell cultures of Marek's disease virus SB1 strain and the PFU of the cultures was calculated and diluted to 1200 PFU/dose before use.

Virus

Marek's disease virus SD2012-1 strain [16], a very virulent MDV, was used to challenge immunized chickens. At first, duck embryo fibroblast (DEF) cell binding live SD2012-1 virus in 10% dimethyl sulphoxide was recovered from -196°C, then was cultured with DEF cell, and after passage of 2 generations, cultures of SD2012-1 were collected and diluted to 4000 PFU/ml for challenge.

Immunization

HVT vaccine, CVI988 vaccine and SB1 vaccine were used alone or were combined as bivalent vaccine and triple vaccine in different doses to immunize chickens. 1-day-old SPF chickens were divided into 15 immunization groups and 2 control groups with 30 chickens in each group. In the 15 immunization groups, chickens were vaccinated with different types and doses of MD vaccines, and the MD vaccines and immunization doses for 15 groups of SPF chickens were shown on Table 1 (In this study, vaccines of HVT, HVT+SB1, CVI988, CVI988+HVT and CVI988+SB1+HVT were used to immunize chickens. Vaccines of SB1 alone and SB1+CVI988 were not used because they did not appear in China). Another 2 groups of chickens with sham vaccine treatment were served as control. All the 17 groups of chickens were housed in separate isolators.

Vaccine	Immunization 1		Immunization 2		Immunization 3	
	Group (30chickens/ group)	Dosage (PFU/dose), excessively diluted concentration	Group (30chickens/ group)	Dosage (PFU/dose), normal field dose	Grou (30chickens/ group)	Dosage (PFU/dose), high concentration
HVT	1-1	400	2-1	2000	3-1	10000
HVT+SB1	1-2	400+240	2-2	2000+1200	3-2	10000+6000
CVI988	1-3	600	2-3	3000	3-3	15000
CVI988+HVT	1-4	600+400	2-4	3000+2000	3-4	15000+10000
CVI988+SB1+HVT	1-5	600+240+400	2-5	3000+1200+2000	3-5	15000+6000+10000

Table 1: MD vaccines, immunization dosage and grouped SPF chickens

Challenge

On the 11th day post immunization, chickens of the 15 vaccinated groups and one of the 2 sham vaccine groups were intradermally infected with 2000 PFU of DEF passaged SD2012-1 MDV(diluted to 4000 PFU/ml). All challenged chickens and control chickens were observed daily throughout the entire experimental period. The dead chickens were removed from the isolators daily until experimental termination.

Post mortem procedure

On Day 150 of the experiment (140 days post challenge), all surviving chickens were killed humanely and examined post-mortem for gross MD lesions. Standard post mortem examination was carried out for all killed and dead chickens [17]. Carcasses were checked for nodular lesions on the skin. Breast and thigh muscles were inspected for lymphoid tumors or diffuse infiltration. After the opening of the carcass, the liver, spleen, gonads, kidney, proventriculus, mesenteries, gastro-intestinal tract, heart and lungs were examined for gross MD lesions. Chickens with small focal lesions on all organs were considered to be MD positive only after histopathological confirmation. In this study, the definition of chickens infected with MDV was determined by gross MD lesions and histopathological confirmation of organs with small focal lesions. The total amount of chickens infected with MDV (Total MD) was determined according to this definition.

PCR detection

In order to investigate the shedding of SD2012-1 in the feather of the challenged chickens, the feather pulps were sampled from dead chickens or chickens killed on Day 150 of the experiment. PCR [16] for amplification of MDV Meq gene was used to detect SD2012-1 viral shedding in the feather.

Primers used in this PCR were 5'GGCACGGTACAGGTGTAAAGAG-3 and 5'-GCATAGACGATGTGCTGCTGAG-3. The target DNA of the PCR for SD2012 was 1081bp long, which was 180bp shorter than that for CVI988.

Total DNA was extracted from feather pulps using sodium dodecyl sulfate (SDS) - proteinase K- phenol/chloroform method. PCR amplification was carried out using 2 ul DNA as template in a total volume of 50 ul containing 25 ul 2× Taq PCR Mixture, 2 ul of 10 uM of each of the two primers, and 19 ul ddH2O. The optimum conditions for PCR were as follows: 94°C for 4 min, 35 cycles at 94°C for 1 min, 56°C for 1 min, 72°C for 1.5 min, and final elongation at 72°C for 10 min. The PCR product was analyzed in 0.9% agarose in Tris-borate-EDTA (TBE) buffer gel containing 0.5 mg/ml ethidium bromide.

Vaccine protective index

Vaccine protective index (PI) was calculated as described below [18]:

PI= (%Total MD in sham vaccinated chickens –%Total MD in vaccinated chickens) *100

%Total MD in sham vaccinated chickens

Results

MD pathologic changes of the challenged chickens

Control chickens of sham vaccine without virus challenge had not any MD symptoms or pathologic changes; challenged chickens of sham vaccine had MD symptoms and pathologic changes. None of the vaccination could completely protect the immunized chickens against SD2012-1. MD appeared in groups with sham vaccine, immunization 1, immunization 2 and immunization 3. In the 16 groups of challenged chickens the earliest death appeared on 42-45 days post challenge on chickens with sham vaccine, HVT and HVT+SB1 vaccine. The peak of death came on 60-85 days post challenge. The main gross lesions of the early dead chickens were degeneration and swelling of organs without development of tumor. Tumors could be found on chickens that died later or on chickens with symptoms later.

Group	Vaccine	Vaccine dosage (PFU/dose)	Total chickens	Total mortality	Live with MD lesions	Total chickens with MD lesions (%)	Protective index
Control 1	Sham vaccine (challenge)	0	30	9	14	23 (76.7%)	/
Control 2	Sham vaccine (no challenge)	0	30	0	0	0	/
01-Jan	HVT	400	30	9	14	23 (76.7%)	0
01-Feb	HVT+SB1	400+240	30	8	15	23 (76.7%)	0
01-Mar	CVI988	600	30	7	9	16 (53.3%)	30
01-Apr	CVI988+HVT	600+400	30	6	9	15 (50.0%)	31
01-May	CVI988+SB1+HVT	600+240+400	30	6	9	15 (50.0%)	31
02-Jan	HVT	2000	30	8	12	20 (66.7%)	13
02-Feb	HVT+SB1	2000+1200	30	8	11	18 (60.0%)	21
02-Mar	CVI988	3000	30	4	6	10 (33.3%)	57
02-Apr	CVI988+HVT	3000+2000	30	4	4	8 (26.7%)	65
02-May	CVI988+SB1+HVT	3000+1200+2000	30	2	6	8 (26.7%)	65
03-Jan	HVT	10000	30	9	14	23 (76.7%)	0
03-Feb	HVT+SB1	10000+6000	30	9	12	21 (70.0%)	9
03-Mar	CVI988	15000	30	4	5	9 (30.0%)	61
03-Apr	CVI988+HVT	15000+10000	30	3	5	8 (26.7%)	65
03-May	CVI988+SB1+HVT	15000+6000+10000	30	2	6	8 (26.7%)	65

Table 2: Protective efficacy of MD vaccines against SD2012-1

PCR detection of the challenged chickens

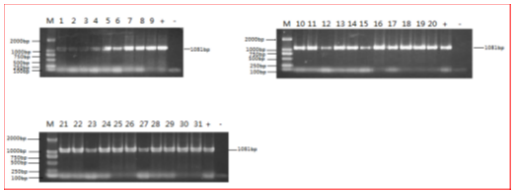

Figure 1: Partial PCR amplification of MD Meq gene from feather pulp collected from living chicken 140 days post challenge with SD2012-1

(M) DL2000 marker; (+) MD2012-1 virus cultured with DEF cells; (-) SPF feather pulp without MD infection.

1, 2 from 1-1 group; 3, 4 from 1-2 group; 5, 6 from 1-3 group; 7, 8 from 1-4 group; 9 from 1-5 group.

10, 11 and 12 from 2-1 group; 13, 14 from 2-2 group; 15, 16 from 2-3 group; 17, 18 from 2-4 group; 19, 20 from 2-5 group.

21, 22 and 23 from 3-1 group; 24, 25 from 3-2 group; 26, 27 from 3-3 group; 28, 29 from 3-4 group; 30, 31 from 3-5 group.

Feather pulp samples of the 450 challenged chickens in immunized groups were detected to be MDV SD2012-1 positive by PCR(in this study, PCR product of Meq gene of SD2012-1 were 1081bp long, which was 180bp shorter than that of CVI988). Feather pulp samples for PCR were collected from chickens that died or that were killed on 140 days post challenge with SD2012-1. Figure 1 showed the PCR

detection results of partial feather pulp samples collected from living chickens killed on 140 days post challenge.

Protective efficacy of vaccines against SD2012-1

In this study, 510 SPF chickens were divided into 17 groups, with 30 chickens in each group. Among them, 15 groups were used as immunization groups immunized with HVT, HVT+SB1, CVI988, CVI988+HVT and CVI988+SB1+HVT individually. 2 groups were used as control groups with sham vaccine. After challenge, chickens in 15 immunization groups and one control group displayed varying degrees of MDV infection. Another normal feeding control group with no challenge did not display MDV infection. The protective efficacy of MD vaccines of different types at different doses against SD2012-1 was shown on Table 2.

Discussion

MDV pathotypes isolated in the first half of the 20th century were probably of moderate virulence causing mainly classic MD [19]. But In the past 40 years, the virulence of MDVs had been increasing gradually [5,20-23]. Vaccine was initially a great success [24] on controlling vMD, and then vvMDV isolates had a greater pathogenicity than those isolates obtained before the introduction of vaccination. A bivalent vaccine (SB1+HVT) was introduced in the mid-1980s in response to the increasing number of MD outbreaks in HVT-vaccinated flocks [25]. But by the 1990s even more virulent vv+MDV pathotypes were being isolated from flocks vaccinated with the bivalent vaccine. In the 1990s, CVI988 vaccines were proved to be very effective on control vv +MDV when used individually or in combination with SB1 and HVT [26,27]. Despite this, recent MDV isolates might be able to break the protection of the CVI988 vaccine [27].

In China, a number of vvMDVs were recently isolated from different areas [14,15,28], and SD2012-1 was a newly isolated vvMDVs which had the high homology with recent Chinese isolates and had different pathological characters from the prevalent strains [16]. It was poorly understood whether the currently available vaccines were able to protect against this very virulent MDVs.

In this study, we demonstrated the protective efficacy of MD vaccine in different doses to protect against SD2012-1. Results (Table 2) showed HVT and HVT+SB1 could hardly protect the immunized chickens against SD2012-1 in any dose.

It was dilution of MD vaccine that could lead to reduced MD protection, reduced relative body weights, reduced vaccine DNA, and increased MDV DNA load [29].

In this study, CVI988 could partially protect the immunized chickens against SD2012-1. The PI values of CVI988 were 30(at immunization dosage 600 PFU/dose), 57 (at immunization dosage 3000 PFU/dose) and 61 (at immunization dosage 15000 PFU/dose). CVI988+HVT had more protective efficacy than CVI988 alone, but the PI values of CVI988+HVT were 31 (at immunization dosage 600 PFU/dose and 400 PFU/dose), 65 (at immunization dosage 3000 PFU/dose and 2000 PFU/dose) and 65 (at immunization dosage 15000 PFU/dose and 10000 PFU/dose) individually. The PI value of excessively diluted vaccine was significantly lower than that of normal concentration. But the PI value of high concentrations of vaccine was essentially the same as that of normal concentration. The protective efficacy of CVI988+SB1+HVT was nearly close to that of CVI988+HVT.

It was reported that MD vaccines were usually effective in controlling clinical disease but did not produce sterile immunity, and once chickens were infected with MDV, infection might persist for life with continuous viral shedding in feather dander [30,31]. In this study, 450 feather pulp samples collected from the chickens dead or alive on Day 150 were detected to be MDV positive by PCR, and the positive rate of MDV were 100%. It should be noticed that the PCR in this study could differentiate SD2012-1 from CVI988. It was interesting that only SD2012-1 positive results were found in 450 feather pulp samples, and no CVI988 positive results were found by the PCR. The existing of the virus in chickens and its excretion to environment could result in reinfection and cycle of the virus in the same area.

Conclusions

In summary, HVT and HVT+SB1 could hardly protect the immunized chickens against SD2012-1. CVI988, CVI988+HVT and CVI988+SB1+HVT could partially protect experimental chickens against SD2012-1. CVI988+HVT and CVI988+SB1+HVT could provide more efficient protection than CVI988. Normal field dose and more dose of commercial CVI988 could provide partial protection against SD2012-1, but excessive dilution of CVI988 resulted in obvious decrease on protection against SD2012-1. The same result was also found in CVI988+HVT and CVI988+SB1+HVT. Feather pulp samples collected from infected chickens were 100% MDV positive by PCR. The existing of the virus in chickens and its excretion to environment could result in reinfection and cycle of the virus in the same area.

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Author’s Contributions

Zhenhua Gong and Kang Zhang: carried out studies design, experiment and interpretation of the data, wrote the manuscript. Lei Li, Hongwei Wang, Jinping Li, Guangyu Hou, Jianmin Yu and Jianlin Wang: participated in experiment and interpretation of the data. Zhiliang Wang: participated in studies design and draft of the manuscript. All authors read and approved the final manuscript.

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