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Safety Assessment of a Turkey Herpes Virus Vector Newcastle Disease Vaccine in Chickens

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Abstract: A recombinant Herpes Virus of Turkeys (HVT) vaccine expressing the key protective antigen of Newcastle Disease Virus (NDV) could facilitate in the prevention and control of both Marek's Disease Virus (MDV) and NDV infections. The VECTORMUNE® HVT NDV vaccine is a recombinant vaccine in which the *F* gene of a NDV lentogenic strain has been inserted into the HVT genome. In the current study, this vaccine was assessed for its safety in chickens. Based on clinical observations, gross pathological examinations, histopathological examinations, virus shedding and tissue distribution, the VECTORMUNE® HVT NDV vaccine was determined to possess a high level of safety in chickens.

Key words: HVT vector, vaccine, Newcastle disease virus, *F* gene, safety assessment

INTRODUCTION

Newcastle Disease (ND) is caused by Newcastle Disease Virus (NDV) belonging to genus Avulavirus in the family Paramyxoviridae (Mayo, 2002; Alexander, 2011). The NDV genome consists of six genes, encoding the Nucleoprotein (NP), Phosphoprotein (P), Matrix (M), Fusion (F), Hemagglutinin-Neuraminidase (HN) and Large RNA-dependent RNA polymerase (L) proteins. What's more an additional V protein and possibly a W protein are expressed by RNA editing of P mRNA (Steward *et al*., 1993). The F protein is responsible for membrane fusion and has been shown to be the major antigen of NDV (Nagai, 1993; Panda *et al*., 2004).

Herpes Virus of Turkeys (HVT) is antigenically related to Marek's Disease Virus (MDV) serotype 3 and has been used extensively as a vaccine against Marek's Disease (MD) since 1971 (Buckmaster *et al*., 1988). The engineering of HVT-based vectors has direct application for recombinant vaccines in the poultry industry began in 1992 and since its inception, HVT vectors expressing protective genes of avian pathogens such as Newcastle disease virus (Morgan *et al*., 1992), Infectious Bursal Disease Virus (IBDV) (Perozo *et al*., 2009), Avian influenza virus (Iqbal, 2012), Infectious laryngotracheitis virus (Cook *et al*., 2013; Esaki *et al*., 2013), Avian leukosis virus (Bublot and Sharma, 2004) and Eimeria (Cronenberg *et al*., 1999) have been developed or shown to be effective vaccines in poultry.

HVT vaccines licensed for commercial use include Merial's VAXXITEK HVT IBD and Ceva's VECTORMUNE HVT IBD which protect against IBDV and Merck's Innovax®-ILT and Innovax®-ND-SB which protect against ILTV and NDV, respectively (Cook *et al*., 2013). All vaccines have to be proven safe and effective. VECTORMUNE HVT NDV is a genetically engineered live HVT vaccine expressing the *F* gene of a lentogenic strain of NDV and is recommended for use in chickens for the prevention of ND and MD and has received commercial licensure in the United States. In the present study, researchers assessed the safety of this vaccine in chickens.

MATERIALS AND METHODS

Vaccine: VECTORMUNE® HVT NDV, produced by Ceva Biomune Animal Health Company (Lenexa, KS) and supplied by CEVA Animal Health Service Company Limited is an HVT vectored vaccine expressing the *F* gene of a lentogenic strain of NDV and is used as an aid in the prevention of ND and MD.

Animals: The 105, 1 day old Specific-Pathogen-Free (SPF) White Leghorn chickens were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All animal research was approved by the Beijing Administration Committee of Laboratory (Approval ID: SYXK (Beijing) 2013-0013). The research

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was conducted in accordance with animal welfare guidelines of the Laboratory Animal Welfare Institutional Review Board of China Agriculture University. Personnel who supervised the care and welfare of the experimental animals were suitably qualified and trained.

Clinicopathologic assessment in chickens: Three groups, each containing 35, 1 day old SPF chickens were inoculated subcutaneously with either one dose of VECTORMUNE HVT NDV, ten doses of VECTORMUNE HVT NDV or Phosphate-Buffered Saline (PBS) as a non-infected control. All chickens were monitored daily for signs of MD which included paralysis, gasping, ataxia, emaciation, coma, blindness, weight loss, paleness and diarrhea (Calnek and Witter, 1997) until 45 days postinoculation. The chickens were observed daily for 21 days for clinical signs of ND including respiratory signs, neurological signs and viscerotropic signs (Alexander, 2000).

Three chickens from each group were randomly euthanized for necropsy on days 1, 3, 5 and 7 postinoculation. Local reactions at the injection site (malabsorption and scab formation) were examined. Necropsy observations focused on changes to the liver, spleen, lung, trachea, proventriculus and duodenum. The liver, spleen, proventriculus, trachea, duodenum and jejunum of chickens killed 5 and 7 days postinoculation were collected, fixed in 10% neutral buffered formalin for histological observations.

DNA extraction and PCR: Cloacal swabs and tissue samples were collected from chickens and analyzed by PCR to determine recombinant virus shedding and tissue distribution. DNA was extracted from biological samples using a DNAVzol kit (Vigorous, China) according to the manufacturer's instructions. PCR was performed as previously described (Zhang *et al*., 2010) using the primer pair: (forward) 5'-CTAGCAGTGGCAGTTGGGAAGAT-3' and (reverse) 5'-GTTAAGGCAGGGGAAGTGATTTGT-3'.

Tissue distribution of recombinant virus: Three chickens from each group were randomly selected on days 3, 5, 7, 9 and 15 postinoculation, euthanized and subjected to necropsy. Tissue specimens from the liver, spleen, lung, trachea, proventriculus and brain were collected for PCR detection.

Virus shedding: Ten cloacal swabs were randomly taken from each group on days 3, 5, 7, 9 and 15 postinoculation. DNA extracted from the swabs was used in PCR.

Serum antibody level detection: Serum samples randomly collected from ten chickens per group at 21 day postinoculation were tested for NDV-specific antibodies using a commercial Enzyme Linked Immunosorbent Assay (ELISA) kit (Synbiotics, ProFlock, San Diego, CA).

RESULTS

Local and systemic reactions: No abnormal reactions such as malabsorption or scab formation were observed at the injection site for any chicken. No birds of any treatment group developed grossly observable lesions of MD or NDV. Histopathological examinations of the liver, spleen, proventriculus, trachea, duodenum and jejunum of chickens failed to show any changes.

Tissue distribution of recombinant virus: Recombinant virus was not detected in any tissue excised from the control chickens. Recombinant virus was demonstrated in the liver of the one dose group on day 7 postinoculation in the spleen of the ten dose group on days 7, 9 and 15 postinoculation in the lung of the one dose group on days 7 and 15 postinoculation and on days 7 and 9 postinoculation for the ten dose group in the trachea of both one and ten dose groups on days 7 and 15 postinoculation and in the proventriculus of the ten dose group on days 3, 7 and 15 postinoculation (Table 1).

Shedding of recombinant virus: The level of shedding of the vaccine strain was assessed from cloacal swabs collected from ten birds per group on days 3, 5, 7, 9 and 15 postinoculation. Recombinant virus was not detected in any of the examined samples for either the control or inoculated chickens.

Table 1: Tissue distribution of recombinant virus in the organs of SPF chickens following subcutaneous inoculation with VECTORMUNE HVT NDV vaccine[a]

	Positivity at specified time postinoculation (No. of positive/total)														
	Control group (days)					1 dose group (days)					10 dose group (days)				
Samples	3	5	7	9	15	3	5	7	9	15	3	5	7	9	15
Liver	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Spleen	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	1/3	1/3
Lung	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/3	0/3	1/3	0/3	0/3	1/3	1/3	0/3
Trachea	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/3	0/3	2/3	0/3	0/3	3/3	0/3	2/3
Proventriculus	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	3/3	0/3	2/3
Brain	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

[a]Detected by PCR targeting of the 170 bp fragment of the NDV *F* gene

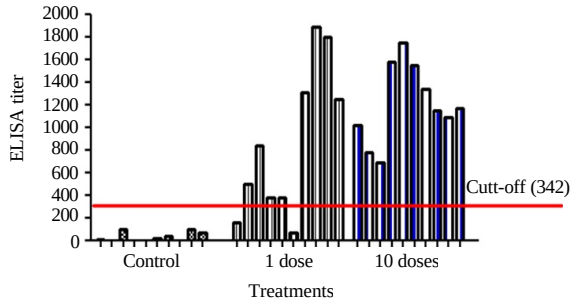

Fig. 1: Serum NDV-specific antibody titers of chickens at nominated times postinoculation. NDV-specific antibody titers were established using the Synbiotics NDV ELISA kit

Serum NDV-specific antibody titers postinoculation: The sera of control chickens were negative for NDV-specific antibody. All sera for the ten dose group were positive for NDV-specific antibodies while 8 out of 10 sera samples for the one dose group were positive (Fig. 1). The results demonstrated that immunization with the recombinant vaccine VECTORMUNE HVT NDV induced NDV-specific antibody production in chickens.

DISCUSSION

The poultry industry of today is constantly dealing with a plethora of avian pathogens with the potential to impact significantly on the economic viability and productivity of this industry. Mortality and condemnation losses resulting from field outbreaks of MD and ND are given greatest priority among a multitude of problems which threaten this industry worldwide.

Oncogenic herpes virus MDV is the etiological agent responsible for MD, a highly contagious lymphoproliferative disease of poultry. MD associated losses to the poultry industry result from paralysis, high mortality and condemnations because of T cell lymphomas and peripheral nerve damage in chickens (Nair, 2005). NDV, an avian paramyxovirus causes a highly contagious disease in numerous bird species including chickens, turkeys, pigeons and waterfowl. The disease may cause death and severe respiratory and central nervous system associated problems.

Vaccination is the most cost effective strategy for the control and prevention of MD and ND. Recently, much progress has been made towards controlling these two diseases using HVT recombinant vaccines. HVT has served as an important live vaccine for the prevention of MD (Witter, 1998; Baigent *et al*., 2006) and more recently as a vector for generating recombinant vaccines and expressing protective genes of avian pathogens (Baigent *et al*., 2006). The HVT vaccine possesses some ideal characteristics for use as a recombinant vaccine vector. Firstly, the HVT genome is approximately 159 kb, a sufficient size for the insertion of multiple foreign genes into its genome (Afonso *et al*., 2001). Secondly, it is non-pathogenic to chickens and other animals. Thirdly, the HVT vaccine can exist stably and persistently in its host, offering the potential for long-term induction of protective immunity against pathogens in animals. Finally, the HVT vaccine is available in a cell-free dry lyophilized form which can be more effectively transported and stored. Compared with conventional vaccines, the recombinant HVT vaccine VECTORMUNE HVT NDV can be used for the prevention and control of both MD and ND via a simple injection. This study demonstrated that vaccination with VECTORMUNE HVT NDV had a high level of safety in chickens. No adverse vaccine reactions, vaccine-induced mortality or clinical signs of MD and ND were observed and all experimental chickens and ducks remained clinically healthy. Pathological and histopathological examinations failed to show any changes between the vaccination group and the control group. Virus shedding was also undetectable in cloacal swabs of birds at measured time points of 3-15 days post vaccination.

To evaluate the expression of NDV *F* gene in SPF chickens, serum samples were collected and ELISA tests performed to detect NDV-specific antibody (Fig. 1). The results showed that the recombinant vaccine VECTORMUNE HVT NDV was able to induce NDV-specific antibody production in chickens. In summary, the study indicates that vaccination with VECTORMUNE HVT NDV is safe for the target animals, chickens. Researchers also predict that the vaccine could be used successfully in chicken flocks to prevent MD and ND.

CONCLUSION

In this report, a recombinant Herpes virus of Turkeys (HVT) vaccine expressing the key protective antigen of Newcastle Disease Virus (NDV) was assessed for its safety in chickens. Based on clinical observations, gross pathological examinations, histopathological examinations, virus shedding and tissue distribution, the VECTORMUNE® HVT NDV vaccine was determined to possess a high level of safety in chickens.

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