

## 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

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### 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 (Bublout 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<sup>a</sup>

Samples	Positivity at specified time postinoculation (No. of positive/total)														
	Control group (days)					1 dose group (days)					10 dose group (days)				
	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

<sup>a</sup>Detected by PCR targeting of the 170 bp fragment of the NDV *F* gene



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