CONSTRUCTION AND EFFICACY OF A RECOMBINANT HVT-ND VACCINE AGAINST NDV AND MDV CHALLENGE IN SPF AND NDV CHALLENGE IN BROILER BIRDS

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ABSTRACT/ INTRODUCTION

Marek's Disease (MD) in chickens is a common cause of condemnations and immune suppression in broilers. The etiologic agent, serotype 1 Marek's disease virus (MDV) is a member of the family Herpesviridae. Herpesvirus of turkeys (HVT), is an avirulent turkey virus that is capable of replication in chickens. HVT has been demonstrated as a useful vector for delivering major avian antigens, as well as an effective vaccine for MDV. NDV (Newcastle disease virus) causes a highly contagious and fatal disease affecting all species of birds. NDV fusion protein (F) is one of the major viral glycoproteins present in the viral envelope and is one of the main immuno-protective NDV antigens. Thirteen HVT-ND recombinants were constructed using various promoters and poly A sequences, and the target gene expression cassettes were inserted at various sites of the HVT genome. A HVT-ND recombinant vaccine was identified and selected by its excellent *in vivo* efficacy (95% protection) against a velogenic NDV challenge on Day 28 in SPF (specific pathogen-free) birds. Efficacy of this vaccine against a virulent MDV challenge was also observed. Furthermore, 100% NDV efficacy was demonstrated in commercial broiler birds on Day 33. Finally, the recombinant vaccine stability was demonstrated with *in vitro* passaged viral culture by PCR, as well as IFA and DNA sequencing. *In vivo* stability was show with viruses recovered from backpassage study.

MATERIALS AND METHODS

HVT-ND vaccine

HVT-ND is a recombinant viral vaccine. An NDV F gene expression cassette was inserted into the HVT genome.

0.05 mL/egg *(in ovo)*, 0.2 mL/bird (subcutaneous injection)

Challenge viruses

Velogenic NDV Texas GB (USDA),

Virulent MDV GA22

SPF Birds

Leghorn CRL (Charles River Labs)

Broiler Birds

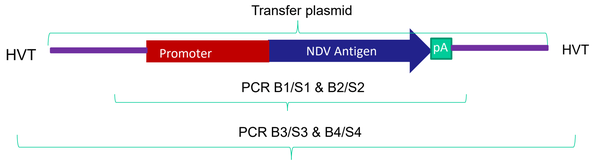
Commercial broiler eggs, straight run

Allotment/ Randomization

All eggs to be allocated for the study came from a single incubator. At the time of transfer and *in ovo* vaccination (E18), eggs were distributed such that each area of the incubator is represented in each flat. Flats were individually numbered and randomized to treatment by the Biometrics representative. Treatments were then transferred to hatchers according to biosecurity constraints and the randomization.

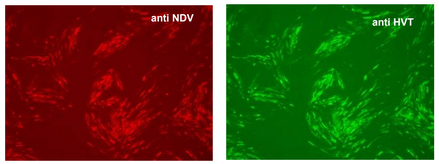
All bird procedures were approved by the Institutional Animal Care and Use Committee.

RESULT - Structure of recombinant HVT-ND



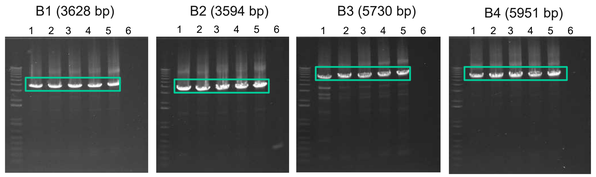
RESULT - Target Antigen Expression by IFA

Target antigen NDV protein expression was demonstrated by Immunofluorescence using either NDV anti-serum, or monoclonal antibody against HVT



RESULT - Stability of HVT-ND *in vitro*

Recombinant HVT-ND was serial passaged in vitro, the stability was tested by PCR.& IFA



DNA (HVT-ND):
Lane **1**. p1; Lane **2.** p5; Lane **3**. p7; Lane **4.** p8; Lane **5.** p9 ; Lane **6.** No DNA

Passage	Stability by IFA (% Pos./ # Plaques examined)
MSV	100% (2000/2000)
MSV+1	100% (700/700)
MSV+2	100% (800/800)
MSV+3	100% (1000/1000)
MSV+4	100% (1000/1000)
MSV+5	100% (1000/1000)

RESULTS – NDV Efficacy in SPF

Against a vologenic NDV challenge

Trt	Vaccine	Route	Challenge (D28)	% Protected (D42)
T01	Non-vaccinated	-	No	**NA** (40/40)
T02	CEF cells	*In ovo*	Yes	**0** (0/40)
T03	HVT-ND	*In ovo*	Yes	**93** (37/40)

Trt	Vaccine	Route	Challenge (D28)	% Protected (D42)
T01	Non-vaccinated	-	No	**NA** (40/40)
T02	CEF cells	Subcutaneous	Yes	**0** (0/40)
T04	HVT-ND	Subcutaneous	Yes	**100** (40/40)

RESULTS – MDV Efficacy in SPF

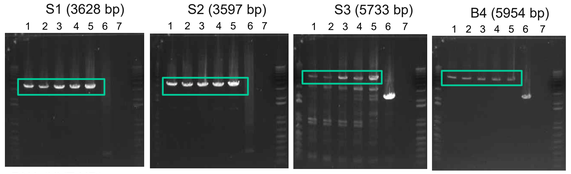
Against a virulent MDV challenge

Trt	Vaccine	Route	Challenge (D5)	% Protected (D54)
T01	Non-vaccinated	-	No	**NA** (30/30)
T02	Placebo (CEF)	*In ovo*	Yes	**10** (3/40)
T04	HVT-ND	*In ovo*	Yes	**83** (25/30)

Trt	Vaccine	Route	Challenge (D5)	% Protected (D54)
T01	Non-vaccnated	-	No	**NA** (30/30)
T02	CEF cells	*Subcutaneous*	Yes	**0** (0/30)
T03	HVT-ND	*Subcutaneous*	Yes	**80** (24/30)

RESULTS - Stability of HVT-ND *in vivo*

Recombinant HVT-ND was re-isolated from backpassage study and tested by PCR and IFA



DNA (HVT-ND):
Lane **1**. p1; Lane **2.** p2; Lane **3**. p3; Lane **4.** p4; Lane **5.** p5 ; Lane **6.** HVT; Lane 7: no DNA

Passage	Stability by IFA (% Pos./ # Plaques examined)
Backpass p1	100% (236/236)
Backpass p2	100% (37/37)
Backpass p3	100% (226/226)
Backpass p4	100% (146/146)
Backpass p5	100% (54/54)

RESULTS – NDV Efficacy in Broilers

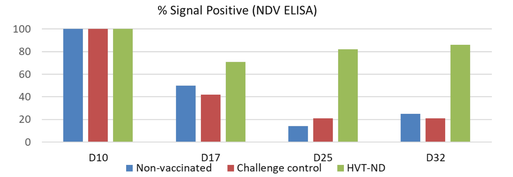
Against a velogenic NDV challenge

Trt	Vaccine	Route	Challenge (D33)	% Protected (D47)
T01	Non-vaccinated	-	No	**NA** (0/12))
T02	Challenge control	-	Yes	**8** (1/12)
T05	HVT-ND	*In ovo*	Yes	**100** (12/12)

RESULTS – NDV Serology ELISA in Broilers

ProFlock NDV Plus ELISA kit

Trt	Vaccine	% Signal Positive			
		D10	D17	D25	D32
T01	Non-vaccinated	100	50	14	25
T02	Challenge control	100	42	21	21
T05	HVT-ND	100	71	82	86



CONCLUSIONS

- ❑ HVT-ND recombinant vaccine was constructed and confirmed for NDV antigen expression
- ❑ NDV efficacy (D28/D42) was demonstrated against a velogenic NDV challenge for both *in ovo* and subcutaneous routes of administration in SPF birds
- ❑ MDV efficacy (D5/D54) was demonstrated against a virulent MDV challenge for both *in ovo* and subcutaneous routes of administration in SPF birds
- ❑ NDV efficacy (D33/D47) was demonstrated against a velogenic NDV challenge for the *in ovo* route of administration in broilers
- ❑ Genetic & phenotypic stability was demonstrated for both *in vitro* and *in vivo* passages.

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