ONSET OF IMMUNITY OF A RECOMBINANT HVT-ND AGAINST A VELOGENIC NDV CHALLENGE IN SPF BIRDS

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ABSTRACT

A recombinant HVT-ND was developed as a bi-valent vaccine for protection against Newcastle disease (ND), a highly contagious and fatal disease affecting all species of birds; and Marek's disease (MD), a common cause of condemnations and immune suppression in broilers. In the first study with SPF leghorns, recombinant HVT-ND was inoculated in eggs on E18. On Days 14, 16 and 19, forty vaccinated birds from each treatment group were challenged with a velogenic NDV, respectively. Protection of 93% (37/40) was observed for the Day 19 challenge. For Day 16 challenge, 85% efficacy (34/40) was observed. For the Day 14 challenge, 75% efficacy (30/40) was observed.

In the second study with SPF leghorns, HVT-ND was inoculated in eggs on E18. On Days 17, 18 and 19, forty vaccinated birds from each treatment group were challenged with a velogenic NDV, respectively. Protection of 93% (37/40) and 98% (39/40) was observed for the Day 19 challenge.

INTRODUCTION

Marek's Disease (MD) in chickens is a common cause of motality and condemnations in broilers. The etiologic agent, Marek's Disease Virus (MDV, GaHV2) is a member of the family Herpesviridae. Herpesvirus of turkeys (HVT, MeHV1, MDV-3), is an avirulent turkey herpesvrus that is capable of replication in chickens. HVT has been demonstrated as a useful vector for delivering major avian antigens as a multivalent vaccine, 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 the main immuno-protective NDV antigens.

MATERIALS AND METHODS

HVT-ND vaccine

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

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

NDV challenge virus

Velogenic NDV Texas GB (USDA)

SPF Birds

Leghorn CRL (Charles River Labs)

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 was 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.

Feather Pulp HVT DNA qPCR (quantitative PCR) **and ddPCR** (digital droplet PCR) **tests**
Total DNA was isolated from feather pulp. Both pPCR and ddPCR were conducted for the same set of DNA.

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

STUDY DESIGN 1

Trt	Vaccine	Challenge	# Birds/ Trt	# Birds/ Pen*	# Pens/ Trt	# Pens chall. D17	# Pens chall. D18	# Pens chall. D19
T01	Non-vaccinated	No	144	24	6	2	2	2
T02	Non-vaccinated	Yes	144	24	6	2	2	2
T03	HVT-ND subcutaneous	Yes	144	24	6	2	2	2
T04	HVT-ND *in ovo*	Yes	144	24	6	2	2	2

RESULTS – NDV EFFICACY (Study 1)

Trt	Vaccine	Route	Challenge (D17, D18, D19)	% NDV Efficacy		
				D31 (D17)	D32 (D18)	D33 (D19)
T01	Non-vaccinated	-	No	NA (40/40)	NA (40/40)	NA (40/40)
T02	Chall control	-	Yes	**0** (0/40)	**0** (0/40)	**0** (0/40)
T03	HVT-ND	Subcutaneous	Yes	**75** (30/40)	**88** (35/40)	**93** (37/40)
T04		*In ovo*	Yes	**100** (40/40)	**88** (35/40)	**98** (39/40)

STUDY DESIGN 2

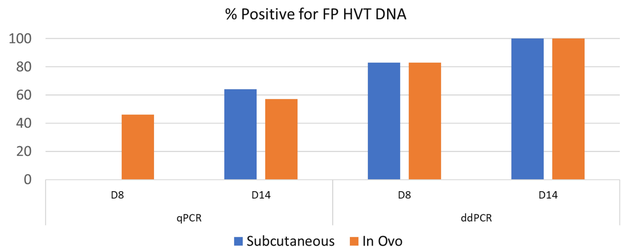
Trt	Vaccine	# Birds/ Trt D0	# Birds/ Pen*	# Pens/ Trt	# Pens chall. D16	# Pens chall. D19
T01	Non-vaccinated	144	24	6	2	2
T02	HVT-ND subcutaneous	144	24	6	2	2
T03	HVT-ND *in ovo*	144	24	6	2	2

RESULTS - NDV EFFICACY (Study 2)

Trt	Vaccne	Route	Challenge (D16, D19)	% NDV Efficacy	
				D30 (D16)	D33 (D19)
T01	Non-vaccinated	-	No	**0** (0/40)	**0** (0/40)
T02	HVT-ND	Subcutaneous	Yes	**70** (28/40)	**95** (38/40)
T03		*In ovo*	*Yes*	**85** (34/40)	**93** (37/40)

RESULTS – Feather Pulp HVT DNA quantitation by qPCR vs. ddPCR

Trt	Vaccine	Route	FP HVT qPCR % Positive		FP HVT ddPCR % Positive	
			D8	D14	D8	D14
T01	Neg Control	-	-	-	-	-
T02	HVT-ND	Subcutaneous	0	64	83	100
T03		*In ovo*	46	57	83	100



CONCLUSIONS

- 93-98% efficacy was observed at Day 19 post vaccination against a velogenic NDV challenge strain for both *in ovo* and subcutaneous routes of administration.
- 93-98% efficacy was observed at Day 19 for both vaccination routes in two independent studies.
- HVT DNA isolated from feather pulp was quantitated by both qPCR and ddPCR (digital droplet PCR) methods. ddPCR method is shown to be more sensitive to be used as a measure of vaccine update.

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