Running title: Queensland V4 and Ulster 2C vaccination by nebulisation

Immunogenicity and Safety of Queensland V4 and Ulster 2C strains of Newcastle Disease Virus Given to Maternally Immune, Newly Hatched Chickens by Nebulisation

Hrvoje Mazija,[AE] Stanko Čajavec,[BF] Neda Ergotić,[B] Irena Ciglar-Grozdanić,[A] Željko Gottstein,[A] and William L. Ragland[C]

[A] Department of Poultry Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

[B]Veterina d.d., 10436 Rakov Potok, Croatia

[C]Institut Ruđer Bošković, 10000 Zagreb, Croatia

[E]Corresponding author. E-mail: hmazija@vef.hr

[F]Deceased

SUMMARY. Commercial chickens with a high level of maternal antibodies for Newcastle disease were vaccinated when newly hatched with Queensland V4 or Ulster 2C NDV strains by nebulisation. The exposure time to a fine aerosol of vaccine produced with an ultrasonic nebuliser was 60 seconds. The chickens were challenged oculonasally with virulent NDV strain Texas GB in weekly intervals up to the 49th day of life. Although protected for several weeks by maternal antibody, they were sufficiently protected thereafter by active immune response to the vaccines. Vaccinal reactions were not observed. Queensland V4 produced higher titers than Ulster 2C and provided better protection to challenge.

Key words: Newcastle disease virus, Queensland V4, Ulster 2C, live virus, vaccination, aerosol, maternally derived antibodies

Abbreviations: ELISA = Enzyme-Linked ImmunoSorbent Assay; ND = Newcastle disease; NDV = Newcastle disease virus; QV4 = Queensland V4; SD = standard deviation; SPF = specific pathogen free; U2C = Ulster 2C; vNDV = virulent Newcastle disease virus

INTRODUCTION

Immunization of newly hatched chickens against ND is usually performed using live vaccines given either by coarse spraying (4), application via drinking water, or by oculonasal instillation, and later boosted using spraying or application via drinking water (14). Aerosol vaccination is an established, effective method for immunizing chickens against Newcastle disease (ND). Vaccination with aerosols has an advantage over other routes of application in that it stimulates both local and cellular immunity (3). Whereas aerosols of differing particle sizes have provided adequate immunity in chickens of various ages, it has suffered from vaccinal reactions, especially when the conventionally used strains of ND virus (NDV), namely B1 and La Sota, were applied to newly hatched chickens (2, 4, 12). The present study was conducted to determine if Queensland V4 and Ulster 2C strains of NDV would provide sufficient protection to viral challenge when they were administered in fine particle aerosols to newly hatched chicks that had maternally derived antibodies for NDV, and the extend of vaccinal reactions, if any.

Appearance of virulent NDV (vNDV) in different parts of the world requires repeated and expensive use of live and/or inactivated vaccines (22). In spite of this, there are continuing reports of considerable economic losses due to mortality and

cost of control of the disease (5, 9, 15, 26). Infection of many farm flocks with virulent field strains of NDV caused epornitics and significant economic losses during years 1992 to 1996 in West European countries (16).

Recently, Mazija *et al.* (19) described safe and successful application of La Sota vaccine to maternally immune, newly hatched commercial chickens using an ultrasonic device. Size of the aerosol-generated particles ranged between 3 and 5 microns, allowing the vaccine virus to reach the surface of the entire respiratory system. Vaccinal reactions were not observed. Immunity developed regardless of the presence of maternal antibodies, and challenge infection performed in weekly intervals up to 49 days of life conferred long-lasting, specific resistance to ND.

The use of asymptomatic enteric, less immunogenic strains, like Ulster 2C (U2C) (20) and Queensland V4 (QV4) (9), to further reduce a chance of vaccinal reactions (21) are attractive alternatives to B1 and La Sota. Gough and Allan (12) were the first to vaccinate chickens by aerosol with U2C, and reported that maternal antibodies interfered with protection to challenge with the Herts 33 strain of NDV. They also reported absence of vaccinal reactions. Van Eck and Goren (23) reported mild, vaccinal reactions in maternally immune chickens (1 to 10 days old) to aerosol vaccination with U2C, as well as 95% protection of birds challenged with Herts 33 at 8 weeks of age (24). Chansiripornchai and Sasipreeyajan (6) reported efficacy of aerosol vaccination of newly hatched

chickens with U2C. They used unvaccinated 1-day-old ROSS-308 broiler chicks obtained from a commercial hatchery, and while one would assume they had maternal antibodies, it was not stated and antibody titers were not measured. Czifra *et al.* (10) reported successful vaccination with aerosol vaccination of maternally immune, newly hatched chickens with an apathogenic NDV strain, designated as NDV-6/10.

Kim and Spradbrow (13) immunized chickens lacking maternal antibodies for NDV by aerosol with QV4 but no one has attempted to vaccinate maternally immune, day old chickens until the present report. Apparently, vaccination of newly hatched chickens was not attempted because Westbury *et al.* (25) reported that maternal antibody for QV4 interfered with immunization. Kim and Spradbrow could not perform challenge experiments because they were prohibited in Australia.

Differences in response of the respiratory system to various strains of NDV are related to viral tropism. Strains targeting epithelial cells lining the respiratory tract will cause more severe respiratory reaction compared to the enterotropic viruses (7). This probably is the main reason for using asymptomatic enteric U2C for mass aerosol application.

MATERIALS AND METHODS

Viruses. APMV-1/chicken/Australia/Queensland/V4/1966 (QV4) and APMV-1/chicken/Northern Ireland/Ulster/2C/1966 (U2C) were kindly provided by Dr. J. C. Pederson, National Veterinary Services Labs repository, Ames, Iowa, in 1992. Both viruses were freeze-dried products. Velogenic APMV-1/chicken/USA/Texas /GB/1948 (Texas GB) strain of NDV was supplied by the Croatian Veterinary Institute, Zagreb.

Experimental design. A total of 485 day-old male chickens of light hybrids (Lohmann Brown) from commercial NDV-vaccinated breeder flocks were used. Groups of 103 day-old male chickens were vaccinated with the asymptomatic enteric strains U2C and QV4 of NDV. Two control groups were used; one group was exposed to aerosol of water, while a non-vaccinated control group was not exposed to water aerosol. The chickens were exposed to the virus for 60 seconds, which corresponded to one dose of the vaccine (approximately $10^{6.0}$ EID_{50} of the virus). Blood samples were collected from 20 non-vaccinated chicks on day 1 and used as a reference for all groups. Ten more of them were bled on day 7, and another 20 were bled each week through the 35th day. Ten chicks were bled on day 7, 15 were bled on day 49, and 20 were bled each of the intervening weeks from the two principle groups and the water control group. From the 7th day of life, 15 chickens were randomly selected from each vaccinated and control groups at

weekly intervals to the 42nd day, except the non-vaccinated group that went through the 35th day, and challenged with virulent NDV.

Vaccination. The NDV strains used in the experiments were suspended in distilled water and given by nebulisation with a Sonovac® 095 ultrasonic nebuliser in a way that one dose is offered per chicken (17, 19). The device was designed for small hatcheries and has a capacity to vaccinate 6,000 to 12,000 day-old chickens per hour, in a way that standard box with 100 chicks can be placed in a cabinet. Chicks of each group were vaccinated at once for each vaccine.

Challenge. The chickens were individually challenged oculonasally with $10^{6.0}$ ELD_{50} of velogenic NDV strain Texas GB. During the course of experiment each chick was observed daily in the challenged groups. Chickens without clinical signs of ND were considered as protected, and clinically diseased or dead birds were considered as not protected. Isolation of challenge virus from 5 carcases in each experiment was performed to confirm the clinical finding of ND. For this purpose, 5 SPF chicken embryos were inoculated with water suspension of brain tissue (1).

Serological methods. Blood for serological tests was taken from the jugular vein of chicks on the day of vaccination and then weekly until the 49th day after vaccination, as well as ten days after challenge. All blood samples were handled in the conventional way, and separated sera were inactivated for 30 minutes at 56ºC.

Sera collected during the experiments, were examined by ELISA for ND (FlockCheck®, IDEXX, Portland, Maine, USA). Sera were investigated for presence of maternal antibodies as well as for the response to vaccinal and challenge virus.

Statistical analysis. Treatment means were compared by rank sums analysis using the JMP program (SAS Institute, Cary, NC). Data for protection to challenge were analysed by log likelihood and Fisher's exact tests. Differences of $p \leq 0.05$ were considered statistically significant.

RESULTS

Vaccinal reactions. No clinical reactions to the vaccines were observed in vaccinated chickens.

Serological response after vaccination. Results of serological examination of vaccinated and unvaccinated groups are presented in Table 1. There were no significant differences in titres among groups until 14 days when QV4 titers were increased. These differences continued to 21 days when the control groups had lower titres because of decline in maternal antibodies. Titres between U2C and QV4 varied thereafter, but QV4 usually had higher titres. Antibody titres declined in every group until 28 days when they began to increase in the vaccinated groups,

158 reaching the highest titre at 35 days. Titres in the control groups continued to
159 decline reaching negligible levels at 21-28 days. The decline was according to a
160 classic decay curve of maternal antibodies (Fig. 1).

161 **Serological response and survival following challenge with Texas GB**
162 **strain.** There was no difference between the results of birds challenged at 21 and
163 42 days post vaccination by QV4 and U2C (Tables 2 and 3), but cumulative
164 mortality among chickens vaccinated with QV4 was less than among those
165 vaccinated with U2C.

166 Since the challenge was performed in weekly intervals, the rise of immune
167 response to challenge virus was detected in birds challenged on the 14th day and
168 continued until the 42nd day, reaching maximum values in both vaccinated groups
169 challenged on the 28th day (Table 2). As confirmation of successful challenge the
170 inoculated 5 SPF embryos died during 72 hours and proved to be positive to Texas
171 GB NDV using RT-PCR and sequencing.

172

173 **DISCUSSION**

174

175 The challenge experiments have demonstrated that aerosol vaccination with an
176 ultrasound nebuliser is a safe and effective way of inducing long-lasting specific
177 resistance to velogenic Texas GB strain of NDV that continues for at least 49 days.

Relatively high level of maternal antibodies for NDV did not interfere with vaccinal immune responses as confirmed by antibody responses and resistance to the Texas GB challenge virus, which was consistent with the observations of other investigators who vaccinated with aerosols (3, 6). Effective responses were probably a result of the vaccine entering deeper into the respiratory tract than by conventional spray vaccination (18). In the report by Chansiripornchai and Sasipreeyajan (6), day-old broiler chicks were injected with inactivated, oil adjuvanted Kimber strain and live U2C administered by aerosol, the concept being that Kimber strain would provide a boost to immunity as the titres from U2C waned. Another group was injected with an inactivated Kimber strain and live B1 administered by aerosol. Chickens were challenged with Herts 33 at 28 days. Chickens in the group given U2C by aerosol had significantly fewer deaths than the group given B1 by aerosol, thus confirming the utility of U2C. Results of our study show, however, that the concomitant vaccination with inactivated vaccine is an unnecessary expense.

Our results with U2C were consistent with those of van Eck *et al.* (24) except they observed vaccinal reactions, but not with those of Gough and Allan (12) who reported interference by maternal antibodies, and confirm that aerosol vaccination with U2C is efficacious. Our results with QV4 were superior to those with U2C. The QV4 strain induced the highest titres, except on day 35, and provided better

protection to challenge. Consequently, QV4 should be afforded more interest as a commercially viable vaccine for aerosol exposure of maternally immune, newly hatched chickens.

Results of various investigators, while consistent, do vary somewhat, particularly in the occurrence of vaccinal reactions. While it is not possible to explain all the differences, it is known that different strains of chickens vary in their response to vaccination (11). Size of particles delivered might be a determining factor for a significant part of these differences. The various instruments used would have delivered aerosols of differing composition, particularly the size and range of sizes of particles delivered. Size of particles delivered by van Eck *et al.* (1991) were 50 ± 2 microns, whereas the Sonovac® delivers particles of 3-5 microns. Surely, the extent of lung exposure and the dose of vaccinal virus delivered deeply into the lung would differ, and could affect the outcome results of vaccination. We have never observed vaccinal reactions with the Sonovac®. We would like to believe this is related to particle size; an intensive investigation of various particle sizes delivered with the Sonovac® on vaccinal reactions and titres achieved should be done.

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Table 1. ELISA ND titers of male chickens of light hybrids after aerosol vaccination for ND.

Treatment (exposure time 60 sec)	Days after vaccination[1]							
	1[2]	7	14	21	28	35	42	49
Ulster 2C	$3151^{A,a}$ ±2033 (20)	$5693^{A,a}$ ±342 (2)	$585^{A,b}$ ±305 (20)	$230^{A,c}$ ±260 (19)	$1151^{A,b}$ ±1408 (20)	$3999^{A,a}$ ±5197 (20)	$882^{A,b}$ ±1549 (20)	$1499^{A,b}$ ±1607 (15)
QV4	$3151^{A,a}$ ±2033 (20)	$3608^{A,a}$ ±2037 (10)	$1497^{B,b}$ ±1454 (20)	$1183^{B,b}$ ±1529 (20)	$1926^{A,a}$ ±1839 (20)	$3291^{A,a}$ ±3199 (20)	$1951^{B,a}$ ±1893 (19)	$2353^{A,a}$ ±1679 (15)
dH_2O control	$3151^{A,a}$ ±2033 (20)	$1380^{B,b}$ ±1530 (10)	$569^{A,c}$ ±475 (18)	$72^{C,d}$ ±58 (19)	$1^{B,e}$ ±2 (20)	$0^{B,e}$ ±0 (18)	$27^{C,f}$ ±29 (15)	$195^{B,g}$ ±139 (15)
Non-vaccinated control	$3151^{A,a}$ ±2033 (20)	$2604^{A,a}$ ±1433 (10)	$615^{A,b}$ ±405 (17)	$76^{C,c}$ ±107 (15)	$2^{B,d}$ ±9 (15)	$3^{B,d}$ ±9 (15)	n.d.[3]	n.d.[3]

[1] Mean ELISA titer to NDV ± SD. Number of birds sampled in parenthesis.

[2] Samples were collected from 20 non-vaccinated birds, and used as a reference for each group.

[3] Not done.

[A,B,C] Means in each column with the same upper case alphabetic superscript are not different at $p \leq 0.05$.

[a,b,c,d,e,f] Means in each row with the same lower case alphabetic superscript are not different at $p \leq 0.05$.

Table 2. ELISA ND titers of male chickens of light hybrids 10 days after challenge with Texas GB strain of NDV.

Treatment	ELISA ND titers[1]					
	Day of challenge					
	7	14	21	28	35	42
Ulster 2C	$1875^{A,a}$ ±2674 (10)	$14113^{A,b}$ ±6272 (10)	$20480^{A,c}$ ±6190 (6)	$26971^{A,d}$ ±4603 (10)	$19413^{A,c}$ ±5776 (10)	$23485^{A,e}$ ±2124 (10)
QV4	$2418^{A,a}$ ±2013 (10)	$19325^{B,b}$ ±3829 (10)	$23226^{A,c}$ ±7347 (10)	$27119^{A,d}$ ±4731 (10)	$14919^{A,e}$ ±6809 (10)	$23033^{A,d}$ ±2707 (10)
Control[2]	$2656^{A,a}$ ±3829 (20)	$11906^{A,b}$ ±4785 (20)	$17752^{A,b}$ ±7183 (15)	$14483^{B,b}$ ±7551 $(8)^3$	$15152^{A.b}$ ±4464 $(3)^3$	$22079^{A,b}$ ±899 $(2)^3$

[1] Mean ELISA titer to NDV ± SD. Number of birds sampled in parenthesis.

[2]Pooled the two control groups.

[A,B] Means in each column with the same upper case alphabetic superscript are not different at $p \leq 0.05$.

[a,b,c,d,e,]Means in each row with the same lower case alphabetic superscript are not different at $p \leq 0.05$.

Table 3. Survival of male chickens of light hybrids 10 days after challenge with Texas GB strain of NDV.

Treatment	Protection against challenge[1]							Cumulative survival (live/total)
	Day of challenge							
	7	14	21	28	35	42	49	
Ulster 2C	$15/15^{A,a}$	$14/14^{A,a}$	$14/15^{A,a,b}$	$10/15^{A,a,b}$	$14/14^{A,a}$	$13/15^{A,a,b}$	$11/15^{A,a,b}$	$91/103^{A}$
QV4	$14/15^{A,a}$	$12/13^{A,a}$	$14/15^{A,a}$	$13/15^{A,B,a}$	$13/15^{A.a}$	$14/15^{A,a}$	$14/15^{A,a}$	$94/103^{A}$
dH_20 control	$13/15^{A,a}$	$14/15^{A,a}$	$13/15^{A,B,a}$	$6/15^{B,b}$	$4/15^{B,b}$	$1/15^{B,b}$	$0/15^{B,b}$	$51/105^{B}$
Unvaccinated control	$12/15^{A,a}$	$14/15^{A,a}$	$10/15^{B,a}$	$7/15^{B,a,b}$	$3/15^{B,b}$	$2/10^{B,b}$	$2/15^{B,b}$	$50/100^{B}$

[1]No. of birds surviving and free of clinical signs /no. of birds challenged.

[A,B] Survival ratios in each column with the same upper case alphabetic superscript are not different at $p \leq 0.05$.

[a,b] Survival ratios in each row with the same lower case alphabetic superscript are not different at $p \leq 0.05$.

346 Figure 1. ELISA ND titre decay curve for non-vaccinated control in Experiment 1

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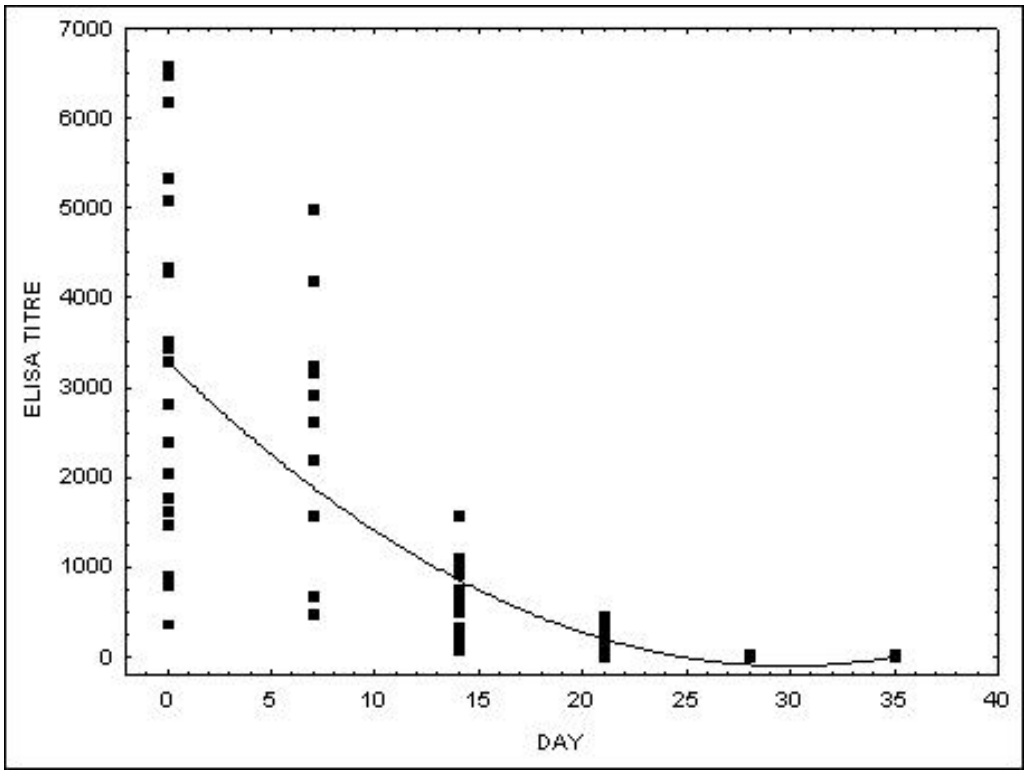

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