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The Effect of Newcastle Disease Vaccination with Strain V4 on the Course of Infection with the Peats Ridge Strain of Newcastle Disease Virus

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by Paul Selleck, Hans Heine, John Bingham, David Boyle & Peter Daniels

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Researcher Contact Details
Peter Daniels
CSIRO, Australian Animal Health Laboratory
Private Bag 24
Geelong, Victoria 3220
Phone: (03) 5227 5000
Fax: (03) 5227 5555
Email: Peter.Daniels@csiro.au

In submitting this report, the researcher has agreed to RIRDC publishing this material in its edited form.

RIRDC Contact Details
Rural Industries Research and Development Corporation
Level 1, AMA House
42 Macquarie Street
BARTON ACT 2600
PO Box 4776
KINGSTON ACT 2604

Phone: 02 6272 4539
Fax: 02 6272 5877
Email: rirdc@rirdc.gov.au
Website: http://www.rirdc.gov.au

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Foreword

Newcastle disease has the potential to cause devastating losses to the poultry industries of Australia. While exotic pathogenic strains of Newcastle disease are of great concern, in recent years Newcastle disease strains that have evolved from endemic strains of the virus have also caused problems for the industries. Outbreaks of Newcastle disease are usually controlled through slaughter-out of infected flocks, leading to potentially crippling losses to the owner.

Vaccination of flocks is an alternative control strategy, if it can be demonstrated to result in effective control of disease. This project examined the efficacy of the commonly-used V4 Newcastle disease vaccine for the control of outbreaks of Newcastle disease once the epidemic is in progress. This report details the methodology, the findings and the implications of a series of trials conducted at the CSIRO Australian Animal Health Laboratory to examine the use of V4 vaccine to limit the spread of Newcastle disease virus during epidemics.

This project was funded from industry revenue which is matched by funds provided by the Federal Government. This report is an addition to RIRDC's diverse range of over 900 research publications, forms part of our established industries – chicken meat R&D program, which aims to support increased sustainability and profitability in the chicken meat industry by focusing research and development on those areas that will enable the industry to become more efficient and globally competitive and that will assist in the development of good industry and product images.

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Simon Hearn
Managing Director
Rural Industries Research and Development Corporation

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Abbreviations

AAHL – Australian Animal Health Laboratory
GMT – geometric mean titre
HI – haemagglutination inhibition
NDV – Newcastle disease virus
PCR – polymerase chain reaction
RT-PCR – reverse transcriptase polymerase chain reaction
EID_{50} – median egg infectious doses

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Executive Summary

Newcastle disease may cause extensive losses in the poultry industry, through illness and death of infected birds and through slaughter-out control policies. In Australia, virulent Newcastle disease virus (NDV) may evolve from avirulent, endemic forms of the virus. Control of virulent and avirulent NDV outbreaks can be carried out through vaccination of flocks, using a live virus vaccine, V4. Vaccination of flocks prior to an NDV outbreak provides protective immunity. However, it is not known if vaccination of flocks in the face of an outbreak is effective at curtailing the progression of the outbreak.

Objectives

The objectives of the study were to deliver experimental data on:

1. Whether prior vaccination with V4 can reduce subsequent infection with an avirulent NDV strain, Peats Ridge strain, and/or reduce the amount and duration of shedding of Peats Ridge strain;
2. Whether V4 vaccination during a Peats Ridge strain infection can modify the course of the Peats Ridge infection; and
3. The serological response to the Peats Ridge strain alone and to Peats Ridge infection following prior vaccination with V4 vaccine.

Methodology

Two separate trial protocols were conducted, each consisting of a trial group and an unvaccinated challenge control group.

1. A vaccinate-challenge trial, where 20 seven-day-old SPF chicks were vaccinated with V4 vaccine and then challenged with Peats Ridge strain 21 days later.
2. A challenge-vaccinate trial, where 20 four-week-old SPF chickens were challenged with Peats Ridge strain and then vaccinated with V4 strain two days later.

Oral-tracheal swabs and cloacal swabs were taken from each bird and tested for the presence of virus by egg inoculation. A selection of virus isolates and original positive swabs were tested by TaqMan PCR technology to determine which of the two virus types – V4, Peats Ridge strain or both – was present in the sample. Serum samples were taken at intervals after challenge and tested for antibody titres.

Results and discussion

The challenge control groups indicated that the Peats Ridge strain was shed via the tracheal route by almost all birds up until day 5, after which the number of tracheal shedders declined. No tracheal shedding was found after day 6. In contrast, shedding via the cloacal route occurred mainly between days 3 and 8, with low numbers of shedders before and after this time. No shedding was detected after day 10.

Prior vaccination with a single dose did not completely protect birds from subsequent Peats Ridge strain infection. However, in birds that had received prior immunisation with V4, shedding was almost exclusively confined to the tracheal route, shedding ceased after day 4 and was detected in a much lower proportion of the birds.

In birds that were vaccinated two days after Peats Ridge strain challenge, the proportion of tracheal shedders on the various days did not differ significantly from those in control groups. Cloacal

shedding in the first week followed a similar trend to that in controls, although after the second week shedding occurred in a lower proportion of birds and some birds in the vaccinated group shed for a longer period.

TaqMan PCR tests indicated that all detected virus shed from the chickens was the challenge Peats Ridge strain. No V4 shedding was detected after V4 vaccine was administered to chickens that had an established Peats Ridge strain infection. This may indicate that the presence of an established infection inhibits the subsequent establishment of a second viral infection.

The serology results indicate that there was 100% seroconversion to Peats Ridge strain challenge. Seroconversion was rapid with over 90% of birds showing seroconversion by day 7.

Implications

The implications of this study can be summarised as follows.

1. Prior vaccination with V4 vaccine is effective at significantly reducing the shedding during subsequent Peats Ridge strain infection.
2. Post-challenge vaccination with V4 vaccine during active Peats Ridge strain infection does not appear to significantly affect the number of birds that shed Peats Ridge strain, nor does it shorten the duration of shedding.
3. Peats Ridge strain infection induces a significant frequency of seroconversion to significant HI antibody levels. Although not tested for in this study, these levels of antibodies are probably protective against subsequent virulent or avirulent Newcastle disease virus infection.

Introduction

Newcastle disease virus varies considerably in its pathogenicity to chickens. Many variants are non-pathogenic whereas others may be devastating to poultry flocks (Alexander 2000). Infection with pathogenic strains may cause severe economic losses due to high mortality ratios of affected flocks, which suffer from neurological, respiratory and gastro-intestinal signs. Mild variants may cause sub-clinical disease or mild pathology of the respiratory and intestinal system, as well as causing decreased egg production. There are also major economic implications resulting from slaughter-out policies associated with outbreaks of the disease.

Australia was considered free of Newcastle disease until a non-pathogenic variant, named V4, was detected in 1966. Other avirulent isolates were detected in the succeeding years and it was realised that ND virus is endemic to Australia (Westbury 2001) and probably maintained by wild birds. In the late 1990s virulent variants of NDV appeared in chicken flocks in New South Wales and it was determined that these variants had evolved from endemic strains by genetic mutation at the fusion protein cleavage site (Kirkland 2000; Westbury 2001; Gould *et al.* 2001). The appearance of endemic virulent variants, and the threat of incursion by exotic virulent strains, has indicated the use of vaccines to assist in preventing outbreaks of Newcastle disease in poultry flocks.

The vaccine that is recommended for use in Australia is the V4 strain, derived from an endemic avirulent variant. It is used as a live virus vaccine, inducing a sub-clinical infection in susceptible chickens that confers cross-protection against infection by virulent virus variants (Spradbrow *et al*. 1978, 1988; Latif Ibrahim *et al.* 1981; Bell *et al.* 1991a, b). In Australia, due to the relative scarcity of virulent Newcastle disease, flocks are not routinely vaccinated. Therefore, when the disease does appear in susceptible, unvaccinated, chicken populations there is a need to vaccinate chickens in the locality of the outbreak. There are no data available that indicate how V4 vaccination, given in the face of an outbreak, will affect the progress of an infection. Under such conditions it is important that a vaccine not only reduces the pathogenic effects of the infection but also reduces the amount and duration of virus shedding. The reduction of the amount of virus infection present in a flock will, in turn, reduce the probability that higher virulence will evolve in the infecting virus.

This project was established to provide data to address these questions. In this study we examined the shedding patterns of Peats Ridge strain, an avirulent endemic Newcastle disease virus that is considered to be the precursor strain of virulent Australian viruses (Westbury 2001). We used an avirulent virus because this is considered to be a suitable model for the infection of more virulent strains; being an endemic virus, it is easier to study, as the experimental containment conditions are simpler. Also, control of avirulent precursor strains is in itself important to reduce the chance of virulent strain emergence. We examined the shedding patterns following challenge just prior to V4 vaccination and we compared this to the alternative situation where infection took place some time after vaccination was given and immunity allowed to develop. The results of this study should be valuable in determining what role vaccination has in determining what control measures need to be put in place for an outbreak of Newcastle disease.

Objectives

The objectives of the project were to deliver experimental data on:

1. Whether prior vaccination with V4 can reduce subsequent infection with Peats Ridge strain and/or reduce the amount and duration of shedding of Peats Ridge strain Newcastle disease virus;
2. Whether V4 vaccination during a Peats Ridge infection can modify the course of the Peats Ridge infection; and
3. The serological response to the Peats Ridge strain alone and to Peats Ridge infection following prior vaccination with V4 vaccine.

Methodology

Trial design

All chickens used in the trials were housed on sawdust litter on the floor. They were provided with food and water *ad libitum*.

Two separate trial protocols were conducted (Table 1).

1. The vaccinate-challenge trial, where 7-day-old SPF chicks were vaccinated with V4 vaccine and then challenged with Peats Ridge strain 21 days later.
2. The challenge-vaccinate trial, where 4-week-old SPF chickens were challenged with Peats Ridge strain and then vaccinated with V4 virus two days later.

Each trial consisted of two groups, each of 20 chickens; the first group received vaccine and challenge viruses and a control group received Peats Ridge challenge virus only.

Due to technical problems, tracheal swabs were not taken in the first vaccinate-challenge trial. Therefore, this trial was repeated in a second vaccinate-challenge trial, where the full set of swabs was taken.

Vaccine

The V4 vaccine (Webster's Newcastle Disease Vaccine "V4 Strain" SPF Living, Fort Dodge) was diluted 1:200 in phosphate buffered saline, pH 7.2 and 40 µl given per bird by eyedrop, according to the manufacturer's instruction.

Challenge

The Peats Ridge strain (isolate 98-1154 no. 4) was used for the challenge inoculum. This strain is derived from an avirulent NDV isolated in 1998 from the Peats Ridge area near Sydney (not to be confused with a virulent isolate made in the same area in 1999). Original infected allantoic fluid was diluted tenfold in phosphate buffered saline, pH 7.2 and a total volume of 250 µl was administered into the eyes, nasal orifices and mouth. A total virus dose of 7.7 $\log_{10}$ median egg infectious doses (EID_{50}) was given per bird.

Table 1. The timeline of the trial events in each of the trial and control groups. Table 1a shows the vaccination, challenge and swabbing events according to the approximate age of the chickens. Swabs consisted of daily oral-tracheal and cloacal samples. Table 1b shows the timeline of the blood samplings, in relation to the day of challenge.

Table 1a.

		Event by Age (Days)				
Trial	Group	7	28	29	30	31 – 42
Vaccinate-Challenge	Vaccinates	V4 vaccine	Challenge with Peats Ridge strain	Swabs	Swabs	Swabs
	Control		Challenge with Peats Ridge strain	Swabs	Swabs	Swabs
Challenge-Vaccinate	Vaccinates		Challenge with Peats Ridge strain	Swabs	V4 vaccine Swabs	Swabs
	Control		Challenge with Peats Ridge strain	Swabs	Swabs	Swabs

Table 1b.

Trial	Group	Days after challenge					
Vaccinate-Challenge 1	Vaccinates	-1	7	14	21	27	34
	Control	-1	7	14	21	28	35
Vaccinate-Challenge 2	Vaccinates	0	7	14	21		
	Control	0	7	14	21		
Challenge-Vaccinate	Vaccinates	0	8	11	15		
	Control	0	8	11	15		

Sample collection

Blood for serum was taken from the wing vein at various days after challenge (Table 1). Virus shedding was tested for by taking oral-tracheal and cloacal swabs daily after challenge. Swabs were placed into transport medium (phosphate buffered saline pH 7.2 with penicillin (500 units/ml), streptomycin (1000 μg/ml) and gentamycin (50 μg/ml) and were frozen at minus 80^{o}C until tested.

Virus detection

Swabs were tested for the presence of viable virus by inoculating 200 μl of swab transport medium into the allantoic sac of each of two eggs. The eggs were incubated at 37^{o}C for 5 days after which allantoic fluid was tested for NDV haemagglutinating activity. Negative allantoic fluid was passed into another two eggs and haemagglutinating activity tested again five days later. Swab samples were considered negative if the second passage was negative for haemagglutinating activity. Only swabs taken from around the period of shedding were analysed for detection of virus: swabs taken two or more days after the last isolate of the group were not analysed. Although virus replication and shedding is known to occur for a substantial period after initial infection (Samuel & Spradbrow 1989), this decision was considered justified because once virus could no longer be detected within the flock for one day then the epidemic period was assumed to be over and no regular detections would subsequently occur.

Characterisation of virus isolates by TaqMan PCR

V4 vaccine and Peats Ridge strains are distinguished by their different fusion protein cleavage sequence. The DNA sequence of a portion of the fusion protein containing the fusion protein cleavage region was determined from a 300 bp PCR product generated from viral RNA isolated from allantoic fluid. The DNA sequence was translated into amino acid sequence to determine the fusion protein cleavage sequence.

The suitability of TaqMan tests to detect and differentiate V4 vaccine and Peats Ridge strain in combined populations was evaluated by mixing V4 and Peats Ridge strain suspensions of known titre (as determined by egg inoculation) and comparing the DNA sequence analysis and TaqMan PCR methods. The TaqMan tests were at least or more sensitive than DNA sequencing to determine which virus was predominant in combined populations. Both viruses could be detected and the majority and minority virus identified in mixtures where one of the strains was in 10-fold excess. Here we assume that biological test quantification (by egg infectious doses) is equivalent to nucleic acid quantification: although this is not necessarily the case, it is the most accurate measure available.

A strategically selected sample of positive first- or second-pass allantoic fluid isolates were tested to characterise the genotype of the virus. The samples selected for this analysis are indicated in Appendix 2 and include isolates from each of the major shedding periods and routes of interest, including the immediate post-vaccinate tracheal route and the period during the second week post-vaccinate cloacal route, both situations in challenge-vaccinate birds where maximal likelihood of dual virus shedding may occur. In addition, a sample of isolates from unvaccinated control birds and the post-challenge tracheal route from previously vaccinated birds were tested.

Testing of allantoic isolates would indicate which virus was present in the swab. This would be highly sensitive in detecting the presence of virus due to the high quality of allantoic fluid, compared with swab medium. However, it may not indicate dual infection if one of the virus types were to be more competitive in embryonated eggs to the point of excluding the less competitive type. If dual infections were to occur, they would be expected in the period after vaccination in the challenge-

vaccinate trial. In order to test for this possibility, a sample of original swabs from the days following vaccination in the challenge-vaccinate trial were also tested by TaqMan methods. In addition, in order to test the relative competitiveness of the two strains when inoculated together into embryonated eggs, mixtures of the two strains at ratios of infectious units of 1:1, 9:1 and 1:9 were inoculated into eggs and TaqMan tests conducted on the mixture itself and the allantoic fluid from the egg following incubation. Both virus strains were detected in the mixtures and in the allantoic fluids. Although preliminary, this information provides greater surety that the presence of dual infections in original swabs will be detected following passage through embryonated eggs.

Real-time reverse-transcription PCR using TaqMan probe chemistry (referred in this report as the TaqMan test) was carried out on the ABI PRISM 7700 Sequence Detection System instrument (Applied Biosystems). Primers and TaqMan probes were designed and evaluated using PrimerExpress design software (Applied Biosytems). Three TaqMan real-time PCR tests were designed to be performed simultaneously under uniform reaction conditions to detect all NDV strains and to discriminate between V4 vaccine and Peats Ridge strain. The design of TaqMan PCR primers and probes was based on nucleotide sequence alignment (obtained following the NDV national survey) of a number of pathogenically diverse Australian NDV strains including V4 vaccine strains, and avirulent and virulent field strains. The TaqMan test NDV-all was based on a conserved sequence region of the F1 portion of the fusion protein and detected all of the NDV strains. The two TaqMan tests NDV-V4 and NDV-avir were designed to discriminate between the V4 vaccine and Peats Ridge strains respectively, utilising conserved forward and reverse primers flanking the fusion protein cleavage region and specific TaqMan probes to bind over the cleavage region. The V4 vaccine specific TaqMan probe NDV-F-V4 differed by 3 nucleotide substitutions from the Peats Ridge strain specific probe NDV-F-avir, allowing for differentiation of vaccine strain from Peats Ridge strain by multiplex analysis of all three TaqMan tests on the same sample. Viral RNA was extracted from allantoic fluid or swabs using the Qiagen Rneasy Mini kit or the automated Roche Magnapure extraction. The TaqMan tests were performed in triplicates in 25 µl volume reactions on 96-well optical plates with amplification for 45 cycles. TaqMan tests were carried out in either a two-step format with separate reactions for cDNA synthesis (using TaqMan reverse transcription reagents and random hexamer cDNA primers) followed by TaqMan reaction (using TaqMan Universal master mix), or in a one-step format combining RT and TaqMan PCR reaction (using TaqMan One-step master mix) using gene-specific primers. The amplification plot of each reaction was generated and the cycle threshold Ct and fluorescence signal (ΔRn) values were used to identify the virus(es) present in a sample.

Serology

The serum samples were tested for the presence of antibodies to NDV by haemagglutination inhibition using a standard protocol (Della-Porta & Spencer 1993).

Table 2a. Newcastle disease virus isolation data, showing the number of swabs out of each group of 20 from which Newcastle disease virus was isolated. This information is demonstrated graphically in Figure 1.
NT = Not tested

			Number of Positive Isolates by Days after Challenge													
Trial	Group	Swab	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Vaccinate-Challenge 1	Vaccinates	Trachea l	NT													
		Cloacal	0	1	0	0	0	0	0	0	NT					
	Control	Trachea l	NT													
		Cloacal	0	15	16	19	16	18	2	13	2	0	NT			
Vaccinate-Challenge 2	Vaccinates	Trachea l	20	2	6	3	0	0	NT							
		Cloacal	0	0	0	NT										
	Control	Trachea l	19	20	20	20	20	8	0	0	NT					
		Cloacal	2	8	19	16	13	6	7	7	0	0	0	NT		
Challenge - Vaccinate	Vaccinates	Trachea l	20	20	20	20	20	9	2	0	NT					
		Cloacal	0	(3)	18	20	20	16	3	1	5	2	1	6	1	NT
	Control	Trachea l	20	20	20	(19)	15	3	0	NT						
		Cloacal	0	4	7	15	12	10	8	17	9	3	0	NT		

Table 2b. Summary of virus isolation data from Table 2a. The data is the same as Table 2a, but ordered for ease of comparison between the different groups.

		Number of Positive Isolates by Days after Challenge														
Swab	Trial group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Comment
Trachea l	V-C 1															NT
	V-C 2	20	2	6	3	0	0	NT								
	C-V	20	20	20	20	20	9	2	0	NT						
	Control 1															NT
	Control 2	19	20	20	20	20	8	0	0	NT						
	Control 3	20	20	20	(19)	15	3	0	NT							

Cloacal	V-C 1	0	1	0	0	0	0	0	0	NT						
	V-C 2	0	0	0	NT											
	C-V	0	(3)	18	20	20	16	3	1	5	2	1	6	1	NT	
	Control 1	0	15	16	19	16	18	2	13	2	0	NT				
	Control 2	2	8	19	16	13	6	7	7	0	0	0	NT			
	Control 3	0	4	7	15	12	10	8	17	9	3	0	NT			

Results

During the trials all birds remained healthy, as would be expected with infection by V4 and Peats Ridge strains.

Virus isolation results are shown in Table 2 and Figure 1. Analysed serology results are shown in Table 3. Raw data tables for virus isolations, genotyping and serology results are displayed in Appendices 1, 2 and 3 respectively.

The three challenge control trials indicated that the Peats Ridge strain was shed by the tracheal route by almost all birds up until day 5, after which the number of tracheal shedders declined (Figure 1). No tracheal shedding was found after day 6. In contrast, shedding via the cloacal route occurred mainly between days 3 and 8, with low numbers of shedders before and after this time. Shedding was no longer detected after day 10. In birds that had received prior immunisation with V4, shedding was almost exclusively confined to the tracheal route, shedding ceased after day 4 and after day 1, shedding was detected in a much lower proportion of the birds. In birds that were vaccinated two days after Peats Ridge strain challenge, the proportion of tracheal shedders on the various days did not significantly differ from those in control groups (Figure 1). Cloacal shedding in the first week appeared to follow a similar trend to that in controls, although after the second week shedding occurred in a lower proportion of birds and some birds shed virus beyond day 10.

All the allantoic virus isolates typed were characterised as Peats Ridge strain. In addition, all original swabs that were tested positive for virus and typed were found to contain Peats Ridge strain (Appendix 2). No V4 strain was detected in any of the allantoic fluids or swabs.

The serology results indicate that there was 100% seroconversion to Peats Ridge strain challenge (Table 3). Seroconversion was rapid with over 90% of birds showing seroconversion by day 7. Maximum detected titres were reported on days 14 and 21.

Table 3a. The geometric means of the serum HI titres of each of the groups. The titre refers to the reciprocal of serum dilution at the endpoint.

		Reciprocal titre by days after challenge						
Trial	Group	0	7/8	11	14/15	21	27/28	34/35
Vaccinate-Challenge 1	Vaccinates	6.96	35.50		45.25	36.76	25.99	25.11
	Control	<2	17.75		45.25	48.50	45.25	38.05
Vaccinate-Challenge 2	Vaccinates	24.25	73.52		93.70	81.57		
	Control	<2	28.84		43.71	36.76		
Challenge-Vaccinate	Vaccinates	<2	21.92	68.59	55.72			
	Control	<2	26.07	76.11	61.82			

Table 3b. Summary of serology data, extracted from Table 3a and Appendix 3. The total number of birds in each group was 20. The groups Control 1, 2 and 3 refer to the control groups of the first and second vaccinate-challenge and challenge-vaccinate groups respectively.

	Vaccinate-Challenge 1	Vaccinate-Challenge 2	Challenge-Vaccinate	Challenge Control 1	Challenge Control 2	Challenge Control 3
Percentage seroconversion to challenge (at least one value equal to or greater than a 4-fold increase over the Day 0 titre)	Not relevant	Not relevant	100	100	100	100
GMT at challenge following V4 vaccination	6.96	24.25	Not relevant	Not relevant	Not relevant	Not relevant
Percentage at challenge with titres greater than 1:4	65 %	90 %	Not relevant	Not relevant	Not relevant	Not relevant
Maximum detected GMT	25.99	93.70	68.59	48.50	43.71	76.11
Day after challenge of maximum detected GMT	7, 14	14	11	21	14	11
Lowest titre at day of maximum GMT	8	16	32	32	8	16
Percentage of birds with lowest titre at maximum GMT	10 % (D14)	5 %	15 %	40 %	5 %	5 %
GMT at Day 7	25.99	73.52	21.92	17.75	28.84	26.07
Lowest titre at Day 7	8	16	8	8	8	2
Percentage of birds with lowest titre at Day 7	5 %	5 %	10 %	5 %	10 %	5 %

Discussion

The first objective of this study was to determine whether prior vaccination with V4 could reduce subsequent infection with and shedding of Peats Ridge strain Newcastle disease virus. The study demonstrates that prior vaccination with a singe dose did not completely protect against subsequent infection, as was seen by the isolations from the oral-tracheal route in a small number of birds up until Day 4. However, prior vaccination appeared to have three effects on reducing the virus shedding. Firstly, it significantly reduced the number of birds shedding the virus: between 2 and 6 birds – compared to all 20 in the control groups – shed virus by the oral-tracheal route. Secondly, it reduced the time period of shedding from 6 days in the control groups to 4 days in the pre-vaccinated groups, a difference that may not be significant. Thirdly, it almost eliminated the shedding of virus from the intestinal tract: only one cloacal swab yielded virus, as compared to the majority of cloacal swabs from the control groups. No data was obtained on the effect of multiple vaccinations on shedding of Peats Ridge virus.

The isolation patterns in the birds vaccinated prior to challenge indicate that there was virus present in all birds at the oral-tracheal site on Day 1, but that this fell such that by Day 2 only few birds had virus present. The isolations at Day 1 may be of residual virus retained from the inoculum at the site. In the subsequent days (Days 2-4) the isolates are most likely to be of virus generated following replication.

The cloacal isolation patterns indicate that intestinal infection is rare in birds that have been vaccinated prior to challenge. Only one isolate was made in the pre-vaccinated birds and this was on Day 2 after challenge. It appears that spread of the infection from the oral-oculonasal inoculation site to other tissues is blocked by the pre-existing immunity.

In comparison, the shedding pattern of the challenge-vaccinate group was essentially little different from that in the control birds, except for more prolonged cloacal shedding in a smaller proportion of the post-challenge vaccinated group. Genotyping indicated that this late shedding was of Peats Ridge strain, so the difference between this group and the controls may not be significant. Interestingly, all the control groups had a relatively high (compared with the challenge-vaccinate group) proportion of day 8 cloacal shedders, but the statistical significance of this finding is also uncertain and may reflect variation in swabbing efficiency. Comparison of the post-challenge vaccinated group and the control groups indicated that oral-tracheal shedding trends were similar in terms of both numbers of birds and shedding periods.

PCR genotyping analysis on the allantoic fluid isolates indicated that Peats Ridge strain was present in both the trachea and the cloaca following challenge in all groups. This is as expected for the vaccinate-challenge group, where the infection cycle of the V4 vaccine would be over and the only virus present would have originated from the challenge inoculum. For the challenge-vaccinate group, it is somewhat surprising that no V4 virus was present at detectable levels. It is possible that the presence of an already established infection, by Peats Ridge strain, may have inhibited the subsequent establishment of a second infection by V4 virus.

Passage of swab samples by inoculation into embryonated eggs may have had the effect, if there was a dual infection, of selecting the competitively dominant strain. Hence, typing of allantoic isolates may indicate what strain was present in the swab, but may not indicate if there was a dual infection. For determining the presence of dual infections, analysis of the original swab sample would be necessary. This testing was conducted on a number of swabs in the post-vaccination period of the challenge-vaccinate group. It was apparent that the PCR technique was at the limits of its detection threshold as many of the swabs that were positive on egg inoculation were negative on TaqMan PCR.

This study indicates that the vaccination of chickens during an infection would not shorten or decrease the number of birds shedding virus. This principle would only apply to the shedding patterns of individual birds; at a population level the same patterns may not apply. Within a flock of birds, the infection status – and therefore the potential response to V4 vaccination – would be heterogeneous. Thus, vaccination at day 2 of an outbreak may result in the establishment of V4 virus infection in some birds prior to them being infected with Peats Ridge strain, and these birds may well have better protection to subsequent infection. In order to comprehensively assess the effect of vaccination at a flock level, it would be necessary to incorporate models of population immunity that take into account the heterogeneous nature of the flock.

The serology results show several notable patterns. Infection with Peats Ridge strain alone (control groups) resulted in 100 % seroconversion of the challenged birds, with nearly all birds seroconverting by Day 7. Maximum group HI titres reached means of between 43 and 76. Even at Day 7 only 1-2 birds per group had titres equal to or lower than 8. When given after V4 vaccination, the Peats Ridge strain challenge induced significant titre increases – by a factor of almost 4 in both trials – when compared to the maximum mean titre. These data would indicate that infection with this strain may cause good protection against subsequent NDV infections and that protection would be present in almost all birds by Day 7 – this would have to be demonstrated conclusively by the appropriate challenge experiments.

Prior vaccination with V4 resulted in the birds having HI antibodies at the time of challenge. Nevertheless, there was no major apparent difference in the group serological trends – that is, the Day 7 and peak mean titres – between the prior-vaccinated and challenge control groups.

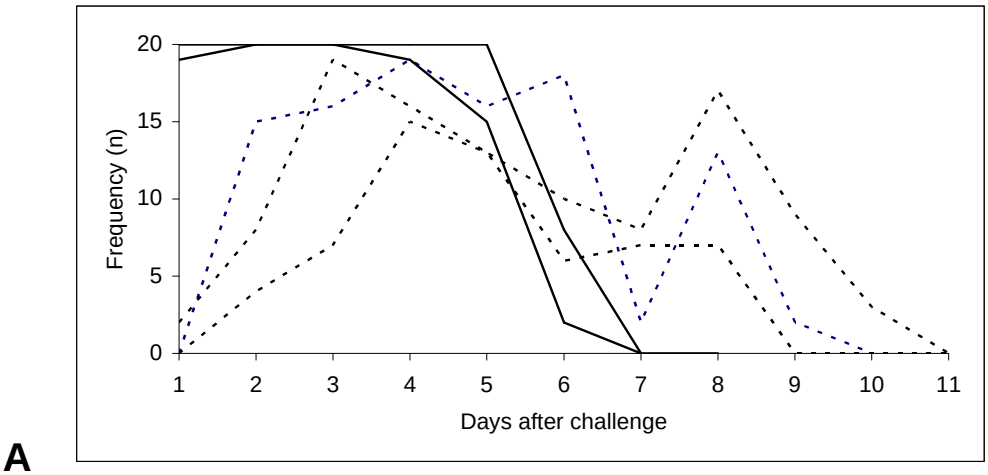
Implications

The implications of this study can be summarised as follows.

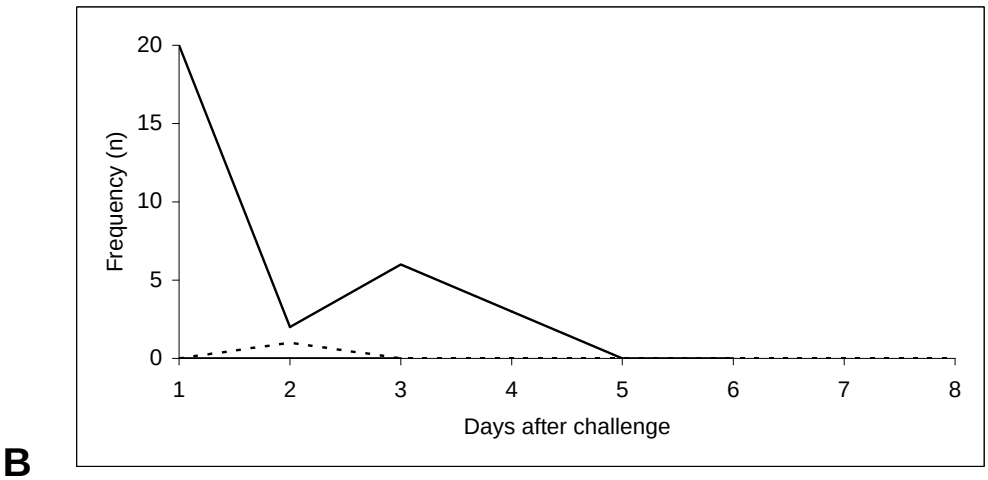
1. Prior vaccination with V4 vaccine is effective at significantly reducing the shedding of virus during subsequent Peats Ridge strain infection.
2. Post-challenge vaccination with V4 vaccine during active Peats Ridge strain infection does not appear to significantly affect the number of birds that shed Peats Ridge strain, nor does it shorten the duration of shedding.
3. Peats Ridge strain infection induces a significant frequency of seroconversion to significant HI antibody levels. Although not tested for in this study, these levels of antibodies are probably protective against subsequent virulent Newcastle disease, but not infection with virus.

Recommendations

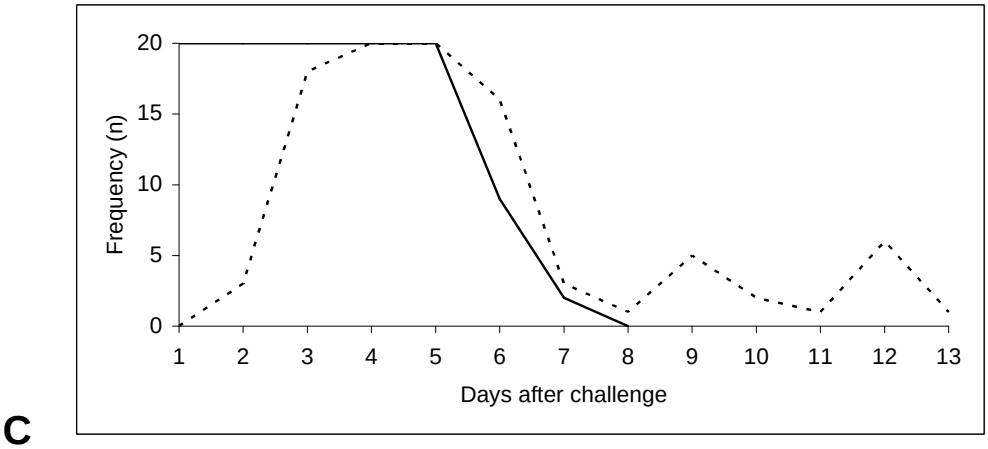
This study indicates that vaccination with V4 vaccine following infection does not appear to influence the shedding patterns of Newcastle disease. Other control strategies should be investigated. Alternatively, if post-infection vaccination is used, its effect at the population level must be considered, as vaccination may be partially effective at limiting epidemics in a heterogeneously-infected bird population.



A



B



C

Figure 1. Trends of Newcastle disease virus shedding from the trachea (continuous lines) and cloaca (dotted lines). See Table 2 for data. The graphs display the number of positive birds shedding virus without the influence of vaccination (A), with challenge three weeks after V4 vaccination (B) and with V4 vaccination two days after challenge (C).

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Appendix 1a (i). Newcastle disease virus isolation data: Vaccinate – Challenge trial 1.

Cloacal swabs

	Virus Isolation Pass (by day)																	
Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
26	-	-	-	-	-	-	-	-										
27	-	-	-	-	-	-	-	-										
28	-	-	-	-	-	-	-	-										
29	-	-	-	-	-	-	-	-										
30	-	-	-	-	-	-	-	-										
31	-	-	-	-	-	-	-	-										
32	-	-	-	-	-	-	-	-										
33	-	-	-	-	-	-	-	-										
34	-	-	-	-	-	-	-	-										
35	-	-	-	-	-	-	-	-										
36	-	-	-	-	-	-	-	-										
37	-	-	-	-	-	-	-	-										
38	-	-	-	-	-	-	-	-										
39	-	-	-	-	-	-	-	-										
40	-	1	-	-	-	-	-	-										
41	-	-	-	-	-	-	-	-										
42	-	-	-	-	-	-	-	-										
43	-	-	-	-	-	-	-	-										
44	-	-	-	-	-	-	-	-										
45	-	-	-	-	-	-	-	-										

Key: '1' – Virus isolated on first pass in eggs
'2' – Virus isolated on second pass in eggs
'-' – No virus isolated after two passes in eggs
'**1**' or '**2**' (bold font) – Isolate or original swab tested by TaqMan genotyping (Appendix 2)

Appendix 1a (ii). Newcastle disease virus isolation data: Vaccinate – Challenge trial 2.

Tracheal Swabs

	Virus Isolation Pass (by day)													
Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
426	1	-	-	-	-	-								
427	2	-	-	-	-	-								
428	2	-	-	-	-	-								
429	2	-	-	-	-	-								
430	2	-	-	-	-	-								
431	1	-	-	-	-	-								
432	2	-	**1**	-	-	-								
433	2	-	-	-	-	-								
434	1	-	-	-	-	-								
435	2	**1**	-	-	-	-								
436	2	-	-	-	-	-								
437	2	-	**1**	-	-	-								
438	1	-	-	**2**	-	-								
439	2	-	-	-	-	-								
440	2	-	**1**	**2**	-	-								
441	2	-	**1**	**2**	-	-								
442	1	-	-	-	-	-								
443	2	**1**	**1**	-	-	-								
444	2	-	**1**	-	-	-								
445	2	-	-	-	-	-								

Cloacal Swabs

Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
426	-	-	-											
427	-	-	-											
428	-	-	-											
429	-	-	-											
430	-	-	-											
431	-	-	-											
432	-	-	-											
433	-	-	-											
434	-	-	-											
435	-	-	-											
436	-	-	-											
437	-	-	-											
438	-	-	-											
439	-	-	-											
440	-	-	-											
441	-	-	-											
442	-	-	-											
443	-	-	-											
444	-	-	-											
445	-	-	-											

Key: '1' – Virus isolated on first pass in eggs
'2' – Virus isolated on second pass in eggs
'-' – No virus isolated after two passes in eggs
'**1**' or '**2**' (bold font) – Isolate or original swab tested by TaqMan genotyping (Appendix 2)

Appendix 1a (iii). Newcastle disease virus isolation data: Challenge – Vaccinate trial.

Tracheal Swabs

	Virus Isolation Pass (by day)													
Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
76	1	1	**1**	**1**	1	2	-	-						
77	1	1	**1**	**1**	**1**	2	-	-						
78	1	1	**1**	**1**	1	-	2	-						
79	1	1	**1**	**1**	1	2	-	-						
80	1	1	**1**	**1**	**1**	1	-	-						
81	1	1	**1**	**1**	1	-	-	-						
82	1	1	**1**	**1**	1	-	2	-						
83	1	1	**1**	**1**	**1**	1	-	-						
84	1	1	**1**	**1**	1	-	-	-						
85	1	1	**1**	**1**	1	-	-	-						
86	1	1	**1**	**1**	1	-	-	-						
87	1	1	**1**	**1**	1	-	-	-						
88	1	1	**1**	**1**	1	2	-	-						
89	1	1	**1**	**1**	1	1	-	-						
90	1	1	**1**	**1**	1	-	-	-						
91	1	1	**1**	**1**	1	2	-	-						
92	1	1	**1**	**1**	1	-	-	-						
93	1	1	**1**	**1**	1	-	-	-						
94	1	1	**1**	**1**	1	-	-	-						
95	1	1	**1**	**1**	1	2	-	-						

Cloacal Swabs

Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
76	-	-	-	**1**	2	2	1	-	-	-	-			
77	-	-	-	2	2	2	-	-	-	-	-			
78	-	-	**1**	2	**1**	2	2	-	-	-	-			
79	-	1	**1**	**1**	2	2	-	-	-	-	-			
80	-	-	2	2	**1**	1	-	-	-	-	-			
81	-	-	2	**1**	1	1	-	**1**	**1**	**1**	**1**	**1**	**1**	
82	-	-	2	2	2	2	-	-	**2**	-	-	**2**	-	
83	-	1	**1**	2	**1**	1	-	-	**2**	-	-	**2**	-	
84	-	-	2	2	2	2	-	-	**2**	-	-	**2**	-	
85	-	-	2	2	1	1	-	-	-	-	-			
86	-	?	2	**1**	1	2	-	-	-	**2**	-	**2**	-	
87	-	-	2	2	2	2	-	-	-	-	-			
88	-	-	2	**1**	**1**	2	-	-	**2**	-	-	**2**	-	
89	-	1	2	**1**	1	2	-	-	-	-	-			
90	-	-	2	2	2	2	-	-	-	-	-			
91	-	-	**1**	2	2	2	-	-	-	-	-			
92	-	-	**1**	2	2	-	-	-	-	-	-			
93	-	-	**1**	**1**	1	-	-	-	-	-	-			
94	-	-	2	**1**	2	-	1	-	-	-	-			
95	-	-	2	2	2	-	-	-	-	-	-			

Key: '1' – Virus isolated on first pass in eggs
'2' – Virus isolated on second pass in eggs
'-' – No virus isolated after two passes in eggs
'**1**' or '**2**' (bold font) – Isolate or original swab tested by TaqMan genotyping (Appendix 2)

Appendix 1b (i). Newcastle disease virus isolation data, Challenge control group. Vaccinate-challenge trial 1.

Cloacal swabs

	Virus Isolation Pass (by day)																	
Bird No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
76	-	2	2	1	1	2	-	2	2	-								
77	-	2	-	1	1	2	-	2	-	-								
78	-	2	2	2	2	2	-	2	2	-								
79	-	-	-	2	2	2	-	2	-	-								
80	-	-	2	1	1	1	-	2	-	-								
81	-	2	-	2	2	-	-	2	-	-								
82	-	2	-	1	1	1	-	2	-	-								
83	-	2	2	-	-	1	-	2	-	-								
84	-	-	1	2	1	2	-	2	-	-								
85	-	2	1	2	1	2	-	2	-	-								
86	-	2	2	1	2	2	-	2	-	-								
87	-	-	2	1	-	2	-	-	-	-								
88	-	2	2	2	-	2	-	-	-	-								
89	-	-	2	1	1	2	-	-	-	-								
90	-	2	2	1	1	2	-	-	-	-								
91	-	2	2	1	2	2	-	2	-	-								
92	-	2	2	2	2	2	-	-	-	-								
93	-	2	2	2	-	2	-	2	-	-								
94	-	2	2	2	2	1	2	-	-	-								
95	-	2	2	2	2	-	2	-	-	-								

Key: '1' – Virus isolated on first pass in eggs
'2' – Virus isolated on second pass in eggs
'-' – No virus isolated after two passes in eggs
'**1**' or '**2**' (bold font) – Isolate or original swab tested by TaqMan genotyping (Appendix 2)

Appendix 1b (ii). Newcastle disease virus isolation data, Challenge control group. Vaccinate-challenge trial 2.

Tracheal Swabs

	Virus Isolation Pass (by day)													
Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
226	1	1	**1**	1	1	1	-	-						
227	1	1	1	1	1	-	-	-						
228	1	1	**1**	1	1	2	-	-						
229	1	1	1	1	1	2	-	-						
230	1	1	**1**	1	1	-	-	-						
231	1	1	1	1	1	-	-	-						
232	1	1	**1**	1	1	1	-	-						
233	1	1	1	1	1	2	-	-						
234	1	1	**1**	1	1	1	-	-						
235	1	1	1	1	1	-	-	-						
236	1	1	1	1	1	-	-	-						
237	-	1	1	1	1	2	-	-						
238	1	1	1	1	1	-	-	-						
239	1	1	1	1	1	-	-	-						
240	1	1	1	1	1	-	-	-						
241	1	1	1	1	1	2	-	-						
242	1	1	1	1	1	-	-	-						
243	1	1	1	1	1	-	-	-						
244	1	1	1	1	1	-	-	-						
245	1	1	1	1	1	-	-	-						

Cloacal Swabs

Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
226	-	-	**1**	1	1	2	-	-	-	-	-			
227	1	1	2	2	1	-	-	2	-	-	-			
228	-	-	**2**	1	1	2	1	2	-	-	-			
229	-	1	1	1	1	-	-	-	-	-	-			
230	-	-	**2**	2	-	-	-	2	-	-	-			
231	2	1	1	1	1	-	-	-	-	-	-			
232	-	1	**1**	1	1	-	-	2	-	-	-			
233	-	-	1	2	1	2	-	-	-	-	-			
234	-	-	**1**	2	1	-	2	2	-	-	-			
235	-	-	2	2	-	-	-	-	-	-	-			
236	-	-	2	2	-	2	2	-	-	-	-			
237	-	1	2	2	2	2	-	-	-	-	-			
238	-	-	2	-	-	-	2	-	-	-	-			
239	-	-	2	-	-	-	-	2	-	-	-			
240	-	-	1	1	1	-	2	-	-	-	-			
241	-	-	-	-	-	-	2	-	-	-	-			
242	-	2	1	1	-	-	-	2	-	-	-			
243	-	1	1	1	1	-	-	-	-	-	-			
244	-	2	2	-	2	2	-	-	-	-	-			
245	-	-	2	1	1	-	2	-	-	-	-			

Key: '1' – Virus isolated on first pass in eggs
'2' – Virus isolated on second pass in eggs
'-' – No virus isolated after two passes in eggs
'**1**' or '**2**' (bold font) – Isolate or original swab tested by TaqMan genotyping (Appendix 2)

Appendix 1b (iii). Newcastle disease virus isolation data, Challenge control group. Challenge-vaccinate trial.

Tracheal Swabs

	Virus Isolation Pass (by day)													
Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
51	1	1	**1**	1	1	-	-							
52	1	1	1	1	1	-	-							
53	1	1	1	1	1	-	-							
54	1	1	**1**	NT	1	-	-							
55	1	1	1	1	1	-	-							
56	1	1	1	1	1	-	-							
57	1	1	**1**	1	1	-	-							
58	1	1	1	1	1	-	-							
59	1	1	1	1	1	-	-							
60	1	1	**1**	1	1	-	-							
61	1	1	1	1	1	-	-							
62	1	1	1	1	1	1	-							
63	1	1	**1**	1	1	-	-							
64	1	1	1	1	-	-	-							
65	1	1	1	1	-	-	-							
66	1	1	1	1	1	2	-							
67	1	1	1	1	-	-	-							
68	1	1	1	1	1	-	-							
69	1	1	1	1	-	-	-							
70	1	1	1	1	-	-	-							

Cloacal Swabs

Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
51	-	-	2	1	1	1	1	1	1	-	-			
52	-	-	-	**1**	2	2	-	2	2	-	-			
53	-	1	1	1	1	2	1	1	1	-	-			
54	-	-	-	1	1	1	1	1	2	2	-			
55	-	-	-	-	-	-	2	-	2	-	-			
56	-	-	-	**2**	-	-	2	2	2	-	-			
57	-	1	2	2	2	2	-	-	-	-	-			
58	-	-	-	2	1	-	-	-	-	-	-			
59	-	-	-	-	-	-	-	2	-	-	-			
60	-	-	-	**1**	1	-	1	1	1	-	-			
61	-	-	1	1	1	1	1	1	1	-	-			
62	-	-	2	1	-	-	-	2	-	2	-			
63	-	-	-	**1**	2	2	-	2	-	-	-			
64	-	-	-	2	2	2	-	2	-	-	-			
65	-	-	-	-	-	-	-	2	-	-	-			
66	-	-	1	**2**	2	2	-	2	2	-	-			
67	-	1	2	1	2	-	2	2	-	-	-			
68	-	-	-	2	-	-	-	2	-	2	-			
69	-	-	-	-	-	-	-	2	-	-	-			
70	-	1	-	-	-	2	-	2	-	-	-			

Key: '1' – Virus isolated on first pass in eggs
'2' – Virus isolated on second pass in eggs
'-' – No virus isolated after two passes in eggs
'NT' – Not tested
'**1**' or '**2**' (bold font) – Isolate or original swab tested by TaqMan genotyping (Appendix 2)

Appendix 2a. TaqMan PCR test results on selected NDV-positive allantoic fluid samples. Avirulent pathotype includes Peats Ridge strain.

Sample Number	Bird Number	Day	Allantoic sample derived from	TaqMan PCR result (Number of positive reactions out of duplicates)			Pathotype detected
				NDV-all	NDV-V4	NDV-avir	
Challenge – Vaccinate trial							
1	76	4	Tracheal	2	0	2	Avirulent
2	79	4	Tracheal	2	0	2	Avirulent
3	82	4	Tracheal	2	0	2	Avirulent
4	88	4	Tracheal	2	0	2	Avirulent
5	85	4	Tracheal	2	0	2	Avirulent
6	77	5	Tracheal	2	0	2	Avirulent
7	80	5	Tracheal	2	0	2	Avirulent
8	83	5	Tracheal	2	0	2	Avirulent
9	76	4	Cloacal	0	0	0	No virus
10	79	4	Cloacal	2	0	2	Avirulent
11	81	4	Cloacal	0	0	1?	No virus
12	86	4	Cloacal	0	0	0	No virus
13	93	4	Cloacal	2	0	2	Avirulent
14	78	5	Cloacal	2	0	2	Avirulent
15	80	5	Cloacal	2	0	2	Avirulent
16	83	5	Cloacal	1	0	2	Avirulent
17	88	5	Cloacal	2	0	2	Avirulent
18	81	8	Cloacal	2	0	2	Avirulent
19	81	9	Cloacal	2	0	2	Avirulent
20	82	9	Cloacal	2	0	2	Avirulent
21	83	9	Cloacal	2	0	2	Avirulent
22	84	9	Cloacal	2	0	2	Avirulent
23	88	9	Cloacal	2	0	2	Avirulent
24	81	10	Cloacal	2	0	2	Avirulent
25	86	10	Cloacal	2	0	2	Avirulent
26	81	11	Cloacal	2	0	2	Avirulent
27	81	12	Cloacal	1	0	2	Avirulent
28	82	12	Cloacal	2	0	2	Avirulent
29	83	12	Cloacal	2	0	2	Avirulent
30	84	12	Cloacal	2	0	2	Avirulent
31	86	12	Cloacal	1	0	2	Avirulent
32	88	12	Cloacal	2	0	2	Avirulent
33	81	13	Cloacal	1	0	2	Avirulent
Challenge trial							
34	51	3	Tracheal	2	0	2	Avirulent
35	54	3	Tracheal	2	0	2	Avirulent
36	57	3	Tracheal	2	0	2	Avirulent
37	60	3	Tracheal	1	0	1	Avirulent
38	63	3	Tracheal	1	0	2	Avirulent
39	52	4	Cloacal	2	0	2	Avirulent
40	56	4	Cloacal	2	0	2	Avirulent
41	60	4	Cloacal	2	0	2	Avirulent
42	63	4	Cloacal	2	0	2	Avirulent
43	66	4	Cloacal	2	0	2	Avirulent

Appendix 2a (continued). TaqMan PCR test results on selected NDV-positive allantoic fluid samples.

Sample Number	Bird Number	Day	Allantoic sample derived from	TaqMan PCR result (Number of positive reactions out of duplicates)			Pathotype detected
				NDV-all	NDV-V4	NDV-avir	
Vaccinate – Challenge trial							
44	435	2	Tracheal	2	0	2	Avirulent
45	443	2	Tracheal	2	0	2	Avirulent
46	432	3	Tracheal	2	0	2	Avirulent
47	437	3	Tracheal	2	0	2	Avirulent
48	440	3	Tracheal	2	0	2	Avirulent
49	441	3	Tracheal	1	0	2	Avirulent
50	443	3	Tracheal	2	0	2	Avirulent
51	444	3	Tracheal	2	0	2	Avirulent
52	438	4	Tracheal	2	0	2	Avirulent
53	440	4	Tracheal	2	0	2	Avirulent
54	441	4	Tracheal	2	0	2	Avirulent
Challenge trial							
55	226	3	Tracheal	2	0	2	Avirulent
56	228	3	Tracheal	2	0	2	Avirulent
57	230	3	Tracheal	2	0	2	Avirulent
58	232	3	Tracheal	2	0	2	Avirulent
59	234	3	Tracheal	2	0	2	Avirulent
60	226	3	Cloacal	2	0	2	Avirulent
61	228	3	Cloacal	2	0	2	Avirulent
62	230	3	Cloacal	2	0	2	Avirulent
63	232	3	Cloacal	2	0	2	Avirulent
64	234	3	Cloacal	2	0	2	Avirulent

Appendix 2b. TaqMan PCR test results on selected swab samples that were NDV-positive on isolation. The swabs were selected from the challenge-vaccinate trial, where it was expected that both strains may occur in the same sample. Avirulent pathotype includes Peats Ridge strain.

Sample Number	Bird Number	Day	Sample	TaqMan PCR result (Number of positive reactions out of triplicates)			Pathotype detected
				NDV-all	NDV-V4	NDV-avir	
1	76	3	Tracheal	2	0	1	Avirulent
2	77	3	Tracheal	3	0	3	Avirulent
3	78	3	Tracheal	3	0	1	Avirulent
4	79	3	Tracheal	3	0	2	Avirulent
5	80	3	Tracheal	3	0	2	Avirulent
6	81	3	Tracheal	3	0	3	Avirulent
7	82	3	Tracheal	3	0	3	Avirulent
8	83	3	Tracheal	3	0	3	Avirulent
9	84	3	Tracheal	3	0	1	Avirulent
10	85	3	Tracheal	3	0	0	Not typed
11	86	3	Tracheal	3	0	1	Avirulent
12	87	3	Tracheal	3	0	2	Avirulent
13	88	3	Tracheal	3	0	2	Avirulent
14	89	3	Tracheal	3	0	2	Avirulent
15	90	3	Tracheal	3	0	1	Avirulent
16	91	3	Tracheal	3	0	3	Avirulent
17	92	3	Tracheal	3	0	3	Avirulent
18	93	3	Tracheal	3	0	2	Avirulent
19	94	3	Tracheal	3	0	3	Avirulent
20	95	3	Tracheal	3	0	2	Avirulent
21	76	4	Tracheal	3	0	2	Avirulent
22	77	4	Tracheal	3	0	3	Avirulent
23	78	4	Tracheal	3	0	2	Avirulent
24	79	4	Tracheal	3	0	2	Avirulent
25	80	4	Tracheal	3	0	2	Avirulent
26	81	4	Tracheal	3	0	3	Avirulent
27	82	4	Tracheal	3	0	2	Avirulent
28	83	4	Tracheal	3	0	1	Avirulent
29	84	4	Tracheal	3	0	1	Avirulent
30	85	4	Tracheal	3	0	0	Not typed
31	86	4	Tracheal	1	0	0	Not typed
32	87	4	Tracheal	3	0	2	Avirulent
33	88	4	Tracheal	3	0	3	Avirulent
34	89	4	Tracheal	1	0	1	Avirulent
35	90	4	Tracheal	3	0	2	Avirulent
36	91	4	Tracheal	2	0	1	Avirulent
37	92	4	Tracheal	3	0	0	Not typed
38	93	4	Tracheal	3	0	1	Avirulent
39	94	4	Tracheal	3	0	1	Avirulent
40	95	4	Tracheal	3	0	1	Avirulent

Appendix 2b (continued). TaqMan PCR test results on selected swab samples that were NDV-positive on isolation.

Sample Number	Bird Number	Day	Sample	TaqMan PCR result (Number of positive reactions out of triplicates)			Pathotype detected
				NDV-all	NDV-V4	NDV-avir	
41	78	3	Cloacal	2	0	0	Not typed
42	79	3	Cloacal	3	0	1	Avirulent
43	83	3	Cloacal	3	0	0	Not typed
44	91	3	Cloacal	0	0	0	No virus
45	92	3	Cloacal	0	0	0	No virus
46	93	3	Cloacal	0	0	0	No virus
47	76	4	Cloacal	1	0	1	Avirulent
48	79	4	Cloacal	3	0	3	Avirulent
49	81	4	Cloacal	0	0	0	No virus
50	86	4	Cloacal	1	0	0	Not typed
51	88	4	Cloacal	2	0	0	Not typed
52	89	4	Cloacal	3	0	3	Avirulent
53	93	4	Cloacal	2	0	0	Not typed
54	94	4	Cloacal	1	0	0	Not typed

Appendix 3a (i). Reciprocal serum HI antibody titres: Vaccinate-Challenge trial 1.

Bird No.	27/6	5/7	12/7	19/7	25/7	1/8
26	4	64	64	32	32	32
27	2	16	8	32	8	16
28	8	64	16	32	16	8
29	8	32	32	64	64	32
30	2	32	32	64	64	64
31	16	64	32	64	8	16
32	16	32	64	64	32	32
33	16	32	64	128	64	64
34	16	32	64	128	64	64
35	4	32	64	16	8	16
36	4	64	32	16	64	16
37	4	32	32	32	16	16
38	8	32	64	32	16	16
39	8	32	64	16	32	32
40	16	64	64	32	32	32
41	8	32	64	32	16	32
42	8	32	64	32	16	16
43	8	64	128	32	64	32
44	8	32	32	16	32	16
45	4	8	64	32	16	32

Appendix 3a (ii). Reciprocal serum HI antibody titres: Vaccinate-challenge trial 2.

Bird No.	4/8/03	11/8/03	18/8/03	25/8/03
426	16	128	128	128
427	32	64	64	32
428	64	64	128	128
429	32	64	64	64
430	32	128	128	64
431	32	64	64	64
432	64	128	256	128
433	16	64	128	128
434	4	16	16	16
435	32	64	128	128
436	16	128	128	128
437	8	64	64	64
438	32	128	128	128
439	32	32	64	64
440	64	128	128	128
441	32	32	64	32
442	32	64	64	64
443	4	64	128	128
444	32	128	128	128
445	32	128	128	128

Appendix 3a (iii). Reciprocal serum HI antibody titres: Challenge-vaccinate trial.

Bird No.	25/8/02	2/9/02	5/9/02	9/9/02
76	<2	64	128	128
77	<2	8	32	64
78	<2	32	64	64
79	<2	32	64	128
80	<2	32	64	128
81	<2	32	128	16
82	<2	16	64	32
83	<2	32	64	128
84	<2	16	128	64
85	<2	16	64	64
86	<2	16	32	16
87	<2	64	128	128
88	<2	16	32	32
89	<2	34	64	128
90	<2	16	64	32
91	<2	16	64	32
92	<2	32	128	64
93	<2	16	64	64
94	<2	8	64	32
95	<2	16	64	32

Appendix 3b (i). Reciprocal serum HI antibody titres: Challenge control group. Vaccinate-challenge trial 1.

Bird No.	24/6	2/7	9/7	16/7	23/7	30/7
76	<2	16	16	32	32	16
77	<2	16	64	64	128	64
78	<2	16	32	32	32	32
79	<2	16	32	64	64	64
80	<2	16	64	32	64	64
81	<2	16	64	64	64	64
82	<2	16	32	64	64	64
83	<2	16	64	64	64	32
84	<2	8	32	64	64	32
85	<2	32	64	64	32	32
86	<2	16	64	64	32	128
87	<2	32	64	64	64	128
88	<2	32	32	64	32	32
89	<2	32	64	32	32	32
90	<2	16	32	32	32	32
91	<2	16	32	32	32	8
92	<2	16	64	64	64	32
93	<2	16	64	32	32	32
94	<2	16	64	64	32	16
95	<2	16	32	32	32	32

Appendix 3b (ii). Reciprocal serum HI antibody titres: Challenge control group. Vaccinate-challenge trial 2.

Bird No.	25/8/02	2/9/02	5/9/02	9/9/02
51	<2	64	128	128
52	<2	32	64	32
53	<2	34	128	128
54	<2	128	128	128
55	<2	8	32	32
56	<2	32	256	128
57	<2	8	32	32
58	<2	64	128	64
59	<2	16	64	64
60	<2	16	256	128
61	<2	64	128	64
62	<2	32	64	128
63	<2	16	32	16
64	<2	32	64	32
65	<2	16	64	64
66	<2	128	64	32
67	<2	32	64	32
68	<2	2	16	32
69	<2	16	128	128
70	<2	32	64	128

Appendix 3b (iii). Reciprocal serum HI antibody titres: Challenge control group. Challenge-vaccinate trial.

Bird No.	4/8/032	11/8/03	18/8/03	25/8/03
226	<2	32	64	32
227	<2	64	32	32
228	<2	64	128	64
229	<2	32	64	32
230	2	32	64	64
231	2	32	128	64
232	2	32	128	64
233	2	16	64	64
234	2	32	16	16
235	<2	16	32	16
236	2	16	16	16
237	2	32	64	64
238	2	128	128	64
239	2	8	16	32
240	2	128	128	128
241	2	8	16	16
242	2	16	8	16
243	2	32	32	32
244	2	16	32	32
245	2	32	32	32