



HAL
open science

Herd immunity to Newcastle disease virus in poultry by vaccination

Michiel van Boven, Annemarie Bouma, Teun Fabri, Elly Katsma, Leo Hartog, Guus Koch

► To cite this version:

Michiel van Boven, Annemarie Bouma, Teun Fabri, Elly Katsma, Leo Hartog, et al.. Herd immunity to Newcastle disease virus in poultry by vaccination. *Avian Pathology*, 2008, 37 (01), pp.1-5. 10.1080/03079450701772391 . hal-00540101

HAL Id: hal-00540101

<https://hal.science/hal-00540101>

Submitted on 26 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Herd immunity to Newcastle disease virus in poultry by vaccination

Journal:	<i>Avian Pathology</i>
Manuscript ID:	CAVP-2007-0109.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	14-Sep-2007
Complete List of Authors:	van boven, michiel; Wageningen University and Research Centre, Animal Sciences Group Bouma, Annemarie; Utrecht University, Faculty of Veterinary Medicine Fabri, Teun; Animal Health Service Katsma, Elly; Wageningen University and Research Centre, Animal Sciences Group Hartog, Leo; Central Institute for Animal Disease Control, Department of Virology Koch, Guus; Central Institute for Animal Disease Control, Virology; CIDC-Lelystad, Virology
Keywords:	reproduction number, herd immunity, vaccination, transmission

SCHOLARONE™
Manuscripts

[Cavp-2007-0109.R1](#)

Herd immunity to Newcastle disease virus in poultry by vaccination

Michiel van Boven^{1#}, Annemarie Bouma², Teun H.F. Fabri³, Elly Katsma¹, Leo Hartog⁴, Guus Koch^{4*}

¹*Animal Sciences Group, Wageningen University and Research Centre, P.O. Box 65, 8200 AB Lelystad, the Netherlands*; ²*Faculty of Veterinary Medicine, Department of Farm Animal Health, Utrecht University, P.O. Box 80151, 3508 TD Utrecht, the Netherlands*; ³*Animal Health Service, Arnsbergstraat 7, P.O. Box 9, 7400 AA Deventer, the Netherlands*; ⁴*Department of Virology, Central Institute for Animal Disease Control, Wageningen University and Research Centre, P.O. Box 2004, 8203 AA Lelystad, the Netherlands*; [#]*present address: Centre for Infectious Disease Control, National Institute for Public Health and the Environment, P.O. Box 1, 3720BA Bilthoven, the Netherlands*

Short Title : Vaccination against Newcastle disease

* corresponding author
Visiting address: Houtribweg 39, 8221 RA Lelystad, the Netherlands
Postal address: P.O. Box 2004, 8203 AA Lelystad, the Netherlands
Tel: +31 320 238609
Fax: +31 320 238668
email: guus.koch@wur.nl

Received: 18 July 2007

- Formatted:** Font: (Default) Times New Roman, Complex Script Font: Times New Roman
- Formatted:** Right
- Formatted:** Font: (Default) Times New Roman, 12 pt, Complex Script Font: Times New Roman, 12 pt
- Formatted:** Font: (Default) Times New Roman, 14 pt, Complex Script Font: Times New Roman, 14 pt
- Deleted:** `
- Deleted:** `
- Formatted:** Font: (Default) Times New Roman, 14 pt, Complex Script Font: Times New Roman, 14 pt
- Formatted:** Font: (Default) Times New Roman, Complex Script Font: Times New Roman
- Formatted:** Font: (Default) Times New Roman, 12 pt, Bold, Complex Script Font: Times New Roman, 12 pt
- Formatted:** Font: (Default) Times New Roman, Complex Script Font: Times New Roman
- Formatted:** Font: (Default) Times New Roman, Italic, Complex Script Font: Times New Roman
- Formatted:** Line spacing: Double
- Deleted:** ¶
- Formatted:** Font: (Default) Times New Roman, Italic, Complex Script Font: Times New Roman
- Deleted:** ¶
- Formatted:** Font: (Default) Times New Roman, Italic, Complex Script Font: Times New Roman
- Deleted:** ¶
- Formatted:** Font: (Default) Times New Roman, Italic, Complex Script Font: Times New Roman
- Deleted:** ¶
- Formatted:** Font: (Default) Times New Roman, Italic, Complex Script Font: Times New Roman
- Formatted:** Font: (Default) Times New Roman, Complex Script Font: Times New Roman
- Deleted:** running head
- Formatted:** Font: (Default) Times New Roman, Complex Script Font: Times New Roman
- Formatted:** Font: (Default) Times New Roman, Italic, Complex Script Font: Times New Roman
- Formatted** ... [1]
- Formatted:** Line spacing: Double
- Deleted:** ... [2]

Cavp-2007-0109.R1

Formatted: Font: 10 pt, Complex Script Font: 10 pt

Herd immunity to Newcastle disease virus in poultry by vaccinationMichiel van Boven^{1#}, Annemarie Bouma², Teun H.F. Fabri³, Elly Katsma¹, Leo Hartog⁴, Guus Koch^{4*}

Formatted: Line spacing: Double

Abstract

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: virus

Newcastle disease (ND) is an economically important disease of poultry for which vaccination is applied as a preventive measure in many countries. Nevertheless, outbreaks have been reported in vaccinated populations. This suggests that either the vaccination coverage level is too low or that vaccination does not provide perfect immunity, allowing the virus to spread in partially vaccinated populations. Here we study the requirements of an epidemiologically effective vaccination program against Newcastle disease in poultry, based on data from experimental transmission studies. The transmission studies indicate that vaccinated birds with low or undetectable antibody titres may be protected against disease and mortality but that infection and transmission may still occur. In fact, our quantitative analyses show that ND virus is highly transmissible in poultry with low antibody titres. As a consequence, herd immunity can only be achieved if a high proportion of birds (>85%) have a high antibody titre (\log_2 haemagglutination inhibition titre ≥ 3) after vaccination. We discuss the implications for the control of ND in poultry by vaccination.

Deleted: v

Deleted: fraction

Deleted: has

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: Newcastle disease virus

Deleted:

16

10/10/07

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Introduction

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Newcastle disease (ND) is a highly contagious viral disease affecting wild and domestic avian species (Seal *et al.*, 2000; Alexander, 2003). The impact of ND is most notable in domestic poultry due to the high susceptibility of poultry, and the severe consequences of outbreaks of virulent strains on the poultry industries. In fact, it has been argued that ND may represent a bigger drain on the world economy than any other animal viral disease (Alexander, 2003), although the current epizootics of H5N1 avian influenza in Southeast Asia are challenging (if not surpassing) this status.

Deleted: Newcastle disease

Deleted: ,

Deleted: Newcastle disease

In response to the threat presented by ND, several countries have put in place vaccination campaigns to prevent epizootics. However, outbreaks have been reported in vaccinated populations despite the fact that vaccination is widely applied (BurrIDGE *et al.*, 1975), as for example in the Netherlands in 1992-1993, the UK in 1997, and USA in 2002 (Alexander, 2003, and references therein).

Deleted: Newcastle disease

Formatted: Indent: First line: 36 pt, Line spacing: Double

Deleted: epidemics

Deleted: et

Deleted: ..

Deleted: (

Deleted:)

Deleted: (

Deleted:)

Deleted: (

Deleted:)

It is known that vaccination of poultry provides an excellent means to lessen clinical signs of infection caused by virulent Newcastle disease virus (NDV) (Alexander, 2003; Senne *et al.*, 2004; Kapczynski and King, 2005). It has also been known for a long time that vaccination itself (with live vaccines based on non-virulent virus strains) may cause disease and reduced growth in vaccinated birds (Alexander, 2003). As a consequence, there has been a trend to use ever less virulent strains as the seed viruses for vaccine production. Although this strategy has reduced the disease rates after vaccination, it also may have contributed to the fact that current vaccines and vaccination campaigns are not maximally effective in preventing infection and transmission (BurrIDGE *et al.*, 1975; Voeten *et al.*, 1987; Alexander, 2003; Senne *et al.*, 2004; Kapczynski and King, 2005). Hence, it is not clear whether the ultimate goal of prevention of major outbreaks after primary virus introductions can be achieved with current vaccines and vaccination programs.

Deleted:

Deleted: Newcastle disease

Vaccination of large numbers of broiler chickens against ND is usually carried out using non-virulent live virus that is administered by spray or atomist, or via drinking water. These administration techniques usually produce considerable variation in the individual antibody immune responses of vaccinated birds, indicating potential variation in the levels of protection after vaccination (Senne *et al.*, 2004). Therefore, a central question in the control of Newcastle disease is whether virulent ND viruses are

Deleted: Newcastle disease

Deleted:

16

10/10/07

able to spread in heterogeneously vaccinated populations, and, more specifically, under which conditions (vaccination coverage level, distribution of antibody titres) epidemic spread can be prevented.

Deleted: Newcastle disease virus

To determine whether poultry flocks are at risk of major outbreaks of ND, we use the concept of 'herd immunity' (Anderson and May, 1991; Diekmann and Heesterbeek, 2000). Epidemiological theory informs us that whether a population is protected against epidemic spread of an infectious agent or not is determined by the reproduction numbers of the infectious agent in populations with high and low levels of protection after vaccination, and by the fraction of animals that have a high level of protection after vaccination. 'Herd immunity' is achieved if the fraction of animals with a high level of protection is equal to or greater than a certain critical fraction of animals with a high level of protection. The critical vaccination fraction is in turn determined by the reproduction numbers of the infectious agent in populations with high and low levels of protection (see Materials and Methods for details). The central aim of the present study is therefore to provide estimates of the reproduction numbers of NDV in bird populations with high and low levels of protection after vaccination.

Deleted: Newcastle disease virus

To this end we have carried out an extensive set of experiments with various virus-vaccine combinations. All experiments were performed with broilers that received two vaccine doses, thereby mimicking vaccination schemes in the field. The experiments were carried out with birds that had a low antibody titre (as determined by the haemagglutination inhibition test) after vaccination (\log_2 titre: 0-2), and with birds that had a high antibody titre after vaccination (\log_2 titre: ≥ 3). Throughout we have used two commonly-used vaccines that are based on the mildly virulent (lentogenic) La Sota and Ulster strains (Alexander, 2003). As challenge virus we used three well-known highly virulent (velogenic) strains, i.e. Herts33/56, Netherlands/93, and California/71. On the basis of the data of these experiments we estimated the reproduction numbers of NDV in broilers living in close-contact groups with low and high antibody titres. We discuss the implications of our findings for the design of vaccines and vaccination campaigns aimed at protection of poultry flocks against major outbreaks of ND.

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: commonly

Deleted: La

Deleted: Newcastle disease virus

Deleted: Newcastle disease

Formatted: Line spacing: Double

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: ¶
Epidemiological considerations

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted:

16

10/10/07

Materials and methods

Epidemiological considerations. Whether or not a major outbreak can occur after a primary introduction of an infectious agent into a population is determined by the (basic) reproduction number, which gives the expected number of secondary infections caused by one infected individual in a large population in the early stages of an outbreak. If the reproduction number (denoted by R) exceeds the threshold value of 1 a chain reaction of infection events is possible, and an epidemic can occur. If, on the other hand, the reproduction number remains below the threshold value of 1 a major outbreak cannot occur. In a standard compartmental SEIR model (where the compartments represent Susceptible, Exposed but not yet infectious, Infected and infectious, and Removed individuals) with one type of infected individual and permanent immunity after infection or vaccination the reproduction number determines the critical vaccination coverage needed to achieve herd immunity. In fact, epidemiological theory states that herd immunity is obtained if the actual vaccination coverage exceeds the critical vaccination coverage p_c , which is given by $p_c = 1 - 1/R$ (Anderson and May, 1991).

Newcastle disease vaccines, however, do not provide unconditional immunity against infection and transmission, and the individual responses to vaccination can be highly variable. As a consequence, we have to adapt the above considerations to allow for variable immunity after vaccination. To keep the analyses manageable we consider a model with two immune classes, one with a low level of protection against infection and disease, and one with a high level of protection against infection and disease. With two types of individuals the overall reproduction number depends on the reproduction numbers of the virus in homogeneous populations with high and low levels of protection (R_{high} and R_{low}) as follows:

$$R = pR_{high} + (1-p)R_{low},$$

where p is the fraction of individuals that has a high level of protection (Diekmann and Heesterbeek, 2000).

The critical fraction of individuals that needs to have a high level of protection in order for the population to achieve herd immunity, p_c , is given by setting $p = p_c$ and solving the above equation for $R = 1$. A straightforward calculation shows that

$$p_c = \frac{R_{low} - 1}{R_{low} - R_{high}}.$$

(1)

Deleted:

16
10/10/07

The above theoretical considerations can be refined and extended in a number of directions, but for the present purposes Equation (1) suffices as a rule of thumb specifying the requirements of an epidemiologically effective vaccination program.

Equation (1) implies that herd immunity cannot be achieved whenever the infectious agent is able to spread epidemically in populations with a high level of protection (i.e. if $R_{high} > 1$), and that no animals need to have a high level of protection whenever the pathogen cannot spread epidemically in populations with a low level of protection (i.e. if $R_{low} < 1$). In the special case that a high level of protection guarantees that no transmission can occur ($R_{high} = 0$) Equation (1) reduces to the familiar $p_c = 1 - 1/R_{low}$.

Experimental approach. To determine the transmission dynamics of NDV in vaccinated and unvaccinated poultry living in close contact groups a number of experimental transmission studies with broiler chickens were carried out. All experiments were performed in a high containment unit under BSL3+ conditions at the Central Institute for Animal Disease Control Lelystad. The experiments complied with the Dutch law on animal experiments and were reviewed by an ethical committee. The design of transmission experiments has been described before (de Jong and Kimman, 1994; Bouma *et al.*, 1996; van der Goot *et al.*, 2005). Briefly, a number of birds that were inoculated with the virus at the start of the experiment were housed with a number of uninfected contact birds. During the course of the experiments the status of the birds (normal, sick, and dead) were recorded, and the serological status of each bird was determined at the start and at the end of the experiments.

Birds were vaccinated at day-old by spraying with commercial vaccines either La Sota (Nobilis® ND clone 30) in experiments 1-3 and 5-7, or Ulster (Poulvac NDW) in experiments 4 and 8, and received a booster vaccination with the same vaccine using an atomist between 8 and 20 days of age. Birds were transported to animal BSL-3+ facilities of the CIDC-Lelystad laboratory between 22 and 26 days of age, well before the start of the actual challenge experiments. In each experiment ten birds were inoculated with virus and housed together at day 0. Subsequently, ten uninfected contact birds were added to the inoculated birds at day 1.

Three different viruses were used for challenge infection (Table 1). For experiments 1, 4, 5, and 8 a velogenic NDV Netherlands/93 (APMV-1/Netherlands/152608/93) was used. This virus was isolated in the Netherlands during the 1992-1993 epizootic from a vaccinated layer flock, and was characterised as a

Deleted: Experimental approach ¶

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: et

Deleted: et

Deleted: placed in a stable

Deleted: day

Formatted: Indent: First line: 36 pt, Line spacing: Double

Deleted: Per

Deleted: put

Deleted: in a stable

Deleted: Table

Deleted: Newcastle disease virus

Deleted: epidemic

Deleted:

16

10/10/07

velogenic virus based on the amino acid residues (RRQKR.F) at the cleavage site of the fusion protein. The virus has an intracerebral pathogenicity index (ICPI) of 1.84. Phylogenetic analysis has placed the virus in clade 5a (Aldous *et al.*, 2003) or genotype VII (Lomniczi *et al.*, 1998). In experiments 2 and 6 Herts33/56 was used as challenge virus. The Herts33/56 virus used in this study had an ICPI of 1.86, and was obtained from the Veterinary Laboratory Agency (UK) in 1973. Herts33/56 is placed within lineage group 3b (Aldous *et al.*, 2003) or genotype IV (or the separate genotype W) (Lomniczi *et al.*, 1998; Czeglédi *et al.*, 2006). In Experiments 3 and 7 a viscerotropic velogenic California/71 virus (California 2098/71) was used. This virus was isolated from a chicken during the Newcastle disease outbreak in California in 1971 and was received from Dr. Hanson of the University of Wisconsin. This virus has an ICPI of 1.8 and is placed in clade 3c or genotype V. Animals were challenged via trachea (0.1 ml) and nasal cavity (0.1 ml) with a suspension containing 10^6 median egg infectious doses (EID₅₀) for experiments 1 and 5, 10^6 EID₅₀ for experiments 2 and 6, $10^{6.4}$ EID₅₀ for experiments 3 and 7, and $10^{3.8}$ EID₅₀ for experiments 4 and 8.

The haemagglutination inhibition (HI) test was performed according to standard EU protocol (EU council directive 92/66/EEC). For the test the amount of Ulster/66 ND antigen was adjusted to 8 haemagglutinating units. Controls on the antigen content in the HI test were carried out using serial twofold dilutions starting at 1:2, 1:3, 1:4, 1:5, and 1:6. The test was considered valid when the amount of antigen in the control lies between 6 and 9 haemagglutinating units; 1 unit being the dilution at which 100% agglutination is still observed. The titre was determined by testing two-fold dilution series of sera, and the titre was expressed as the highest dilution showing complete inhibition of agglutination. In each HI test run a negative control, and sera with low, mean and high titre were included. Test results are considered valid only when the titre of these reference sera did not deviate more than one dilution step up or down from the mean.

Throughout we classified birds with log₂ antibody titres in the range 0-2 as having a low level of protection, and birds with log₂ antibody titres ≥ 3 as having a high level of protection. This classification scheme was chosen on the basis of earlier evidence which suggested that log₂ antibody titres ≥ 3 were sufficient to protect against disease (not shown), the fact that log₂ antibody titres ≥ 3 are very unlikely to result from non-specific reactions, and the fact that the Dutch legislature asks that at least 90% of vaccinated birds of four weeks and older have a log₂ antibody titre of at least 3.

Birds that died with signs of infection or showed an increase of the HI titre of at least two log steps were considered to be infected and infectious after challenge.

Deleted: et
Deleted: Czeglédi
Deleted: et
Deleted:
Formatted: Line spacing: Double
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Deleted: is
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman
Formatted: Indent: First line: 36 pt, Line spacing: Double
Deleted: a
Deleted: ... [8]

Deleted: ¶
Statistical analyses

Formatted: Line spacing: Double

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: et

Deleted: et

Deleted: Table

Statistical analyses. The analyses of the transmission experiments follow the line of analyses of earlier transmission experiments with pseudorabies virus in pigs and highly pathogenic avian influenza virus in poultry (de Jong and Kimman 1994; Bouma *et al.*, 1996; van der Goot *et al.*, 2005). Throughout, the analyses are based on the final size of the transmission experiments i.e. on the number of initially uninfected animals that have been infected during the course of the experiment (Table 1). Here we assume an exponentially distributed infectious period, which gives high estimates of the reproduction number and broad confidence intervals in comparison with models that assume less variation in the infectious period (Ball, 1986). As a consequence, the method also yields a fairly high estimate of the critical fraction of animals that needs to have a high level of protection to obtain herd immunity.

Formatted: Indent: First line: 36 pt, Line spacing: Double

Deleted: et

Confidence intervals of the estimated parameters are calculated as reported by Velthuis *et al.*, (2007). In short, exact confidence bounds of the parameter estimates are determined by calculating all possible values r of the reproduction number R for which the hypothesis $H_0: R=r$ is not rejected, i.e. for which the p-value is larger than 0.05. In the case where all contact animals are infected (Experiments 3 and 4) no estimate of the reproduction number is calculated ($\hat{R} \rightarrow \infty$), and only the lower bound of the (one-sided) 95% confidence interval is given.

Deleted: Experiments

Formatted: Line spacing: Double

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Results

Deleted: ¶
NDV infection and disease

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: pathogenic

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: experimentally

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: Table

Deleted:

16
10/10/07

NDV infection and disease. All three challenge viruses used in this study are highly virulent as measured by the Intracerebral Pathogenicity Index (ICPI >0.7), and are able to kill experimentally-infected, susceptible chickens within two days. In our transmission experiments with vaccinated birds, however, only a minority of the birds died during the experiments. In fact, in the experiments with birds that had high antibody titres after vaccination (\log_2 titre: ≥ 3 ; Experiments 5-8) 27 of the 80 contact birds showed signs of infection (Table 1), and just 1 of these 27 infected contact birds died. In the experiments with birds that had low antibody titres (\log_2 titre: 0-2; Experiments 1-4) the majority of the contact birds had signs of infection (54 out of 60), but still only a minority of these infected contact birds died (10 out of 54). Overall, these results indicate that vaccination provides excellent protection against mortality after a natural

infection with virulent Newcastle disease virus when vaccinated birds have high antibody titres, and that vaccination is still quite effective in reducing mortality rates in birds with low antibody titres. On the other hand, vaccination does not seem to provide substantial protection against infection, especially in birds having low antibody titres.

NDV transmission. The data in [Table 1](#) indicates that for most virus-vaccine combinations the virus is able to spread extensively in birds with low antibody titres (\log_2 titre: 0-2). In fact, in Experiment 3 (one trial) and Experiment 4 (two trials) all contact birds became infected, and in Experiment 1 (two trials) only 1 of the 20 contact birds escaped infection. The results of Experiment 2 were somewhat different as 5 of 10 contact birds escaped infection.

The results of the formal analyses are presented in [Table 2](#). The quantitative analyses of Experiments 1-4 yield estimates of the reproduction number R_{low} varying from $\hat{R}_{low}=0.95$ [95% CI=(0.27-2.8)] at the low end in Experiment 2 to $\hat{R}_{low}>2.1$ with 95% confidence at the high end in Experiment 4. If all transmission experiments with birds which had a low antibody titre at the start of the experiment are combined we find that $\hat{R}_{low}=3.1$ (1.8-4.3), and we conclude that, in general, **NDV** is able to spread epidemically in groups of vaccinated broilers with low antibody titres. It should be noted that this conclusion rests on the assumption of no systematic differences in the transmission characteristics of the different viruses. Unfortunately, with the current data this assumption cannot be validated or falsified.

In groups of birds with high antibody titres there was more variation in the outcome of the experiments ([Table 1](#)). This is mirrored by the statistical analyses which show considerable variation in the estimates of the reproduction number R_{high} . At the low end $\hat{R}_{high}=0.19$ (0.023-0.78) in [Experiment 6](#) to $\hat{R}_{high}=1.5$ (0.50-3.9) at the high end in [Experiment 8](#). Notice that all experiments indicate that at least some spread is possible in vaccinated birds with high antibody titres, and that [Experiments 5](#) and [8](#) do not exclude the possibility of epidemic spread in populations with high antibody titres (i.e. $R_{high} > 1$). If, however, we assume that differences between different sets of experiments are the result of chance and combine all experiments with birds having high antibody titres the estimate of the reproduction number is $\hat{R}_{high}=0.72$ (0.41-1.0), and we conclude that epidemic spread is unlikely in groups of birds having high antibody titres.

Deleted: ¶
NDV transmission

Deleted: Table

Deleted: suggest

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted:

Deleted:

Deleted:

Deleted: Tables 2 gives t

Formatted: Indent: First line: 36 pt, Line spacing: Double

Deleted: Newcastle disease virus

Formatted: Line spacing: Double

Deleted: Table

Deleted: Experiment

Deleted: Experiment

Deleted: Experiments

Deleted:

16

10/10/07

Deleted: ¶
'Herd immunity' against NDV by vaccination

Deleted: '

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: Newcastle disease virus

Deleted: Equation

Deleted: Table

Herd immunity against NDV by vaccination. Herd immunity' to NDV is obtained whenever the virus is unable to cause a prolonged chain of infections, i.e. if no epidemic can unfold after a primary virus introduction (Diekmann and Heesterbeek, 2000). In our context 'herd immunity' is achieved if the fraction of birds that have a high antibody titre after vaccination exceeds the critical fraction of birds with a high antibody titre specified by Equation (1). For the combined analysis of the experiments (Table 2) and assuming that 100% of the birds are vaccinated, the critical fraction is estimated at $p_c = 0.88$, i.e. at least 88% of the birds need to have a high antibody titre after vaccination to prevent a major epidemic. We would like to stress that there is considerable uncertainty surrounding this estimate. For instance, if we take the lower and upper bounds of the confidence intervals of \hat{R}_{low} and \hat{R}_{high} , and insert these values in Equation (1) we obtain $p_c = 0.58$ and $p_c = 1$ as lower and upper bounds of the critical fraction of birds with high level of protection. In other words, p_c may be as low as 58% or as high as 100%. It should be noted, however, that these bounds are conservative in view of our estimation method that yields broad confidence intervals for the reproduction numbers (see Materials and Methods).

Formatted: Indent: First line: 36 pt, Line spacing: Double

Also shown in Table 2 is substantial variation in the estimates of reproduction numbers of the individual experiments. As a consequence, there is also considerable variation in the estimates of the critical vaccination fraction if we focus on experiments with identical virus-vaccine combinations (Experiments 1+5, Experiments 2+6, Experiments 3+7, Experiments 4+8). For Experiments 2+6 the critical vaccination fraction is estimated to be 0%, indicating that epidemic spread of virus is unlikely even in vaccinated populations of birds with low antibody titres ($\hat{R}_{low} < 1$). For Experiments 3+7 the lower bound of the estimate of the critical vaccination fraction is 46%, and for Experiments 1+5 and Experiments 4+8 herd immunity cannot be achieved even if all birds have a high antibody titre after vaccination since the virus is expected to be able to spread epidemically even in populations with high antibody titres ($\hat{R}_{high} > 1$).

Formatted: Line spacing: Double

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted:

16

10/10/07

Discussion

Deleted: Newcastle disease

In this paper we have presented quantitative analyses of NDV transmission in vaccinated chickens. Our results indicate that although vaccination in general provides good protection against disease and mortality, it may not provide sufficient protection against virus transmission so as to be able to prevent or halt epidemics of ND. This finding is of considerable interest as it brings into question the epidemiological effectiveness of current vaccination campaigns against ND. Our analyses indicate that a high fraction of birds (>85%) needs to have a high antibody titre (\log_2 titre: ≥ 3) after vaccination to ensure that no epidemic spread is possible in vaccinated populations.

Deleted: Newcastle disease virus

Deleted: Newcastle disease virus

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Indent: First line: 36 pt, Line spacing: Double

Deleted: Newcastle disease

Deleted: attenuated

In view of our results a central question is whether it is possible at all to obtain consistently high antibody titres using the current administration techniques (by spraying or via the drinking water) of ND vaccines based on viruses of low virulence. Unfortunately, to date there are no systematic studies that have investigated the distribution of antibody titres after vaccination of large populations of poultry. A pilot experiment in The Netherlands suggests that it may be possible to obtain high antibody titres in the majority of birds, but only if strict preconditions on the vaccine content and administration techniques are met (data not shown). It should also be noted that in the absence of circulation of virulent virus in a region there may be an incentive for farmers to use vaccination schemes and procedures that are not epidemiologically optimal because of the negative side-effects of vaccination.

An additional issue that needs attention is the possibility that the virus might spread unnoticed in partially vaccinated populations where it would be detected quickly in unvaccinated populations, thereby increasing the period in which the virus can be transmitted unchecked. Whether this theoretical possibility might become a real problem is not known at present. Certainly, our results and those of Kapczynski and King (2005) have shown that infection, shedding, and transmission of virulent NDV in vaccinated birds may occur without overt disease signs. Given this possibility we believe that if preventive vaccination programs are to be implemented they should go together with a monitoring program ensuring that sufficient flock immunity levels are achieved. Similar views have recently been expressed for highly pathogenic avian influenza viruses in poultry (Capua and Marangon 2006; Capua and Alexander, 2006; Savill *et al.*, 2006).

Deleted: Newcastle disease virus

Deleted: symptoms

Deleted: ,

Deleted: ,

Our results have shown some variation in the transmission levels using different combinations of vaccine and virus (especially in groups of birds with a high antibody titre). On the one hand, in view of the limited amount of data for each particular virus-vaccine combination it may be that the variation in

Deleted:

16

10/10/07

transmission is a result of chance. On the other hand, it may also be that our data have revealed a trend that hints at variable efficacy of different vaccines against different viruses [see also Kapczynski and King (2005)]. In this respect, it is of note that better protection appears to be obtained against challenge viruses that were isolated in the 1930s and 1970s (i.e. Herts33/56 and California71), and which are genetically more closely related to current vaccine strains than the more recently isolated and genetically more distinct Netherlands/93 virus (Lomniczi *et al.*, 1998). This is in line with recent results (Czegledi *et al.*, 2006), which indicated that current vaccines induced better protection against viruses that were isolated in past epizootics than against viruses that are currently circulating. With this in mind it would be interesting to investigate the antigenic relationship between past and current circulating viruses, and the effect of the antigenic match between virus and vaccine on the level of protection conferred against disease, shedding, and transmission. From a practical perspective, since the effectiveness of ND vaccines is ultimately determined by their ability to curb or halt epizootics of ND we believe that vaccine development should be focussed on providing vaccines that protect against infection and shedding, rather than disease.

Deleted: *et*Deleted: *et*

Deleted: Newcastle disease

Deleted: Newcastle disease

Formatted: Line spacing: Double

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Acknowledgments

We thank Don Klinkenberg, Jo van Eck, and Jeanet van der Goot for discussion, and Dennis Alexander and two anonymous reviewers for providing valuable comments. This work was supported by the Dutch Ministry of Agriculture, Nature, and Food Quality.

Deleted:

16

10/10/07

References

- Aldous EW, JK Mynn, J Banks, DJ Alexander (2003) A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. *Avian Pathology* **32**, 239-56
- Alexander D (2003) Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. Pp 63-99 in *Diseases of Poultry* (eds. YM Saif, HJ Barnes, JR Glisson, AM Fadly, LR McDougald, D Swayne). Iowa State University Press
- Anderson RM, RM May (1991) *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press, Oxford, pp. 766
- Ball F (1986) A unified approach to the distribution of total size and total area under the trajectory of infectives in epidemic models. *Advances in Applied Probability* **18**, 289-310
- Bouma, A, MCM De Jong, TG Kimman (1996) Transmission of two pseudorabies virus strains, that differ in virulence and virus excretion, in groups of vaccinated pigs. *American Journal of Veterinary Research* **57**, 43-47
- Burridge MJ, HP Riemann, WW Utterback, EC Sharman EC (1975) The Newcastle disease epidemic in southern California, 1971--1973: descriptive epidemiology and effects of vaccination on the eradication program. *Proceedings of the Annual Meeting of the United States Animal Health Association* **79**, 324-333
- Capua I, S Marangon (2006) Control of avian influenza in poultry. *Emerging Infectious Diseases* **12**, 9 (<http://www.cdc.gov/ncidod/EID/vol12no09/06-0430.htm>)
- Capua I, DJ Alexander (2006) The challenge of avian influenza to the veterinary community *Avian Pathology* **35**, 189-205
- Czegledi A, D Ujvari, E Somogyi, E Wehmann, O Werner, *et al.* (2006) Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. *Virus Research* **120**, 36-48
- de Jong MCM, TG Kimman (1994) Experimental quantification of vaccine-induced reduction in virus transmission. *Vaccine* **12**, 761-766

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Line spacing: Double

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Indent: Before: 0 pt, Hanging: 28.35 pt, Line spacing: Double

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted:

16

... [9]

Diekmann O, JAP Heesterbeek (2000) *Mathematical Epidemiology of Infectious Diseases*. Wiley, Chichester, pp. 303

Kapczynski DR, King DJ (2005) Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. *Vaccine* **23**, 3424-3433

Lomniczi B, E Wehmann, J Herczeg, A Ballagi-Pordany, EF Kaleta EF, *et al.* (1998) Newcastle disease outbreaks in recent years in western Europe were caused by an old (VI) and a novel genotype (VII). *Archives of Virology* **143**, 49-64

Savill NJ, SG St Rose, MJ Keeling, ME Woolhouse (2006) Silent spread of H5N1 in vaccinated poultry. *Nature* **442**, 757

Seal BS, DJ King, HS Sellers (2000) The avian response to Newcastle disease virus. *Developmental and Comparative Immunology* **24**, 257-268

Senne DA, DJ King, DR Kapczynski (2004) Control of Newcastle disease by vaccination. *Developments in Biologicals* (Basel) **119**, 165-70

van der Goot J, G Koch, MCM de Jong, M van Boven (2005) Quantification of the transmission characteristics of highly pathogenic avian influenza A virus (H7N7) in vaccinated and unvaccinated chickens. *Proceedings of the National Academy of Sciences USA* **102**, 18141-18146

Velthuis, AGJ, A Bouma, WEA Katsma, G Nodelijk, MCM de Jong (2007) Design and analysis of small-scale transmission experiments with animals. *Epidemiology and Infection* **135**, 202-217

Voeten AC, van Eck JH, Davelaar FG, Kouwenhoven B (1987) Comparison of the effect of live Newcastle disease vaccine Clone 30 in broilers administered at day 1 or at day 7 and the effect of H120 vaccination at 17 days of age: a field experiment. *Veterinary Quarterly* **9**, 38-48

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Not Bold, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Line spacing: Double

Deleted:

16

10/10/07

Table 1. *Overview of the experimental transmission studies.*

experiment	vaccine	virus	HI titre (log ₂)	number of contact birds	
				infected	dead
1	La Sota	NL93	0-2	9,10	0,1
2	La Sota	Herts33/56	0-2	5	0
3	La Sota	California71	0-2	10	1
4	Ulster	NL93	0-2	10,10	4,4
5	La Sota	NL93	≥3	0,4,10	0,0,0
6	La Sota	Herts33/56	≥3	0,2	0,0
7	La Sota	California71	≥3	1,3	0,1
8	Ulster	NL93	≥3	7	0

Experiments 2, 3, and 8 consist of one trial, Experiments 1, 4, 6 and 7 contain two trials, and Experiment 5 contains three trials. Each trial initially contained ten infected birds and ten uninfected contact birds. The vaccines are based on the La Sota (Nobilis Clone30) and Ulster (NDW Poulvac) viruses. The challenge viruses are NL93 (APMV-1/chicken/Netherlands/152608/93), Herts33/56, and California71. The column under the heading 'HI titre' gives the range of HI titres of the birds just before the start of the experiment. The last two columns give the number of contact birds that were infected and died or were infected per trial.

Formatted: Font: (Default) Times New Roman, Bold, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Bold, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: La

Deleted: La

Deleted: La

Deleted: La

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: La

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: La

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted:

16

10/10/07

Table 2. Overview of the statistical analyses.

experiments	\hat{R}_{low}	\hat{R}_{high}
1+5	3.4 (1.5-8.4)	1.1 (0.46-1.7)
2+6	0.95 (0.27-2.8)	0.19 (0.023-0.79)
3+7	>1.8*	0.38 (0.044-0.98)
4+8	>2.1*	1.5 (0.50-3.9)
all	3.1 (1.8-4.3)	0.72 (0.41-1.0)

Shown are the combined results of experiments 1 and 5 (vaccine: La Sota, virus: NL93), 2 and 6 (vaccine: La Sota, virus: Herts33/56), 3 and 7 (vaccine: La Sota, virus: California71), 4 and 8 (vaccine: Ulster, virus: NL93), and all experiments taken together. \hat{R}_{low} and \hat{R}_{high} represent the maximum likelihood estimates of R_{low} and R_{high} , which are calculated using the infection data of Table 1. The 95% confidence intervals are given between brackets. *: only the lower bound of the (one-sided) 95% confidence interval is given.

Formatted: Font: (Default) Times New Roman, Bold, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Italic, Complex Script Font: Times New Roman

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: .

Deleted: .

Deleted: .

Deleted: .

Formatted: Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Deleted: La

Deleted: La

Deleted: La

Deleted: Table

Formatted: Font: Times New Roman

Deleted:
16
10/10/07

Page 1: [1] Formatted	Verity	10/28/2007 3:06:00 PM
------------------------------	---------------	------------------------------

Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Page 1: [2] Deleted	Verity	10/28/2007 3:40:00 PM
----------------------------	---------------	------------------------------

16

10/10/07

Page 7: [3] Formatted	Verity	10/28/2007 3:06:00 PM
------------------------------	---------------	------------------------------

Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Page 7: [4] Formatted	Verity	10/28/2007 3:06:00 PM
------------------------------	---------------	------------------------------

Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Page 7: [5] Formatted	Verity	10/28/2007 3:06:00 PM
------------------------------	---------------	------------------------------

Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Page 7: [6] Formatted	Verity	10/28/2007 3:06:00 PM
------------------------------	---------------	------------------------------

Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Page 7: [7] Formatted	Verity	10/28/2007 3:06:00 PM
------------------------------	---------------	------------------------------

Font: (Default) Times New Roman, Complex Script Font: Times New Roman

Page 1: [8] Deleted	Verity	10/28/2007 3:40:00 PM
----------------------------	---------------	------------------------------

16

10/10/07

Page 1: [9] Deleted	Verity	10/28/2007 3:40:00 PM
----------------------------	---------------	------------------------------

16

10/10/07