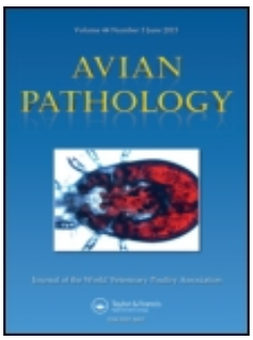

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Transmission of infectious bronchitis virus within vaccinated and unvaccinated groups of chickens

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The aim of this study was to determine whether vaccination against infectious bronchitis virus (IBV) reduces virus transmission, i.e. to test whether IBV transmission among vaccinated chickens is significantly reduced compared to that among unvaccinated chickens. In two vaccinated and two unvaccinated groups of SPF chickens, a standard measure for virus transmission, the reproduction ratio (R) was determined. R is defined as the average number of new infections caused by one typical infectious individual during its entire infectious period.

A single vaccination by eye-drop with IBV H120 reduced the transmission of the IBV challenge virus among the vaccinated chickens (estimated $R = 0.69$, s.e. $= 0.33$) significantly ($P < 0.05$) compared to the transmission among the unvaccinated chickens (estimated $R = 19.95$, s.e. $= 12.41$).

The possible implications for further study, including selection or development of vaccines are discussed.

Introduction

Infectious bronchitis virus (IBV) is a major cause of economic losses in the poultry industry. Vaccination in order to reduce the detrimental effect, cannot prevent sub-clinical infections occurring. Much research has been done on quantifying the individual immune responses, for example, the reduction in virus replication of challenge virus following vaccination (Hitchner *et al.*, 1964; Hofstad, 1967; Winterfield & Fadly, 1971, 1972, 1975; Burke & Luginbuhl, 1972; Winterfield *et al.*, 1972; Winterfield, 1983; Darbyshire, 1985) and the presence or absence of ciliary activity (Darbyshire, 1980; Andrade *et al.*, 1982; Marquardt *et al.*, 1982; Snyder *et al.*, 1983; Darbyshire & Peters, 1984, 1985). Generally, these studies focus on characteristics of the infection that are important for the individual chicken. In the poultry industry, however, one is particularly interested in the immunity of the flock as a whole (herd immunity). Whether IBV may spread in a population depends on the infectivity and susceptibility of the individual chickens in the population and on the contact rate between the chickens (de Jong *et al.*, 1995). By studying the influence of different factors on the transmission of IBV, understanding of how transmission occurs and what it is influenced by, will increase. Eventually, this might lead to measurements that contribute to a better prevention of the damage caused by IBV infections.

A small-scale animal experiment was developed by De Jong & Kimman (1994), with a statistical method for estimating the vaccine-induced herd immunity of Aujeszky's disease virus (pseudorabies virus) in pigs. In these experiments a standard measure for virus transmission, the reproduction ratio (R) is determined. R is defined as the average number of new infections caused by one typical infectious individual during its entire infectious period. Generally, for an unvaccinated population the reproduction ratio is called the basic reproduction ratio or R_0 (de Jong & Diekmann, 1991). If R is less than one, the infection will fade out, resulting in a low percentage of infected animals (minor outbreak). If R is greater than 1, minor and major outbreaks can occur. The probability and the size of a major outbreak increase as R increases. Thus, vaccination preferably should not only reduce transmission significantly, but it should also reduce the R to less than 1.

Our aim was to test whether vaccination against

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IBV induced herd immunity, i.e. to test whether transmission of IBV challenge virus among vaccinated chickens was significantly reduced compared to that among unvaccinated chickens.

Materials and Methods

Animal experiments

Chickens. Four groups (V1, V2, C1 and C2) of 14 one-day-old SPF White Leghorn chickens obtained from the former Poultry Health Centre, Doorn were used.

Vaccination. Vaccination was performed at day 1 by eye-drop application. One dose of vaccine (IB vaccine Nobilis H120, Intervet Nederland BV) was used for each bird in groups V1 and V2. The actual vaccination dose per bird, as determined by egg-titration of inocula immediately following vaccination was $10^{4.8}$ median embryo infectious doses (EID_{50}). Groups C1 and C2 served as unvaccinated controls.

Experimental design. Birds in each of the four groups were marked individually, and housed under similar conditions in identical negative pressure isolators, V1, V2, C1 and C2, with a wire floor of 1.2 m^2. Half of the floor was covered with sterilized cardboard. The ventilation was the same in all four isolators during the experiment, being 9 m^3/h. Pelleted feed from a commercial source and tap-water were provided *ad libitum*. On day 21, seven birds from each group were moved. without being in contact with the open air, into four other negative pressure isolators. In these four isolators the challenge took place. Twenty-four hours later (day 22) all animals were reunited with their previous companions, thereby contact-exposing these companions to IBV excreted by the challenged birds.

Challenge. The challenge IBV strain, M41, was obtained from the former Poultry Health Centre, Doorn, the Netherlands. Challenge was performed by the oral and intratracheal routes. Each bird received 0.9 ml in the pharynx and 0.1 ml in the trachea using a syringe and a soft rubber tube. The actual challenge dose per bird, as determined by egg-titration of inocula immediately following challenge, was $10^{3.5}$ EID_{50} for groups C1 and V1, and $10^{5.0}$ EID_{50} for groups C2 and V2.

Sampling. Tracheal and cloacal swabs were taken on 18, 21, 22, 24, 26, 28, 31, 35 and 38 days post-challenge (d.p.c.). Directly after sampling, swabs were stored individually in 2 ml Hank's medium (containing 40 000 IU benzylpenicillin, 4 mg streptomycin and 4 μg fungizone per ml) at -20°C until titration. Blood samples were collected on days 21 and 38 and stored at -20°C.

Virus titration. The swab samples were titrated for IBV content by inoculating 0.2 ml of a series of 10-fold dilutions [undiluted, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} in Hank's medium (see above)] of swab fluid into the allantoic cavity of five 10-day-old embryonated SPF eggs for each dilution. Thereafter, the eggs were checked daily for embryo mortality. Mortality within 24 h was considered to be non-specific. When mortality had not occurred by the seventh day, the eggs were cooled and the embryo was checked for the presence of IBV-specific abnormalities. Virus titres were calculated according to Reed & Muench (1938). The total amount of virus excreted by a chicken (expressed in EID_{50}) was calculated by addition of the titre of the IBV isolated from the tracheal and cloacal swabs.

Haemagglutination Inhibition (HI) test. The HI test using IBV strain M41 as antigen was performed as described by Alexander & Chettle (1977). Serum dilutions ranged from 1:8 to 1:2048. All HI titres were expressed as $\log_2$ of the reciprocal of the highest serum dilution showing complete haemagglutination inhibition.

Total antibody ELISA. Serum samples were assayed in single (1:500) dilutions using a commercial total antibody ELISA (IDEXX Corporation, Westbrook, Maine, USA) according to the manufacturer's instructions.

Serum-to-positive ratios (S/P-ratios) were calculated, using the formula:

$$\text{SP ratio} = \frac{\text{OD sample} - \text{OD negative control}}{\text{OD positive control} - \text{OD negative control}}$$

where OD stands for optical density. From these S/P-ratios, individual serum titres, expressed as $\log_2$ values, were calculated using a regression formula (IDEXX software).

Data analysis

Estimation of R. The results were statistically analysed according to the stochastic susceptible-infectious-recovered (SIR) model described by Becker (1989). Martingale estimator was used to estimate the reproduction ratio R:

$$R = N/[Pt(1-z)] \sum_{i=S_T+1}^{S_0} 1/i$$

The variables of the Martingale formula are the number of susceptible chickens at the start (S_0) and the end (S_T) of the infection chain, and the total number of contact-infected chickens (Pt). A chicken is considered to be contact infected when IBV is isolated or when a seroconversion (at least a four-fold rise in antibody level) occurs. When all susceptible chickens become infected ($S_T = 0$), the variable z is included, because a part of the infectivity is 'wasted', when there are no more susceptible chickens left to infect (Becker, 1989). This z is the average fraction of the infectivity that is released from all chickens after the last susceptible chicken has been infected. Not using z would then result in an underestimation of R. Here, the value z was estimated as follows: The amount of virus shed after the last contact infection, which was assumed to have taken place 24 h before the last chicken started excreting virus, was divided by the total amount of virus shed during the experiment. For calculation of virus amounts, area measurements were used (Figures 1 and 2).

For the estimation of R, it is assumed that both the inoculated and the contact-infected chickens were equally infectious.

Hypothesis testing. To evaluate the vaccine effect it is not only relevant to estimate R with and without vaccination, but it is also important to test statistically whether the effect of vaccination is significant. Thus, the comparison between the following two hypotheses is relevant:

H_0: $R_{\text{vaccinated group}} = R_{\text{control group}}$,
H_a: $R_{\text{vaccinated group}} < R_{\text{control group}}$.

To carry out the test, the distribution of the appropriate test statistic under the null hypothesis (H_0) is needed. For the stochastic SIR model an algorithm to calculate the probability of each observable outcome expressed as number of contact infections was given by De Jong & Kimman (1994). These probabilities depend on the number of susceptible animals at the start (S_0), the number of infectious animals at the start (I_0), the total number of animals at the start (N_0) and on the value of the unknown R. Hence, the hypotheses can be compared with, as test statistic, the difference in number of contact infections in the control group minus the number of contact infections in the vaccinated group. The probability distribution of this test statistic can now be calculated for each possible value of R when starting conditions are given. Here, in contrast to the method used in De Jong & Kimman (1994), we calculated P-values for all different values of R for the observed difference or a larger difference. The P-value for the test is then the maximum value of P for all possible values of R (B. Kroese & M. C. M. De Jong, unpublished).

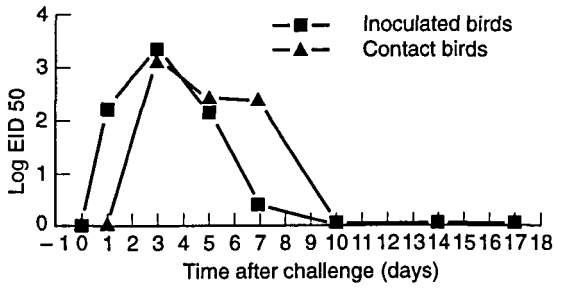

Figure 1. *Average titre (log_{10} EID_{50} per 0.2 ml swab medium) of virus excreted by IBV-infected chickens in unvaccinated group C1.*

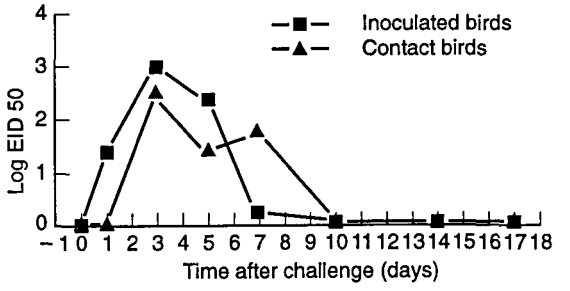

Figure 2. *Average titre (log_{10} EID_{50} per 0.2 ml swab medium) of virus excreted by IBV-infected chickens in unvaccinated group C2.*

Results

Experiments

Unvaccinated control groups C1 and C2. No virus was isolated from any samples from all birds on the day of challenge or from any future contact-exposed bird on the day of reunion (Tables 1 and 2). IBV was isolated from all challenged birds in both groups from 1 until 5 or 7 d.p.c. IBV was also isolated from all contact-exposed birds of both groups from 3 until 7 d.p.c.

All animals in both groups showed at least a four-fold rise (seroconversion) in antibody level in Hl test and ELISA between 0 and 17 d.p.c. (not shown).

Vaccinated groups V1 and V2. No virus was isolated from samples of any birds on the day of challenge or from any future contact-exposed birds on the day of reunion (Tables 3 and 4).

IBV was isolated from five out of seven challenged birds of group V1, four of these showed seroconversion by ELISA, one of them also by HI (Table 3). In two inoculated birds, no virus or immune response was detected, probably because they were totally immune as a result of the vaccination. Therefore, these two chickens were considered not to be infected by the challenge. So, group V1 consisted of five infectious (instead of seven) and nine (instead of seven) contact-exposed chickens (Table 5). Three of these nine contact-exposed birds became infected. The contact infections were detected by VI and serology (all three by ELISA, one by HI test). IBV was only isolated from the cloaca.

In group V2, IBV was isolated from all seven challenged birds (Table 4). Six of them showed a seroconversion by ELISA and three also by HI test. Three of the seven contact-exposed birds in group V2 became infected. The infection was detected by V1 at 3 d.p.c. (two birds) or 5 d.p.c. (one bird). IBV was only isolated from the cloaca. Two of the three contact-infected birds showed a seroconversion by ELISA, none by HI test.

Data analysis

Estimation of R. Three further conditions had to be met before the data could be used for estimating transmission: (a) the chains of infections should have stopped before counting the number of infections, (b) virus excretion of inoculated and contact-infected chickens must be similar, and (c) chickens that escaped infections should have been as susceptible as those that were infected.

In the control groups C1 and C2, the infection had stopped because all contact chickens became infected. At the moment that all contact birds were infected, birds still excreted virus. As a consequence, a part of the infectivity could not be used anymore for infecting chickens, resulting in an underestimated R. Therefore, z had to be introduced. For estimating z, the moment of infection of the last susceptible chicken (between reunion at 1 d.p.c. and sampling at 3 d.p.c.) has to be known. All contact-exposed chickens of both groups already showed high virus titres in the trachea at 3 d.p.c. (Tables 1 and 2). Therefore, we assumed that the infection of the last contact-exposed chicken happened at 2 d.p.c. Therefore, the fraction of excreted IBV that was excreted after the last infection was estimated from 2 d.p.c., being 86 and 88% for groups C1 and C2, respectively (Figures 1 and 2). There was no significant difference in amounts of IBV excreted by the inoculates and the contact-exposed birds.

In the vaccinated groups V1 and V2, all inoculated and contact-infected chickens had stopped excreting detectable amounts of IBV at least 7 days before the end of the experiment (Tables 3 and 4). Therefore, we concluded that the virus transmission had stopped before all susceptible birds were infected. Except for several chickens in group V2 at 1 d.p.c., there was no significant difference in excreted amounts of IBV by the infectious inoculates and the infectious contact-exposed birds.

Table 1. *Results of virus isolation after challenge with $10^{3.5}$ EID_{50} of M41 of unvaccinated chickens in group C1*

Bird number	Amount of virus from trachea/cloaca isolated on days post challenge[a]							
	0	1	3	5	7	10	14	17
Challenged								
1	–/–[b]	2.5/–	2.4/–	2.0/–	–/–	–/–	–/–	–/–
2	–/–	1.8/–	–/–	1.5/1.0	–/0.6	–/–	–/–	–/–
3	–/–	2.3/–	>3.5/–	2.2/–	–/–	–/–	–/–	–/–
4	–/–	1.6/–	2.4/–	1.8/–	–/–	–/–	–/–	–/–
5	–/–	2.5/–	>3.5/0.2	2.1/–	0.4/–	–/–	–/–	–/–
6	–/–	1.8/–	>3.5/–	2.0/–	0.8/–	–/–	–/–	–/–
7	–/–	1.5/0.2	>3.5/–	1.5/0.5	0.5/–	–/–	–/–	–/–
Contacts								
8	–/–	–/–	>3.5/–	2.8/–	0.5/–	–/–	–/–	–/–
9	–/–	–/–	>3.5/–	1.8/–	2.1/–	–/–	–/–	–/–
10	–/–	–/–	3.2/–	2.0/–	2.0/–	–/–	–/–	–/–
11	–/–	–/–	2.3/–	1.5/–	1.8/–	–/–	–/–	–/–
12	–/–	–/–	2.0/0.2	2.4/–	1.5/–	–/–	–/–	–/–
13	–/–	–/–	2.2/–	1.5/–	1.5/1.5	–/–	–/–	–/–
14	–/–	–/–	2.5/–	2.8/–	1.8/–	–/–	–/–	–/–

[a] Log_{10} EID_{50} per 0.2 ml swab medium.
[b] No virus isolated.

Table 2. *Results of virus isolation after challenge with $10^{5.0}$ EID_{50} of M41 of unvaccinated chickens in group C2*

Bird number	Amount of virus from trachea/cloaca isolated on days post challenge[a]							
	0	1	3	5	7	10	14	17
Challenged								
1	–/–[b]	1.6/–	>3.0/–	1.7/–	–/–	–/–	–/–	–/–
2	–/–	0.6/–	2.0/–	1.5/–	–/–	–/–	–/–	–/–
3	–/–	0.8/–	>3.0/–	1.3/1.0	–/0.5	–/–	–/–	–/–
4	–/–	1.2/–	2.0/–	1.5/1.0	–/–	–/–	–/–	–/–
5	–/–	1.4/–	>3.0/0.2	1.7/–	–/–	–/–	–/–	–/–
6	–/–	1.2/–	2.3/–	2.4/–	–/–	–/–	–/–	–/–
7	–/–	0.8/–	2.6/–	2.2/–	–/–	–/–	–/–	–/–
Contacts								
8	–/–	–/–	1.5/–	0.8/–	0.8/–	–/–	–/–	–/–
9	–/–	–/–	2.1/–	0.4/–	0.5/–	–/–	–/–	–/–
10	–/–	–/–	2.8/–	1.2/–	2.3/–	–/–	–/–	–/–
11	–/–	–/–	2.8/–	1.3/–	0.8/0.5	–/–	–/–	–/–
12	–/–	–/–	1.7/–	1.0/–	1.4/–	–/–	–/–	–/–
13	–/–	–/–	0.4/–	1.3/–	1.4/–	–/–	–/–	–/–
14	–/–	–/–	0.5/–	1.8/–	0.9/–	–/–	–/–	–/–

[a] Log_{10} EID_{50} per 0.2 ml swab medium.
[b] No virus isolated.

The transmission parameter R could be estimated for both the vaccinated and control groups. The R values of the two unvaccinated control groups did not differ significantly ($P = 0.6$). The combined R for both unvaccinated control groups (R_0) was estimated as 19.95 (s.e. = 12.41), using the estimated amount of infectivity that was excreted after infection of the last susceptible animal, of 87% [(86 + 88)/2].

Vaccinated group V1 started with five infected and nine susceptible chickens, and ended with three contact infections. Group V2, started with seven infected and seven susceptible chickens, and ended with three contact infections. The R values of the two vaccinated groups did not differ significantly ($P = 0.6$). The combined R for both vaccinated groups was estimated as 0.69 (s.e. = 0.33).

Table 3. *Results of virus isolation and serology after challenge with $10^{3.5}$ EID_{50} of M41 of vaccinated chickens in group V1*

Bird number	Amount of virus from trachea/cloaca isolated on days post challenge[a]								Detection of seroconversion by	
	0	1	3	5	7	10	14	17	ELISA	HI
Challenged										
1	–/–[b]	–/–	–/–	–/–	–/–	–/0.2	–/–	–/–	Pos[c]	Pos
2	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	n[d]	n
3	–/–	0.8/–	–/–	–/–	–/–	–/–	–/–	–/–	Pos	n
4	–/–	–/–	0.2/–	–/–	–/–	–/–	–/–	–/–	Pos	n
5	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	n	n
6	–/–	0.2/0.6	0.2/–	–/–	–/–	–/0.5	–/–	–/–	n	n
7	–/–	0.2/0.2	–/–	–/–	–/–	–/–	–/–	–/–	Pos	n
Contacts										
8	–/–	–/–	–/0.4	–/–	–/–	–/0.2	–/–	–/–	Pos	n
9	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	n	n
10	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	n	n
11	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	n	n
12	–/–	–/–	–/–	–/–	–/–	–/0.2	–/–	–/–	Pos	n
13	–/–	–/–	–/0.2	–/–	–/–	–/–	–/–	–/–	Pos	Pos
14	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	n	n

[a] Log_{10} EID_{50} per 0.2 ml swab medium.
[b] No virus isolated.
[c] At least four-fold rise in titre.
[d] No seroconversion.

Table 4. *Results of virus isolation and serology after challenge with $10^{5.0}$ EID_{50} of M41 of vaccinated chickens in group V2*

Bird number	Amount of virus from trachea/cloaca isolated on days post challenge[a]								Detection of seroconversion by	
	0	1	3	5	7	10	14	17	ELISA	HI
Challenged										
1	–/–[b]	0.8/–	–/–	–/–	–/–	–/0.4	–/–	–/–	Pos[c]	Pos
2	–/–	2.2/0.2	–/–	–/–	–/–	–/–	–/–	–/–	Pos	n[d]
3	–/–	1.5/–	–/–	–/–	–/–	–/–	–/–	–/–	n	n
4	–/–	0.6/0.4	–/–	–/–	–/–	–/–	–/–	–/–	Pos	n
5	–/–	0.6/–	–/0.2	–/–	–/–	–/–	–/–	–/–	Pos	n
6	–/–	2.4/–	–/–	–/–	–/–	–/–	–/–	–/–	Pos	Pos
7	–/–	0.8/–	–/–	–/–	–/–	–/–	–/–	–/–	Pos	Pos
Contacts										
8	–/–	–/–	–/–	–/0.4	–/–	–/–	–/–	–/–	Pos	n
9	–/–	–/–	–/0.2	–/–	–/–	–/–	–/–	–/–	n	n
10	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	n	n
11	–/–	–/–	–/0.4	–/–	–/–	–/–	–/–	–/–	Pos	n
12	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	n	n
13	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	n	n
14	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	n	n

[a] Log_{10} EID_{50} per 0.2 ml swab medium.
[b] No virus isolated.
[c] At least four-fold rise in titre.
[d] No seroconversion.

Hypothesis testing. The probability of observing the outcomes (or more extreme outcomes) of group C1 versus V1 (challenge $10^{3.5}$ EID_{50}) when H_0 (no effect of vaccination on transmission) was true, was $P = 0.10$. The probability of observing the outcomes (or more extreme outcomes) of group C2 versus V2 (challenge $10^{5.0}$ EID_{50}) when H_0 was true, was $P = 0.09$. Because of the similarity of the number of infections in both vaccinated and unvaccinated groups, the probability of the outcomes of the combined results of groups C1 and C2 versus the combined

Table 5. *Detected IBV transmission after challenge of vaccinated and unvaccinated chickens*

Group	Experimental design: Vaccination at day 1	Experimental design: Challenge dose (EID_{50})	Number of birds at day of challenge: Infectious	Number of birds at day of challenge: Susceptible	Number of contact infections	Virus excretion[a] (log_{10} EID_{50}): Inoculates	Virus excretion[a] (log_{10} EID_{50}): Contacts
V1	H120	$10^{3.5}$	5	9	3	0.7	0.4
C1	no	$10^{3.5}$	7	7	7	3.5	3.2
V2	H120	$10^{5.0}$	7	7	3	1.9	0.3
C2	no	$10^{5.0}$	7	7	7	2.9	2.4

[a]Arithmetic mean titre for those chickens that excreted IBV.

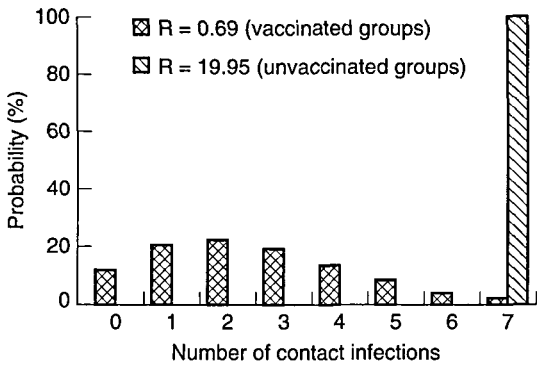

Figure 3. *The final size distribution of the stochastic SIR model ($S_0 = 7$, $I_0 = 7$) for two values of the reproduction value* R.

results of groups V1 and V2 were calculated, resulting in a $P < 0.05$. Therefore H_0 was rejected, meaning that the vaccination had reduced the transmission of challenge virus.

The probability of the possible outcomes (0, 1, 2, 3, 4, 5, 6 or 7 contact infections) in unvaccinated and vaccinated groups of chickens ($S_0 = 7$ and $I_0 = 7$) was calculated for $R = 19.95$ and $R = 0.69$. For $R = 19.95$ only major outbreaks are to be expected because R is greater than 1. This implies that in an infinite population, a considerable part of the population will eventually become infected. For the small finite population in this study, $R = 19.95$ implies that almost certainly $P = 0.99993$) all contact animals become infected (Figure 3). In contrast, for $R = 0.69$ only minor outbreaks are expected as R is less than 1. Which implies, for an infinite population, that the number of eventually infected animals is negligible. For this particular experiment, $R = 0.69$ implies that the chance that all contact animals become infected is very small ($P = 0.008$). Hence, it can be understood that the probability of observing the difference in number of contact-infected animals between the vaccinated and the control group is very unlikely under the assumption that in fact the values of R are not different (the H_0 above).

Discussion

In our experiments R of the vaccinated groups was calculated based on the results of virus isolation after challenge. The challenge virus could be re-isolated from the trachea of five out of seven and seven out of seven inoculated chickens from group V1 and V2, respectively. From seven (2 and 5 in group V1 and V2, respectively) of these 12 chickens, virus was re-isolated only at 1 d.p.c. (there was no sampling at 2 d.p.c.). A re-isolation of challenge virus from the trachea shortly after inoculation could have been residual challenge virus that was only passively present in the lumen of the trachea, indicating that the bird was not really replicating the virus, and was therefore not really infected. Six of the seven birds, from which challenge virus was re-isolated only 1 d.p.c., seroconverted. Therefore, we considered these six birds to have been infected. It cannot be excluded that the lack of seroconversion in the seventh bird was not caused by a low antibody response after infection, but that the re-isolated virus was only passively present in the lumen of the trachea, indicating that the bird was not really infected. If this inoculated bird was considered not to be infected by the challenge, the estimated R value for group V2 was 0.68, meaning that this bird hardly influenced the results of the experiment.

From 10 of the 28 vaccinated birds (two inoculated and eight contact-exposed birds) in groups V1 and V2, no challenge virus was isolated, nor did they respond serologically to challenge. It is not likely that these vaccinated birds that escaped an infection were less susceptible than the other contact-exposed chickens, since they were selected randomly, originated from the same SPF flock, and had similar ELISA and HI titres on day 22 (data not shown).

From the contact-infected birds of the vaccinated groups, virus was isolated from the cloacas only and in very low titre. Probably, no virus was isolated from the tracheas of these birds because IBV was not present or only for a short time because of the local protection induced by the eyedrop vaccination.

For the estimation of R, both the inoculated and the contact-infected chickens should be equally infectious. In general, this condition was fulfilled, except for several chickens in group V2 (high challenge dose), from which a relatively high titre

of IBV was isolated at 1 d.p.c., compared to the titres obtained from the contact-infected chickens. This could result in an over-estimation of R of group V2, meaning that the estimated $R = 0.69$ of both vaccinated groups combined could also be over-estimated. However, the resemblance in number of contact infections between groups V1 versus V2, and between groups C1 versus C2 (also note the resemblance of both values of z), suggests that the two different challenge doses did not influence the degree of transmission. Moreover, the use of these two different challenge doses indicates that the results of the experiment are repeatable.

For estimating z in the unvaccinated groups, we assumed that the infection of the last contact-exposed chicken happened at 2 d.p.c., because all contact-exposed chickens of both groups yielded virus in high titre from the trachea at 3 d.p.c. Because it cannot be excluded that infection of the last susceptible chicken could have taken place earlier or later than 2 d.p.c. (24 h after reunion), we also estimated the fraction of excreted IBV for a last infection at 1.5 d.p.c. (12 h after reunion) and 2.5 d.p.c. (36 h post-reunion and 12 h before sampling). The estimated loss of infectivity in group C1 for 1.5 and 2.5 d.p.c. was 92 and 79%, respectively, and for group C2 93 and 80%. This resulted in an estimated R_0 value for the combined unvaccinated groups of 36 (s.e. = 40) and 12 (s.e. = 9) for 1.5 and 2.5 d.p.c., respectively. Both are significantly higher than $R = 0.69$.

These experiments showed that transmission of IBV can be measured. Because virus transmission is a more relevant measure of vaccine efficacy, experimental quantification of transmission is preferable to tests comparing individual protection against challenge. At present, before an IBV vaccine is authorized in the European Community, it has to meet the requirements of the European Pharmacopea. According to these requirements, a vaccine complies if the virulent challenge virus, which is administrated intratracheally at a dose of 10^3 EID_{50} per bird, can be re-isolated from the trachea (in one sampling between the fourth and seventh day after challenge) from not more than 20% of the birds. These requirements only look at the infectivity of individual chickens. If reduction of transmission of IBV is thought to be important, the present requirements would have to be adjusted.

In this study we compared the transmission of IBV between groups of SPF chickens using one vaccine, applied by eye-drop, and one homologous challenge strain. In order to understand more about the mechanisms that cause the reduction in transmission, variations to this study, such as the vaccine used, the way of vaccine application, other challenge strains (homologous and heterologous), presence of antibodies at the time of vaccination, needed to be studied. This model could be a tool for selecting or developing IBV vaccines that induce such a level of protection that they reduce the transmission of IBV significantly, or better reduce the reproduction ratio to below 1.

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RÉSUMÉ

Transmission du virus de la bronchite infectieuse au sein de groupes de poulet vaccinés ou non

Le but de cette étude a été de déterminer si la vaccination contre le virus de la bronchite infectieuse (IBV) réduisait la transmission du virus, c'est-à-dire de vérifier si la transmission de l'IBV chez les poulets vaccinés était significativement réduite comparée a celle des poulets non vaccinés. Dans quatre groupes de poulet SPF, deux vaccinés et deux non vaccinés, une mesure standardisée de la transmission virale a été déterminée par le taux de multiplication (R). R est défini comme étant le nombre moyen de nouvelles infections induites par individu typiquement infectieux durant toute la période d'infectiosité.

Une seule vaccination avec la souche H120 administrée par instillation oculaire réduit significativement ($P < 0{,}05$) la transmission de l'IBV d'épreuve chez les poulets vaccinés ($R = 0{,}69$, SE = 0,33) comparée à celle des poulets non vaccinés ($R = 19{,}95$, SE = 12,41).

Les implications possibles, pour une étude ultérieure incluant la sélection ou le développement de vaccins, sont discutées.

ZUSAMMENFASSUNG

Bronchitisvirus-Übertragung innerhalb vakzinierter und nicht vakzinierter Kükengruppen

In dieser Studie sollte festgestellt werden, ob die Vakzinierung gegen das Virus der infektiösen Bronchitis (IBV) die Virusübertragung reduziert, d.h., ob die IBV-Übertragun unter vakzinierten Küken im Vergleich zu der unter nicht vakzinierten Küken signifikant vermindert ist. In zwei vakzinierten und zwei nicht vakzinierten Gruppen von SPF-Küken wurde der Reproduktions-Quotient (R) als ein Standardmaß für die Virusübertragung bestimmt. R ist definiert als die durchschnittliche Anzahl neuer Infektionen, die durch ein typisches infektiöses Einzeltier während seiner gesamten infektiösen Periode verursacht wird.

Eine einzige Augentropf-Vakzinierung mit IBV-H120 reduzierte signifikant ($P < 0{,}05$) die Übertragung des IBV-Testvirus unter den vakzinierten Küken (Schätzwert von $R = 0{,}69$. SE = 0,33) im Vergleich zu den nicht vakzinierten Küken (Schätzwert von $R = 19.95$, SE = 12,41).

Die möglichen Konsequenzen dieser Ergebnisse für weitere Untersuchungen einschließlich der Auswahl oder Entwicklung von Vakzinen werden diskutiert.

RESUMEN

Transmisión del virus de la bronquitis infecciosa en grupos de pollos vacunados y no vacunados

La finalidad de este estudio fue determinar si la vacunación contra el virus de la bronquitis infecciosa (IBV) disminuía la transmisión vírica, es decir, si la transmisión de IBV entre pollos vacunados se reducía significativamente en comparación con los no vacunados. Se determinó la ratio de reproducción (R), una medida estándar para la transmisión vírica, en dos grupos de pollos SPF vacunados y dos grupos sin vacunar. Se entiende por R, el número medio de nuevas infecciones producidas por un individuo infectado durante todo el proceso infeccioso. Una sola vacunación con IBV H120 vía intraocular, redujo significativamente ($P < 0.05$) la transmisión del virus entre los pollos vacunados (R estimada = 0.69, SE = 0.33) en comparación con la transmisión entre los no vacunados (R estimada = 19.95, SE = 12.41).

Se discuten las posibles consecuencias para posteriores estudios, incluyendo la selección y desarrollo de vacunas.