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Vaccination against foot and mouth disease reduces virus transmission in groups of calves

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Abstract

The aim of vaccination during an epidemic of foot and mouth disease (FMD) is not to induce clinical protection, but to reduce virus transmission. Since no quantitative data were available on the effectiveness of vaccination in cattle, we investigated whether a single vaccination against FMD could reduce virus transmission in groups of calves by estimating the reproduction ratio *R*, i.e. the average number of secondary cases caused by one infectious animal in a susceptible population. We performed two experiments with six groups of either four vaccinated or four non-vaccinated calves each. Vaccination was carried out with O_1 Manisa vaccine. Two weeks after vaccination, two calves per group were inoculated intra-nasally with FMDV field isolate O/NET2001. The two other calves were contact-exposed to the inoculated calves. Contact infections were observed by clinical inspection, virus isolation and RT-PCR on heparinised blood, oro-pharyngeal fluid and probang samples and antibody response to non-structural proteins. In all six non-vaccinated groups, transmission to contact-exposed calves was recorded; in the vaccinated groups, virus transmission was observed to one contact-exposed calf. In the non-vaccinated groups R_c was 2.52 and significantly above 1, whereas in the vaccinated groups $R_v = 0.18$ and significantly below 1, indicating that vaccination may successfully be applied as additional intervention tool to reduce virus transmission in a future epidemic of FMD.
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1. Introduction

Foot and mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals and has a great potential for causing severe economic loss. The Office International des Epizooties (OIE) established an official list of free countries and zones and recognizes the European Union (EU) as free of FMD without vaccination [1]. A massive epidemic of FMD, however, occurred in Great Britain in 2001, and the virus spread to Ireland, France and The Netherlands [2,3]. Various control measures such as zoösanitary measures, killing of infected herds and pre-emptive culling of contiguous farms were implemented. In The Netherlands, emergency vaccination of all susceptible species was applied as additional tool, because soon after the first outbreak, the culling and destruction capacity was insufficient to depopulate all farms [4]. All vaccinated animals, however, were destroyed within the following weeks after the last outbreak. The epidemic not only had an enormous economic effect, but also resulted in a public and political discussion about alternative control strategies such as a 'vaccination to live' policy without destruction of uninfected vaccinated animals.

Before changing the non-vaccination policy of the EU, more veterinary evidence is needed about the effectiveness of emergency vaccination and the risks that are associated with animal movement and trade after emergency vaccination. Until 1991, mass annual prophylactic vaccination for FMDV has been applied in all cattle and the number of

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outbreaks was successfully diminished [5], suggesting that vaccination can prevent the spread of FMDV between farms. This campaign, however, consisted of multiple prophylactic vaccinations for several years, whereas an emergency vaccination during an epidemic will most likely be applied only once. Therefore, it is not clear whether a single vaccination can provide sufficient herd immunity to reduce virus transmission.

Field data like from the Dutch FMD epidemic in 2001 cannot provide this information, because exact information about virus introduction and transmission after vaccination is lacking. Experimental data are also not suitable, since most vaccines were experimentally tested for their capacity to induce clinical protection against a challenge infection [6], and not for their capacity to reduce virus transmission, which is essential when applied as emergency vaccine. Cox et al. carried out a transmission experiment, and showed that vaccinated calves became infected after exposure to FMDV inoculated calves. However, they determined transmission qualitatively, in a heterogeneous population: the inoculated calves were not vaccinated. This would probably result in an underestimation of the effectiveness of vaccination, as during an emergency campaign all animals in a herd will be vaccinated [7].

Consequently, transmission should be determined in a homogeneous group in which all animals are vaccinated. We therefore determined the effectiveness of a single vaccination against FMDV by comparing virus transmission within groups of vaccinated and non-vaccinated calves with the reproduction ratio R. This reproduction ratio is of biological relevance: when R is above 1, major outbreaks may occur; when R is below 1, only minor outbreaks will occur and an epidemic will fade out [8].

2. Materials and methods

2.1. Animals

We performed two experiments, each with 26 conventionally raised Holstein Friesian calves, 8–10 weeks of age. They were fed on milk twice a day from one through, concentrates and grass pellets, and were housed in free mingling groups in the bio-security facilities at CIDC-Lelystad. In this way, direct and intensive physical contact between the calves within a group was possible.

2.2. Vaccine and challenge virus

The vaccine was a commercially available O_1 Manisa vaccine with 11 PD_{50} (Animal Sciences Group of Wageningen UR, Lelystad), prepared in a double-oil-in-water emulsion (DOE). Virus inoculation was carried out using 1500 cattle-ID_{50} of the first cattle passage of the FMDV field isolate O/NET2001 [9].

2.3. Experimental design

In both experiments, the sexes were randomly allocated to six groups of four calves and one group of two calves at 16 days before inoculation (−16 dpi). Three groups of four calves were vaccinated subcutaneously according to the manufactures standards with a DOE O_1 Manisa vaccine, two weeks before inoculation (−14 dpi).

The group of two calves was vaccinated at the same time and served as a vaccine control group. The other three groups of four calves remained unvaccinated.

At the day of inoculation (0 dpi), two calves per group of four were removed to a separate unit, and sedated with xylazine and subsequently inoculated intra-nasally with 1.5 ml of FMD virus suspension per nostril. Twenty hours after inoculation, these calves were reunited with their original group (1 dpi), thereby contact-exposing the two remaining, not inoculated calves.

The experiment ended at 30 dpi, which was assumed to be a sufficient time span for a possible contact-infection and subsequent rise of antibody titer against structural and non-structural proteins to occur. Also, the detection of carriers was possible, while persistently infected animals are defined as presence of detectable virus for at least 28 days after infection.

2.4. Sampling procedures

Daily rectal temperature of the calves and clinical signs were recorded from 0 till 14 dpi, and also when the physical condition of the calves made further clinical investigation of their health status necessary.

Heparinised blood (10 ml per calf) was collected daily from 0 dpi till day 14 dpi from the jugular vein of all animals. Clotted blood (10 ml per calf) for serology was taken at −14, 0, 4, 7, 11, 14, 21 and 28 dpi, also from the jugular vein. Oro-pharyngeal fluid (OPF) was taken daily from day 0 to 14 dpi by inserting a cotton mouth swab in a forceps of 25 cm long and rubbing the surface of the oro-pharyngeal cavity. Probang samples were collected at 28, 29 and 30 dpi by scraping the oro-pharynx with a probang sampler. Samples were treated and stored as described by Moonen et al. [10].

2.5. Laboratory tests

All OPF, probang and heparinised blood samples were tested for the presence of virus by plaque titration on monolayers of secondary lamb kidney cells [9]. RT-PCR was performed by isolating RNA from OPF, heparinised blood and probang samples using the Magna Pure LC total Nucleid Acid Isolation kit (3 038 505) in the MagNA Pure® system (Roche). The isolates were tested in a Light Cycler based RT-PCR with use of Light Cycler RNA Master Hybridization Probes (3 0180954), all in accordance with the manufacturers instructions (Roche). The primers and probes used are described by Moonen et al. [11].

Virus neutralization test (VN-test) was performed as described by Dekker and Terpstra [12] using O_1 Manisa virus and secondary porcine kidney cells. We considered a four-fold higher antibody titer compared to the mean titer of the vaccine control group as an indication for infection. Antibodies against FMDV non-structural proteins were detected using a commercial NS ELISA (Cedi-test®), performed in accordance with the instructions of the manufacturer, to discriminate between infection and vaccination [13,14].

2.6. Quantification of transmission and statistical methods

We used a stochastic *SIR* model (susceptible-infectious-recovered model) as described by De Jong and Kimman [15], in which S is the total number of susceptible, I the total number of infectious and R the total number of recovered animals. In the model we classified the calves as infected (I) when either clinical signs were present or when a sample tested positive in the virus isolation (VI) assay, the RT-PCR or NS ELISA or when a four-fold increase in VNT titer was observed. The animals were classified recovered at the end of the experiment after being infectious at the start. Their carrier status was not taken into account.

The experimental period was sufficient for all infected or contact animals to recover from infection; therefore, it was valid to use the final size of the experiment to calculate the reproduction ratio. The value for R that maximizes the likelihood function is called the maximum likelihood estimate (MLE) of R. With the final size of infection and by means of this MLE, R can be estimated for the vaccinated groups (R_v) and non-vaccinated groups (R_c) We tested one-sided whether R_v was below 1 and R_c above 1 to quantify the effect of vaccination. To compare the strategy with and without vaccination, we also tested if R_c and R_v were significantly different [16,17].

3. Results

3.1. Clinical signs

In the non-vaccinated groups, vesicles in the mouth or on the coronary band or in the interdigital spaces were observed in 10 out of 12 inoculated calves, where seven contact-exposed calves showed clinical signs (Table 1). The clinical signs appeared very mild and did not have effect on feed intake. In the vaccinated groups, neither the inoculated nor the contact-exposed calves showed clinical signs. In one inoculated vaccinated calf, a small lesion was observed in the mouth for more than 14 days (animal no. 33, Table 2).

3.2. Virus isolation assay

In the non-vaccinated groups, 10 of 12 inoculated calves tested positive in the virus isolation of the OPF, and 5 of 12 contact-exposed calves tested positive. Six of the blood samples of inoculated and two of the blood samples of contact-exposed calves were positive. In the probang samples, five inoculated and two contact-exposed non-vaccinated calves tested positive.

In the vaccinated groups, 9 of 12 inoculated and none of the contact-exposed calves tested positive in the OPF. No positive blood samples were found. In the probang samples, 2 of 12 inoculated vaccinated calves tested positive, and none of the contacts.

3.3. RT-PCR

In the non-vaccinated groups, RT-PCR of the OPF samples showed 11 positive results in the inoculated calves, and eight in the contact-exposed calves. Blood samples of 10 inoculated calves and two contact-exposed calves tested positive. In the probang samples, five inoculated and three contact-exposed calves were positive.

In the vaccinated groups, OPF of 11 inoculated calves were positive, and none of the contact-exposed calves were positive. No positive samples were found in the heparinised blood samples of the vaccinated calves, either inoculated or contact-exposed. In the probang samples, three inoculated vaccinated calves tested positive.

3.4. Serology

In the non-vaccinated groups, all inoculated and eight contact-exposed calves developed a VN titer higher than 0.3 (^{10}log). In the NS-ELISA, 10 inoculated and 5 contact-exposed calves tested positive.

In the vaccinated groups, all 12 inoculated and 11 contact-exposed calves showed a rise in neutralizing antibodies in the VNT 14 days after vaccination (0 dpi). No four-fold higher antibody titer was observed after inoculation as compared to the vaccine control group. Six inoculated and one contact-exposed calf developed NS-antibodies.

The calves that served as vaccine controls tested negative for all tests with exception of the virus neutralization test.

All major test results are summarized in Tables 1 and 2.

3.5. Quantification of virus transmission

We classified the calves as susceptible, infectious or recovered using the results from the clinical signs and laboratory tests, as visualized in Fig. 1. In one of the six groups of vaccinated calves virus transmission occurred. This resulted in a R_v of 0.18 (0.01:1.2) and was significantly below 1 in a one-sided test ($p = 0.05$).

In the groups of non-vaccinated calves, transmission occurred in all groups. This resulted in a R_c of 2.52 (1.13; 52.1), which was significantly above 1 ($p = 0.01$) when tested one-sided. When comparing the strategies we proved that the R-values of both groups were significantly different ($p = 0.003$).

Table 1
Test results from non-vaccinated groups of animals

Animal number	Clinical signs	NS ELISA	VI plasma	RT-PCR plasma	VI OPF	RT-PCR OPF	VI probang	RT-PCR probang	Classification
1	+	+	−	+	+	+	+	+	*R*
2	+	+	+	+	+	+	−	−	*R*
3*	−	+	−	−	+	+	−	−	*R*
4*	+	+	−	−	+	+	+	+	*R*
5	+	+	+	+	+	+	+	+	*R*
6	+	+	+	+	+	+	−	−	*R*
7*	+	+	−	−	+	+	−	+	*R*
8*	+	+	+	+	+	+	+	+	*R*
9	+	+	−	+	+	+	−	−	*R*
10	−	+	−	+	+	+	−	−	*R*
11*	−	−	−	−	−	−	−	−	*S*
12*	−	+	−	−	−	+	−	−	*R*
13	+	+	−	+	−	+	−	−	*R*
14	+	−	−	−	−	−	−	−	*R*
15*	+	−	−	−	−	−	−	−	*R*
16*	−	−	−	−	−	+	−	−	*R*
17	+	+	+	+	+	+	−	−	*R*
18	+	+	+	+	+	+	+	+	*R*
19*	+	−	−	+	−	+	−	−	*R*
20*	−	−	−	−	−	−	−	−	*S*
21	+	+	+	+	+	+	+	+	*R*
22	−	−	−	−	+	+	+	+	*R*
23*	+	−	−	−	−	−	−	−	*R*
24*	+	−	+	−	+	+	−	−	*R*

In this table all major test results of the non-vaccinated animals are summarized. Definition of symbols: *, contact-exposed animal; *S*, susceptible; *I*, infectious; *R*, recovered; +, (at least one) positive test result; −, negative test result (no positive test results).

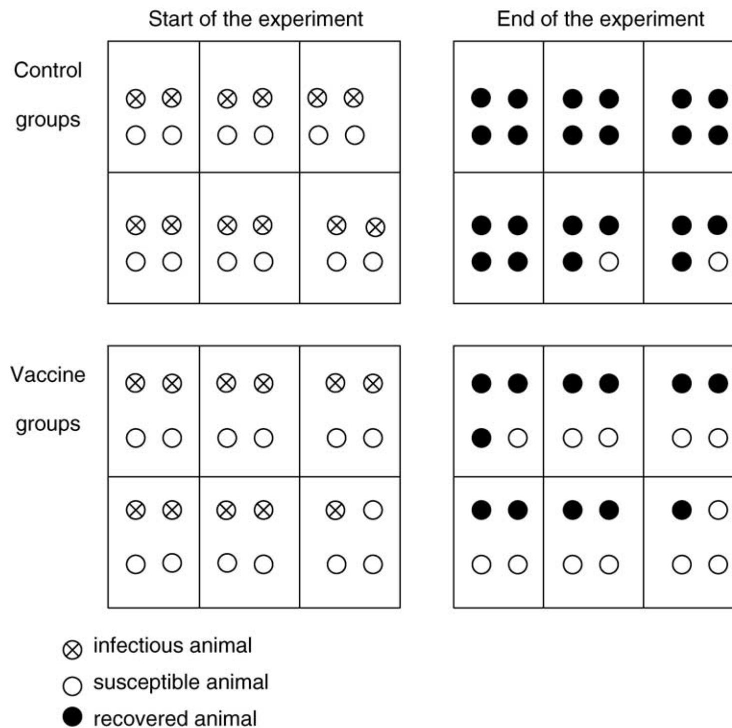

Fig. 1. Final size of infection in the *SIR* model. In this figure the start and end situation per group in the *SIR* model is visualised. Definition of symbols: (⊗) infectious animal; (◯) susceptible animal; (●) recovered animal.

Table 2
Test results from vaccinated groups of calves

Animal number	Clinical signs	NS ELISA	VI plasma	RT-PCR plasma	VI OPF	RT-PCR OPF	VI probang	RT-PCR probang	Classification
25	−	−	−	−	+	+	−	−	*R*
26	−	+	−	−	+	+	+	+	*R*
27*	−	−	−	−	−	−	−	−	*S*
28*	−	+	−	−	−	−	−	−	*R*
29	−	−	−	−	−	−	−	−	*S*
30	−	−	−	−	+	+	−	−	*R*
31*	−	−	−	−	−	−	−	−	*S*
32*	−	−	−	−	−	−	−	−	*S*
33	+	+	−	−	+	+	−	+	*R*
34	−	+	−	−	+	+	−	−	*R*
35*	−	−	−	−	−	−	−	−	*S*
36*	−	−	−	−	−	−	−	−	*S*
37	−	−	−	−	+	+	+	+	*R*
38	−	−	−	−	−	+	−	−	*R*
39*	−	−	−	−	−	−	−	−	*S*
40*	−	−	−	−	−	−	−	−	*S*
41	−	+	−	−	+	+	−	−	*R*
42	−	−	−	−	−	+	−	−	*R*
43*	−	−	−	−	−	−	−	−	*S*
44*	−	−	−	−	−	−	−	−	*S*
45	−	+	−	−	+	+	−	−	*R*
46	−	+	−	−	+	+	−	−	*R*
47*	−	−	−	−	−	−	−	−	*S*
48*	−	−	−	−	−	−	−	−	*S*
Vaccine control									
49	−	−	−	−	−	−	−	−	
50	−	−	−	−	−	−	−	−	
51	−	−	−	−	−	−	−	−	
52	−	−	−	−	−	−	−	−	

In this table all major test results of the vaccinated animals are summarized. Definition of symbol: *, contact-exposed animal; *S*, susceptible; *I*, infectious; *R*, recovered; +, (at least one) positive test result; −, negative test result (no positive test results).

In Fig. 2a and b the predicted number of contact infection with the estimate for R are calculated and compared to the number of observed contact infected animals. Those figures show that the model does predict the number of contact infections rather well.

4. Discussion

In this study, we investigated whether single vaccination against FMDV could significantly reduce virus transmission in groups of calves compared to transmission in groups of non-vaccinated calves, and whether vaccination could reduce R to a value below one. The overall reproduction ratio in the vaccinated groups was 0.18, which was significantly below 1, and significantly different from the reproduction ratio in non-vaccinated groups (2.52). Our findings implicate that single vaccination in a population of calves can reduce transmission and that it might be sufficient to eradicate the virus during an epidemic of FMD. It may therefore be an effective additional intervention tool during an epidemic.

In our model despite inoculation, two calves never tested positive, thereby suggesting that they were not infected at all. Probably, vaccination with O_1 Manisa 14 days before inoculation protected the calf against a challenge infection, or the calves were not susceptible to FMD virus inoculation intranasally. Another explanation is that the inoculation method failed. However, within the unvaccinated groups, infection occurred in all inoculated calves and virus spread to nearly all contact-exposed calves. This indicates that our experimental design was appropriate to measure virus transmission and allowed us to quantify the reproduction ratio and the effect of vaccination on virus transmission. Moreover, in an analysis with all inoculated calves defined as infectious, we found that the impact of different assumptions for the definition of I (infectious) as a result of inconsistent test results did not have great impact on the reproduction ratio ($R_v = 0.17$ ($p = 0.009$) and $R_c = 3.3$ ($p = 0.04$)).

With a reproduction ratio significantly below 1, vaccination might be successfully applied in the field during an epidemic. Extrapolation to the field, however, should always be done carefully. For instance the reproduction ratio is influ-

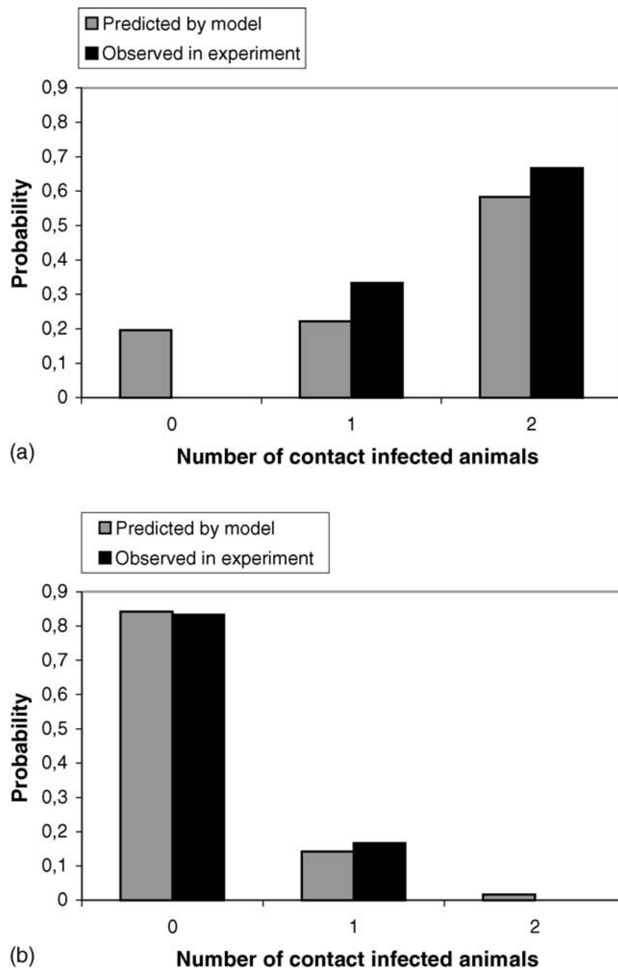

Fig. 2. (a) Comparison of observed vs. expected probabilities of contact infections in control group ($R_c = 2.52$). (b) Comparison of observed vs. expected probabilities of contact infections in vaccinated groups ($R_v = 0.18$).

enced by heterogeneity: the infectivity and susceptibility of the individual animal, the virus strain, the species involved, animal density and interaction etc. [18]. Possibly, vaccination is less effective in other species or in adult cattle, since they might differ in infectiousness or susceptibility from groups of calves [19]. When extrapolating to the field, R_v within the herd, measured in the experiment, below 1 implicates that at herd level the epidemic can be stopped, since the number of contacts between animals at different farms is far less than between animals at the same farm [20].

We chose to study transmission in a homogeneous population and quantified the transmission of infection from vaccinated to vaccinated animals and from non-vaccinated to non-vaccinated animals. This allowed us to model the results under the assumptions of homogeneity. This situation is most likely to occur during an epidemic of FMD with transmission through direct contact. Either all animals in the area are being vaccinated, or all animals are non-vaccinated.

Information about the mechanism of herd immunity induced by vaccination might be gained from the effect of vaccination on infectiousness if vaccinated animals are inoculated and placed in contact with non-vaccinated animals. In the vice versa situation susceptibility of the animal after vaccination can be studied if a vaccinated animal is placed in contact with a non-vaccinated inoculated animal. But in these heterogeneous situations the animals must be housed pair wise to make quantification of the transmission possible. In our study the animals were housed in groups of 4; 2 animals inoculated and 2 animals contact-exposed.

Only infection on the first generation can be observed in our study design. Infection of 2nd and higher generation should be observed in large groups. Since differences in infectivity between inoculated and contact infected animals can be expected, our method is conservative and will overestimate the R.

The diagnosis of FMD infection was based on several diagnostic tests, which mainly showed similar results. By adding RT-PCR techniques to the standard virus isolation assay we were able to detect more positive samples from different animals and also for a longer period of time within one animal. Also two more animals could be diagnosed as carrier animals in probang samples. RT-PCR is known to be a more sensitive technique and in our experiment it proves to be of additional value. However, the results of the PCR did not alter the conclusions about the effectiveness of the vaccination.

As mentioned before, contact structure might affect the transmission of FMDV. In our experiments, we used a loose housing system, in which the calves could mingle freely. Moreover, intense contact was possible by feeding them milk from one through. In this setting, R was significantly above 1 in the group of non-vaccinated calves. As Bouma et al. [9] did not observe transmission between individually housed calves, not even when they had direct contact, these findings indicate that the intense contact structure could have increased the transmission of FMD virus. The results indicate that contact structure is important for transmission of this FMDV virus strain. The observed difference in transmission also suggests that it could be considered not applying vaccination to calves that are housed individually.

The clinical signs in the calves were rather mild, in contrast to described clinical signs of FMD in cattle [21]. Our observations of mild clinical signs in calves is consistent with the study of Bouma et al. [9]. It might be possible that animals with mild signs of FMD are less infectious than animals with severe signs of FMD. Donaldson and Kitching showed that challenge of cattle 14 days after vaccination led to sub-clinical infection in susceptible in-contact animals. This sub-clinical disease was manifested by short-term transient viraemia. No positive virus isolation from blood samples was found to confirm transmission. In contrast, in our experiment viraemia only occurred in clinically diseased animals and was confirmed by laboratory tests [6]. Moreover, the mild clinical appearance in calves may be important, because it might be difficult to recognize FMD in calves. Our experiment shows that these calves do transmit FMDV to in-contact calves. As a consequence, calves might be looked at as

'real hazard' like sheep and goat because of this sub-clinical appearance [22,23].

Data as shown here will provide necessary scientific background for FMD control strategies for disease free countries. With more information about effect of intervention tools, future outbreaks can be stopped in a more efficient way.

Acknowledgements

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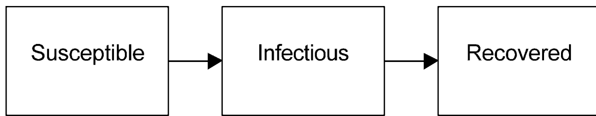
Appendix A. The Model and statistical methods used; in more detail

The stochastic *SIR* model



The *SIR* model is based on several assumptions. All animals within the population have random contact; every class consists of a homogeneous population; the infection rate is constant during the infectious period and each recovered animal is totally immune to infection [18]. The model does not include the possible infectivity of carriers whilst this is expected to be relatively low compared to direct contact infections. There are no a priori arguments not to assume 'true mass action' for our model [24,25].

This stochastic *SIR* model is used to describe the probability distribution of the outcome of a transmission experiment (the final size) in terms of R. The final size of our experiment is illustrated in Table 3. Estimations for R are based on a known distribution over all the possible final size outcomes, i.e. the final size distribution.

Table 3
Final size of infection in the *SIR* model

Groups	N	S_0	I_0	S_t	R_t	I_t	x_i	f
Control	4	2	2	0	4	0	2	4
	4	2	2	1	3	0	1	2
Vaccine	4	3	1	3	1	0	0	1
	4	2	2	2	2	0	0	4
	4	2	2	1	3	0	1	1

N, total number of animals in group; S_0, total number of susceptible animals at start; I_0, total number of infectious animals at start; S_t, total number of susceptible animals at end; I_t, total number of infectious animals at end; R_t, total number of recovered animals at end; f, number of repetitions; x_i, number of contact infections.

The transmission model is described by De Jong and Kimman [15,16]

$$\text{Infection } [S; I] \rightarrow [S-1; I+1] \quad \text{rate}: \frac{\beta \times SI}{N}$$

$$\text{Recovery } [S; I] \rightarrow [S; I-1] \quad \text{rate}: \alpha \times I$$

in which β is the transmission rate parameter and α is the recovery rate parameter.

De Jong and Kimman derived an algorithm to calculate the probability $f(x|R, S_0, I_0)$ for each outcome of x contact infected animals. The final size of infection including the number of contact infections (x_i) are summarized in Table 3.

The value of R that maximises the likelihood function is called the maximum likelihood estimate (MLE) of R. This MLE-function expressed as

$$R_{\text{mle}} = \max_R \prod_{i=1}^{n} f(xi, R|N, S_0, I_0)$$

with x_i = number of contact infections and i observations: maximize the product (π).

The hypothesis tested is whether H_0: $R_v = R_c$ against the one-sided alternative $H_1: R_v < R_c$. R_v is the reproduction ratio in the treated groups and R_c in the control groups [15,16].

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