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**New Insights about vaccine effectiveness: Impact of attenuated PRRS-strain
vaccination on heterologous strain transmission**

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Abstract

Vaccination is the main tool for controlling infectious diseases in livestock. Yet current vaccines only provide partial protection raising concerns about vaccine effectiveness in the field.

Two successive transmission trials were performed involving 52 pigs to evaluate the effectiveness of a Porcine Reproductive and Respiratory Syndrome (PRRS) vaccinal strain candidate against horizontal transmission of a virulent heterologous strain. PRRS virus, above the specified limit of detection, was observed in serum and nasal secretions for all but one pig (the exception only tested positive for serum), indicating that vaccination did not protect pigs from becoming infected and shedding the heterologous strain. However, vaccination delayed the onset of viraemia, reduced the duration of shedding and significantly decreased viral load throughout infection. Serum antibody profiles indicated that 4 out of 13 (31%) vaccinates in one trial had no serological response (NSR).

A Bayesian epidemiological model was fitted to the data to assess the impact of vaccination, and presence of NSRs, on PRRS virus transmission dynamics.

Despite little evidence for reduction in the transmission rate, vaccinated animals were on average slower to become infectious, experienced a shorter infectious period and recovered faster. The overall PRRSV transmission potential, represented by the reproductive ratio R_0 was lower for the vaccinated animals, although there was substantial overlap in the credibility intervals for both groups. Model selection suggests that transmission parameters of vaccinated pigs with NSR were more

similar to those of unvaccinated animals. The presence of NSRs in a population, however, seemed to only marginally affect the transmission dynamics.

The results suggest that even when vaccination can't prevent infection, it can still have beneficial impacts on the transmission dynamics and contribute to reducing a herd's R_0 . However, biosecurity and other measures need to be considered to decrease contact rates and lower R_0 below 1.

Key words: Vaccination; Transmission; Bayesian; Porcine Reproductive and Respiratory Syndrome; R_0

Introduction

For decades, vaccination has constituted one of the main tools for preventing and controlling infectious disease in livestock. The major aims of veterinary vaccines are to improve the health of animals and prevent or reduce pathogen transmission, thereby increasing production of livestock in a cost-effective manner [1]. However, the potential of a vaccine to control an infectious disease in livestock is controversial as many vaccines are leaky [2] and may not protect all vaccinated animals from disease which may compromise vaccine effectiveness in the field [3]. This is pertinent for Porcine Reproductive and Respiratory Syndrome (PRRS) which, despite wide-spread vaccination, remains one of the most costly diseases afflicting the global swine industry [4], directly in terms of the economic loss on affected farms and indirectly due to bacterial complications that require the use of antibiotics [5].

The annual losses due to PRRS have been estimated at \$2.5 Billion in the US and Europe alone [6]. The disease is characterized by reproduction issues, including late abortions, early farrowings and stillbirths, as well as respiratory disease, fever, and poor growth in pigs of all ages.

PRRS virus (PRRSV), the causative agent of PRRS, is a small, enveloped, positive-strand RNA virus in the *Arteriviridae* family, a family known for large genetic and antigenic variability within each species of virus [7]. The source of genetic variation is the virus' ability to rapidly mutate and create new variants [7]. As a result, the clinical pathology can vary substantially between PRRSV isolates [8]. Although PRRSV is not considered zoonotic [9], outbreaks in pigs are associated with increased susceptibility to secondary bacterial [10] and viral [11] infections. PRRSV was first isolated in the late 1980s and was divided into the genotypes PRRSV-1 (European origin) and PRRSV-2 (North American origin), based on genetic, antigenic and pathogenic differences [12]. PRRSV has a high mutation rate and, over time, the genetic diversity of the virus has increased [13,14]. Fast evolution and high genetic diversity severely compromise the ability of both natural and vaccine-induced immunity to provide full protection from infection and disease.

Although the first PRRS vaccine has been commercially available and widely used for over two decades, the prevalence of PRRSV infection in herds remains high as no fully effective vaccine (i.e. that totally prevents disease and virus spread) has been developed [15,16]. Failure of commercial vaccines to confer sterilizing immunity against many PRRSV field strains may promote mutation of PRRSV to adapt to new immune environments of the host animals [17,18].

All current PRRS vaccines are leaky [19]. There is also increasing awareness of substantial heterogeneity in vaccine response [20,21]. In a theoretical modelling study, Bitsouni et al. [3] demonstrated that even leaky vaccines can substantially reduce the risk of disease invasion and spread in a herd, if the vaccine reduces host infectivity or the duration of the infectious period. However, their model also predicts that the presence of vaccinated pigs with no serological response compromises effective vaccine coverage in a herd and can substantially increase the transmission potential of the infection (R_0).

As PRRSV continues to spread rapidly all over the world, with more virulent strains emerging [22,23], concerns regarding the evaluation of vaccine effectiveness in the field start to increase. Whilst vaccine trials routinely assess protective efficacy of vaccines and their effects on diverse immunological, virological and pathological parameters, less is known about how PRRS vaccines affect the transmission dynamics of PRRS within a herd [4,24].

The aim of this study was to evaluate the impact of attenuated PRRS-strain vaccination on heterologous strain transmission, including the effect of vaccinated pigs with no serological response (NSR), on transmission dynamics using a vaccination-contact animal experiment. This trial mimics the natural horizontal transmission in field conditions.

Methods

Animals and housing

Two successive transmission trials were performed (Fig. 1A&B) at the Faculty of Veterinary Medicine at Ghent University. Fifty-two 3 to 5-week-old conventional pigs (twenty-six for each independent experiment) were obtained from a PRRSV negative farm. No other relevant pathogens (SIV, PCV2) were detected in the animals. The pigs were randomly allocated into two groups (vaccinated, unvaccinated), based on body weight. All pigs were housed in separated stables in a biosafety level 2 (BSL2) facility and their health status was monitored closely on a daily basis.

The study was conducted in compliance with the provisions of KB 29/05/2013 (Belgian implementation of the European Directive 2010/63/EU). The study was evaluated by the local Ethical Committee of the Faculty of Veterinary Medicine and Bioscience Engineering and approved with number 2017/110 (Annex 7).

Vaccination and challenge viruses

The Modified Live Virus (MLV) Flanders08att was attenuated by serial passaging on MARC-145 as described previously [25]. The attenuated strain was thawed and diluted in PBS (pH 7.4) to a concentration of 10^5 TCID₅₀ per dose. Vaccination was done by intramuscular (IM) injection in the neck with a single 2 ml dose [26,27]. Challenge was performed with 10^5 TCID₅₀ of the PRRSV strain Flanders13, a highly virulent strain (see Supplementary Appendix A) with 84% sequence similarity with Flanders08att.

Inoculation experimental design

On 34 days post vaccination (dpv), three pigs (shedders) from each group were transferred to another unit and inoculated with PRRS virus (PRRSV strain Flanders13) intranasally, 1 ml of inoculum per nostril (Fig. 1A). In the vaccinated group the method of selection varied between experiments. In Trial 1, 4 out of 13 pigs (31%) of the

vaccination group had no serological response (NSR) on 28 dpv (Fig. 1B; Supplementary Appendix A). One NSR pig and 2 pigs with serological response were randomly selected for direct inoculation (shedders). This was done as it was thought to mimic the natural proportions of pigs with and without serological response. All vaccinated pigs had a serological response in Trial 2 so the selection of pigs was done at random in both groups.

The intranasally inoculated shedders were re-introduced (35 dpv / 0 days post contact (dpc)) to their original units comprising 10 PRRSV-negative pen mates (contact pigs). After reintroduction, sampling (blood and nasal secretions) was done every three days until 30 dpc and lastly on day 35 dpc. At 35 dpc, the pigs were humanely euthanized by intravenous injection of pentobarbital. During sampling, effort was made to reduce transmission between contact groups. Any piglet bleeding after sampling was isolated until the bleeding stopped. In between sampling of pigs, gloves were removed and replaced with new ones in order to prevent cross-contamination between pigs.

Sampling: Nasal secretions & blood

Sampling of nasal secretions was done using dry cotton swabs (one swab per nostril). Nasal swabs were placed into 1 ml of virus transport medium [26,27], vortexed, collected and stored at -70°C for subsequent virus determination. Sampling of nasal secretions was done every three days from 35 dpv (0 dpc) up to 27 dpc (Fig. 1B).

Blood samples (3 - 10 ml) were collected from the pigs by the *vena cava cranialis* puncture method as described previously [26]. After collection, the blood was centrifuged at 3000 rpm for 10 minutes at 4 °C, collected and stored at -70 °C for either virus or antibody determination. Blood samples for antibody titre measurements were

collected at arrival (-7 dpv), vaccination (0 dpv) and every 7 days up to 35 dpv (Fig. 1B; Supplementary Appendix A). Blood sampling for virus determination was done every three days until 30 dpc and lastly at 35 dpc (euthanasia) (Supplementary Appendix A).

Antibody and viral titre determination

Antibody titres for serum samples were determined using the immunoperoxidase monolayer assay (IPMA) (Supplementary Appendix A). Virus titres were determined by virus titration of the serum and nasal samples collected post contact (Supplementary Appendix A). The limit of detection (LOD) was 0.8 logTCID₅₀ per ml (serum) for viraemia and 2.5 logTCID₅₀ per g (nasal secretion) for nasal shedding.

Data management and Statistical analysis

PRRS viral titer (expressed as TCID₅₀ per ml (serum) or per g (nasal secretion)) was log-transformed for subsequent analysis. Pigs were classified as vaccinated, unvaccinated or vaccinated with no serological response (NSR) for the purposes of analysis. NSRs were identified by antibody profiles. As there were only 4 NSR pigs (Trial 1: 1 shedder pig and 3 contact pigs) no formal statistical comparison was performed using these individuals as the power was considered too low, however, they are included in all figures and tables for comparison with vaccinated and unvaccinated pigs. Differences between vaccinated and unvaccinated pigs were assessed by examining the viral shedding patterns and viral load. Analysis of viral shedding profiles was performed using a Generalised Linear Mixed Model (GLMM) (Proc Glimmix, SAS v 9.4). For a quantitative analysis of viral load, the Area under

the viral curve (AUC) for nasal and serum samples of all shedder and contact pigs was generated using the trapezoidal rule. Values below the limit of detection (LOD) were treated as LOD/2. Differences between trial and vaccination status were analysed using a General Linear Model (GLM) (Proc GLM, SAS version 9.4). All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC) with $p < 0.05$ as the level of significance.

Estimating the impact of vaccination on PRRSV transmission dynamics

To assess the impact of vaccination with Flanders08att on transmission of the heterologous PRRSV (Flanders13) strain, a compartmental epidemiological model was fit to data from each contact group in the transmission experiment. Individuals were considered to be in one of 5 states: susceptible to infection (S), exposed *i.e.* infected but not yet infectious (E), infectious (I), latent *i.e.* infected but no longer infectious (L), or “recovered” (R) (Fig. 2A). Transitions between states (as indicated by the arrows) were assumed to be Markovian (*i.e.* they occur with a certain probability per unit time, irrespective of the history of the individual). Note, R doesn't represent a permanently recovered state, as the model allows for transitions back to L, hence it can be thought of as a secondary latent state. The disease status of individuals at the beginning of each trial was assumed known, with shedder pigs in the E state and contact pigs in the S state.

The identification of states and transition routes in this epidemiological model was based on the experimental data (Fig. 3). For example, the necessity of including the exposed state E was determined by the serum and nasal swab viral measurements of the shedder pigs, which were LTLOD for the first few days post infection (Fig. 3).

The reason for the “L” state is because the data shows that serum viraemia levels can persist at a detectable level for significantly longer than the nasal swab measurements (Fig. 3). The reverse transitions in Fig. 2A are incorporated to account for the observed rebound [29,30] in virus above LOD of a number of pigs (n=20/52 38.5%) (Fig. 3).

Model fitting was carried out using a Bayesian approach which generated posterior samples for the model parameters $\theta = \{\beta_V, \lambda_V, \pi_V, \gamma_V, \kappa_V, \delta_V\}$ (where V takes the values “Vac” for vaccinated, “Unvac” for unvaccinated and “NSR” for no serological response) and unknown transition events ξ (where each event e represents a transition that an individual undergoes, e.g. $S \rightarrow E$, with corresponding event time t_e) (Fig. 2A). Note, the total collection of all events on all individuals ξ represents a realisation of the theoretically possible event sequences occurring in the trials. Although ξ is unknown it can be sampled from the posterior, so generating a collection of probable event sequences consistent with the data.

Data y used for model fitting comprised both the nasal swab and the viraemia test results of each individual (specifically, binary +ve/-ve diagnostic test results were generated with the cut-off being set by the detection limit of the tests). These binary measures were used to assign individuals into the appropriate epidemiological model compartments (S, E, I, L or R) at the observation times, as specified in Fig. 2B. Application of Bayes’ theorem to this data implies that the posterior is given by

$$\pi(\theta, \xi | y) \propto \pi(y | \xi) L(\xi | \theta) \pi(\theta), \quad (1)$$

where the observation model $\pi(y | \xi)$ takes the values one or zero depending on whether ξ is consistent with y or not, and the latent process likelihood is given by [31,32].

$$L(\xi | \theta) = \prod_{z=1}^Z \left[\prod_{e=1}^{E_z} r_e e^{-\Lambda_e \times (t_e - t_{e-1})} \right], \quad (2)$$

where z goes over all contact groups ($Z=4$) and e goes over events within each contact group (up to total of E_z events). The quantity r_e takes the value of the transition rate corresponding to event e (i.e. transition $E \rightarrow I$ of an unvaccinated individual would lead to $r_e = \lambda_{\text{unvac}}$) and Λ_e gives the sum of the transition rates for all possible transitions on all individuals in contact group z immediately prior to time t_e . The prior $\pi(\theta)$ in Eq.(1) consists of largely uninformative uniform distributions between 0 and 1 for each of the model parameters. Further details about the general approach used above are given in section 5.3 of [33].

Bayesian inference was performed using Monte Carlo Markov Chain (MCMC) with a large number of iterations to ensure accurate estimates were generated (with effective sample size exceeding 8000 for each parameter after an initial 20% burn-in period) from four randomly initialised chains (used to confirm global convergence of parameters). Details of this procedure along with MCMC diagnostics are given in Supplementary Appendix B.

Because of relatively few NSRs in this study (only 4) it was not possible to accurately estimate transmission parameters for this particular class of individuals. Therefore, the following parameterisation was implemented:

$$\beta_{\text{NR}} = a\beta_{\text{Unvac}} + (1-a)\beta_{\text{Vac}}, \quad (3)$$

with corresponding expressions for each of the other parameters in θ . Here a is a new model selection parameter (with flat prior between 0 and 1) used to choose between two hypotheses: when $a=0$ NSRs behave like vaccinated individuals, and

when $\alpha=1$ they behave like unvaccinated individuals. Thus, α can be used to perform model selection between these two hypotheses. Inference was performed assuming a flat prior for α between 0 and 1.

R_0 estimates were calculated for the vaccinated and unvaccinated groups using the following formula

$$R_{0,V} = \frac{\beta_V(N-1)}{\pi_V}, \quad (4)$$

where $N=13$ is the number of individuals in each contact group and the subscript V is either “Vac” or “Unvac” (note, this expression ignores the potential rebound of individuals from L to I , which are later shown to be relatively infrequent, and so actually represents a lower bound for the true R_0). Samples for R_0 derived from posterior samples for θ were used to generate the plots (here NSRs were assumed to behave the same as unvaccinated individuals, *i.e.* $\alpha=1$).

Results

Antibody response

All vaccinated pigs ($n=26$) over the 2 trials developed antibodies except 4 pigs in Trial 1 (1 shedder pig and 3 contact pigs), which were consequently denoted as having ‘No Serological Response’ (NSR). There were no NSRs in Trial 2.
(Supplementary Appendix A Fig. A1)

Viral titres

PRRS virus GTLOD was detected in all contact pigs except for the nasal samples of one piglet, although the piglet had one serum sample GTLOD, suggesting it was infected (Fig. 3). The proportion of sampling points with virus GTLOD was highest for the serum samples of contact pigs in the unvaccinated contact group. By 9 dpc all contact pigs in the unvaccinated contact group were infected, i.e. had virus GTLOD (85% by 6 dpc) whereas most contact pigs in the vaccinated contact group were not infected until 21 dpc (only 18% by 9 dpc). The pattern was similar for the onset of nasal shedding although the proportion of pigs GTLOD was lower for contact pigs in both the vaccinated and unvaccinated contact groups when compared to viraemia.

Infection profiles.

The proportion of animals GTLOD over the course of the study is summarised for nasal shedding (Fig. 4A) and viraemia (Fig. 4B) with 95% confidence intervals obtained from GLMM. Contact pigs in the unvaccinated group were infected (Fig. 4B) earlier and shed (Fig. 4A) virus earlier and longer than contact pigs in the vaccinated group. The infection profile of NSRs was more similar to contact pigs in the unvaccinated contact group but this could not be tested statistically as a result of the low number of NSRs. Statistically significant differences in the proportion of contact pigs GTLOD between those in the vaccinated and unvaccinated contact group was observed for both nasal shedding (Fig. 4A) and viraemia (Fig. 4B).

Virus load.

AUC for shedder and contact pigs in the vaccinated, unvaccinated and NSR (Trial 1 only) group for nasal shedding and viraemia is shown in Fig. 5. Although NSRs were

not included in the statistical analysis, the AUC for NSRs were generally more similar to the contact pigs in the unvaccinated group. The AUC of contact pigs in the unvaccinated group was significantly higher than contact pigs in the vaccinated group (nasal shedding, $p < 0.001$; viraemia, $p < 0.001$) (Supplementary Table A2). There was a significant difference in the AUC between Trials (Trial 2 > Trial 1, $p = 0.0013$) for nasal shedding, but not viraemia (Supplementary Table A2).

Impact of vaccination and vaccine responsiveness on transmission dynamics

The mode of the posterior distribution for the parameter a in Eq. (3) is close to $a = 1$ (Fig. 6), which strongly suggests that NSRs closely resemble unvaccinated individuals in their contributions to the PRRSV transmission dynamics. In fact, 97% of posterior samples are closer to $a = 1$ than $a = 0$ and the Bayes factor between the models corresponding to $a = 1$ and $a = 0$ (calculated by the ratio of the posterior probability at either value of a) exceeds 100, implying decisive evidence in support of the first model [34]. Hence NSRs were considered as unvaccinated pigs in the subsequent model parameter estimations.

Fig. 7 shows the posterior probability distributions for the various model parameters from the compartmental model in Fig. 2A (means and 95% credibility intervals for the model parameters are shown in Table 1). Due to the large overlap in the posterior distributions for the transmission parameter β associated with vaccinated and unvaccinated individuals (Fig. 7A), it was not possible to establish whether vaccination with Flanders08att reduced PRRSV transmission or not. However, pigs in the vaccinated contact group were slower to become infectious once exposed (Fig. 7B; parameter λ), had a shorter infectious period (Fig. 7C; parameter π) and

recovered faster (Fig. 7D; parameter γ), as shown by the fact that the posterior distributions are substantially separated (*i.e.* there is little overlap in credible intervals). Some unvaccinated pigs rebound from the L to I state but the data is consistent with no such rebound for vaccinated pigs (Fig. 7E; parameter κ). In fact, there is a Bayes factor of 22 between models without and with this transition for vaccinated pigs, providing strong evidence that $L \rightarrow I$ transitions do not happen under vaccination. On the other hand, vaccination did not prevent “recovered” R pigs reverting to the L state (Fig. 7F; parameter δ). Both model parameters (κ and δ) occurred at a relatively low rate for both types of individual. Supplementary Table 3 summarises posterior distributions for the numbers of different types of transition. These reflect the parameter values in Table 1 (in particular, forward transitions $E \rightarrow I \rightarrow L \rightarrow R$ are significantly more common than reverse transitions $R \rightarrow L \rightarrow I$).

A large part of the parameter uncertainty observed in Fig. 7 comes from confounding, which manifests as posterior correlations between different parameters (see Supplementary Appendix B). For example, confounding between the transmission rates β_{Vac} and β_{Unvac} and incubation rates λ_{Vac} and λ_{Unvac} , arises because of uncertainty as to whether individuals become infected at a fast rate and incubate at a slow rate or vice versa. As seen later, this adversely affects the precision with which R_0 estimates can be made. Weaker correlations between π/κ and γ/δ were also observed corresponding to uncertainty in the number of $L \leftrightarrow I$ and $R \leftrightarrow L$ transition pairs between observed time points.

Fig. 8 shows posterior estimates for the time trends of the number of individuals in each model compartment for the unvaccinated and vaccinated contact groups in both trials. The trials are presented separately as this may show the effect of the presence of NSRs on the population; NSRs were only present in the vaccinated

contact group in Trial 1 (Fig. 8A). The infection process was slower in the vaccinated contact groups (Fig. 8 A&C) as opposed to the unvaccinated contact groups (Fig. 8 B&D). The unvaccinated contact group was infected earlier, with almost all of the population infected by day 5. Recovery was faster in the vaccinated contact group where 50% of the population had recovered by approximately 18 days as opposed to approximately 25 days in the unvaccinated contact group (Fig. 8). Despite the small number of animals used, the dynamic patterns for each compartment seen in trial 1 (Fig. 8 A&B) are accurately reproduced in trial 2 (Fig. 8 C&D) suggesting that the effect of vaccination is systematic rather than coming from stochastic variation across trials. In particular, the presence of NSRs does not drastically affect the transmission dynamics, although the rate of recovery in the vaccinated contact group in Trial 1 was approximately 3 days longer than in Trial 2 (Fig. 8 A&C).

R_0 was calculated for the vaccinated and unvaccinated contact groups, assuming NSRs as unvaccinated (Fig. 9). As expected, given that all the pigs in both the unvaccinated and vaccinated contact groups became infected, our estimate of R_0 is large and excludes the threshold value of $R_0 = 1$ for both groups. The mode or the most likely estimate of R_0 for the vaccinated contact group was approximately 5.0, one half of that observed for the unvaccinated contact group (mode $R_0=10$), although there was considerable overlap in the posterior distributions of both groups (95% credible intervals: vaccinated contact group, 2.43-39.7; unvaccinated contact group, 5.93-32.3). The overlap may be partly due to the presence of NSRs in one of the trials.

Discussion

Vaccination is an important weapon in the fight against infectious disease, both in human and livestock populations. Veterinary vaccines are used in livestock and poultry to improve the animal health, prevent or reduce pathogen transmission, thereby increasing production of livestock in a cost-effective manner [1]. More efficient animal production and better access to high-quality protein are essential to feed the growing population. Furthermore, with the increasing global threats of antimicrobial resistance in both animals and humans, vaccines are very important tools to reduce antimicrobial use and thereby slow down the emergence and spread of antimicrobial resistance. However, it is well established that the majority of veterinary vaccines only reduce, not prevent infection and pathogen shedding [35]. This raises concerns about the effectiveness of a vaccine to control or eradicate disease in the field.

It is estimated that veterinary vaccines are available for over 400 diseases affecting mammals, birds and fish including farm animals, pets and wildlife [36]. Given the enormous scale and implications of vaccine use in both health and economics, it is clearly important that their effectiveness be thoroughly evaluated [37]. Most vaccine trials focus on the evaluation of vaccine efficacy or safety but the impact of vaccination on the spread of infection in livestock populations is less understood. Only few studies have evaluated virus transmission using contact models (PRRSV [5,38,39]; Marek's Disease [40]; FMD [41]; Avian flu [42]; Swine flu [43]).

Examining vaccine effectiveness is particularly important for PRRS due to their failure to reduce PRRS prevalence and their risk to promote viral evolution to higher virulence. The protocol developed in this study was a contact model designed to mimic a natural situation involving the introduction of a highly pathogenic heterologous strain (only 84% sequence similarity) into a population.

Despite the small sample size in this study, there were clear differences in the infection profiles and viral load of the pigs in the vaccinated contact group. Although vaccination did not prevent infection, it significantly reduced the viral load and shortened the duration of viraemia and nasal shedding. Nasal shedding is a key parameter responsible for transmission. Pigs can be infected by either direct or indirect contact through respiratory routes and cause primary infection in the nasal mucosa.

An epidemiological model embedded into a Bayesian inference framework was used to estimate the effects of vaccination on underlying transmission parameters from the experimental data. One of the advantages of Bayesian methods is that they can explicitly handle uncertainties surrounding assumptions, data and parameters, making them thus ideal for analysing small datasets. Despite a high degree of uncertainty in some parameters (e.g. the transmission rates β and subsequent R_0), the model results reveal significant beneficial effects of vaccination in some key parameters affecting PRRSV transmission dynamics within a population, such as the onset and duration of the infectious period. Similar positive effects of vaccination have been observed in other studies [5,45]. Unlike other studies, however, the credible intervals on R_0 estimated in this study were large for both vaccinated (2.43-39.7) and unvaccinated (5.93-32.3) contact pigs with considerable overlap. Vaccination and PRRSV transmission has been reviewed [4] including estimates of R_0 for similar PRRSV transmission studies. Rose et al. [5] estimated R_0 for unvaccinated pigs as 5.42 (CI_{95%} 2.94–9.04) and vaccinated pigs as 0.30 (CI_{95%} 0.05–0.96). Pileri et al. [45] estimated R_0 for unvaccinated pigs as 2.78 (CI_{95%} 2.13–3.43) and vaccinated pigs as 0.53 (CI_{95%} 0.19–0.76). Differences in R_0 can be due to many factors including: the genetic difference between the vaccination

strain and challenge strain (7.3% (ORF5)/ 4.9% (ORF7) [5] versus 18.7% (ORF5)/ 12% (ORF7), this study); behavioural differences between challenge strains; and the environmental circumstances within a trial (e.g. space per pig, ventilation, social behaviour) may have an impact on the transmission. A larger trial involving more contact groups would likely help to reduce the large credibility intervals observed for other key parameters, such as the transmission rate β and the transmission potential R_0 , and thus to obtain more conclusive estimates for the impact of vaccination on these pigs.

In contrast to previous vaccination transmission experiments, this study identified pigs with no serological response (NSR) in one of the trials comprising 31% (4/13) of the vaccinated animals. Heterogeneity in vaccine serological response with PRRSV has been reported in previous studies [20,21] although it is likely underreported as the NSRs are often removed before any analyses are carried out. In one study [21] NSRs represented 12% of all vaccinated pigs, however, group-level prevalence of NSRs varied from 0% to 40%. Based on viral load and infection profile the NSRs in this study were more similar to unvaccinated contact pigs. This was confirmed by the epidemiological model using objective model selection methods. Although the conclusion was that the NSRs were more similar to the unvaccinated contact pigs there was a degree of uncertainty surrounding this result. This uncertainty may reflect a direct effect of vaccination, i.e. that vaccination did offer some level of protection despite the lack of measurable antibody titre due to cell-mediated immunity. Alternatively, it could reflect indirect benefits provided by the fact that NSRs are in the same contact-group as vaccinated pigs, which may confer some protection as a result of lower viral load shedding due to vaccination. Such beneficial indirect effects of vaccination on non-vaccinated contact individuals have been

reported in other species [38]. Unfortunately, in this study we cannot distinguish between the two plausible explanations.

In a purely theoretical study Bitsouni et al [3] demonstrate that even vaccines with no or low levels of sterilizing immunity, or less than 100% effective coverage, when appropriately applied can prevent, eliminate or largely reduce the prevalence of PRRSV infections, as long as the vaccine sufficiently speeds up recovery and reduces pathogen shedding. The results of this study largely confirm these model predictions. In particular, the vaccinal strain used in this study was shown to reduce nasal viral load and thus likely also host infectivity, as well as the duration of the infectious period with likely subsequent effects on R_0 . However, the results of our study also suggest that incomplete effective vaccine coverage may have less impact on the transmission dynamics than predicted by theory, as viral shedding and thus potentially infectivity of non-vaccinated individuals may be reduced if their infectious contacts are vaccinated [40]. Such indirect effects of vaccination are currently not incorporated in typical epidemiological prediction models.

One of the main reasons for applying vaccines in livestock is to minimize production loss. In particular, in Europe killed PRRSV vaccines are administered to sows to prevent reproduction losses caused by PRRSV infection [19]. This study did not consider the impact of vaccination on production traits as the objective was to examine the impact of vaccination on transmission. In addition, previous research using the same PRRSV challenge strain as in this study has shown it to be highly virulent (Supplementary Appendix A). Hence, one would expect that the observed vaccine-induced reductions in viral load would also result in reduced production loss.

Similarly, whilst this study provides new important insights into the impact of vaccines on viral shedding and the transmission dynamics, their impact on virus evolution still needs to be examined to get a more complete understanding of how vaccines alter the pathogen and disease landscape. Such investigations are currently in progress.

Conclusion

In the coming decades, new human and animal diseases will continue to emerge. As a result, veterinary vaccines will continue to be an important tool to protect human health, animal health, food safety and food security [46]. This study used a vaccinal strain, which like most PRRSV vaccines, did not prevent pigs from getting infected with a heterologous strain and conferred heterogeneous response to vaccination. However, the vaccinated contact groups had lower viral load, shorter infectious period and faster recovery in comparison to the unvaccinated contact groups, thus reducing the overall transmission potential R_0 , although probably not enough to control or eradicate PRRSV in the field. Biosecurity and other measures (for example closed herds, genetic selection for PRRS resistance) need to be considered to decrease contact rates and lower R_0 below 1. Future evaluation of veterinary vaccines would benefit from including transmission experiments coupled with epidemiological models to more accurately predict vaccine effectiveness and possibly also vaccine safety in the case of MLVs with high recombination rates [36] in the field.

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Author contribution

The SAPHIR Study investigators include HN, ADW, SG, BB, JX, IT, CB, KR, VB and MCT. HN and ADW conceived the study design and analyses. SG and BB contributed to the study design and interpretation of data. The transmission trials were performed by JX, IT, CB and KR. CP performed the Bayesian modelling. MCT statistically analysed the data and prepared the manuscript for publication with JX. Statistical advice provided by HB. RB and VB helped with data interpretation and graphics. All authors reviewed the manuscript for intellectual content and approved the final version of the manuscript.

Competing interests

SG is employed by the Virbac pharmaceutical group. The authors HN, JX, IT, CB and KR performed studies on the efficacy of the vaccinal strain with the Virbac pharmaceutical group. The authors did not receive any salary or receive any personal economic compensation for those studies. The remaining authors have no conflict of interest in this paper.

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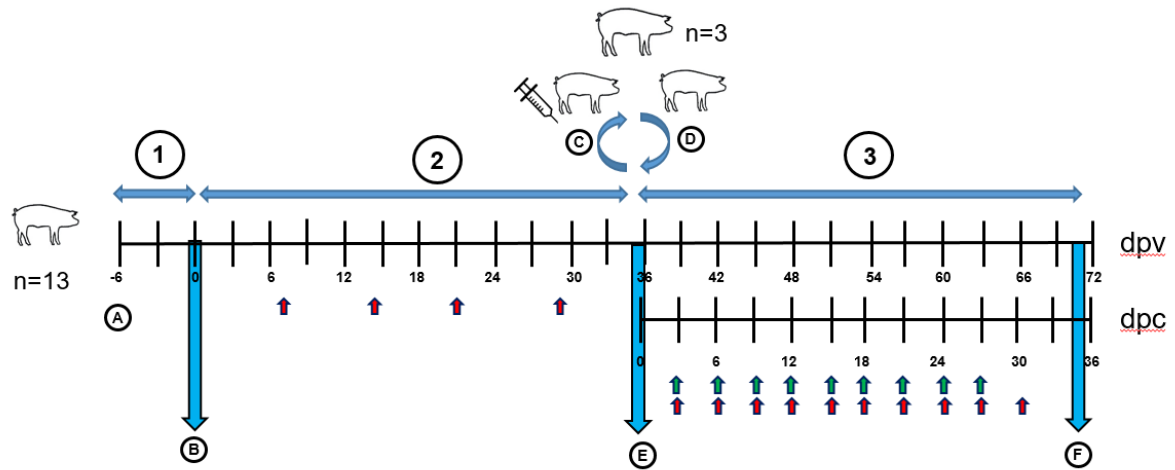


Fig. 1. Transmission experiment.

A. Timeline of the experiment. This represents 1 replicate of the experimental design comprising 13 pigs (3 shedder pigs and 10 contact pigs). Numbers represent periods of time: 1 Pre-vaccination; 2 post vaccination; and 3 post contact. Letters represent events: A arrival; B vaccination; C removal of shedders (n=3) for inoculation with virus; D infected shedder pigs placed back in contact with pen mates (contact pigs, n=10); E beginning of post-contact sampling period; F termination of the experiment. Arrows show when blood (red) and nasal swabs (green) samples were taken, respectively. dpv, days post vaccination. dpc, days post contact. Note that the diagram represents the complete number of samples. For certain replicates/trials not all samples were collected (see text).

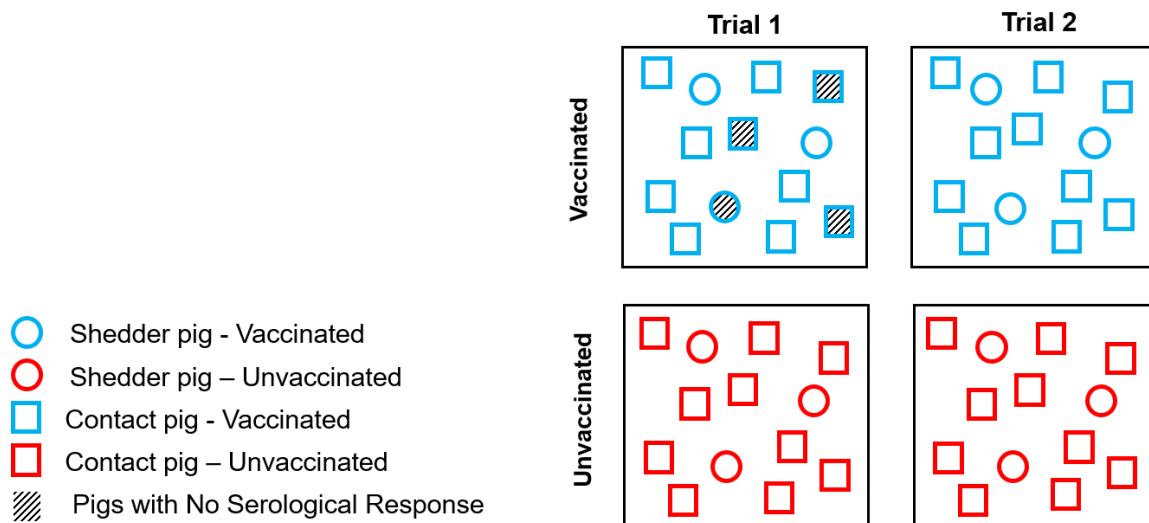


Fig. 1. Transmission experiment.

B. Experimental design. Two consecutive replicates of a transmission experiment (Trial 1 and Trial 2) were performed involving altogether 52 pigs of similar age and weight. Each trial comprised two contact groups, one consisting of vaccinated (Flanders08att strain) pigs only, and the other one consisting of unvaccinated pigs. In each contact group, 3 pigs were designated as shedders, and inoculated with PRRSV strain Flanders13 according to the schedule in Fig. 1A, before put back into contact with their corresponding pen mates (n=10). The experimental design was the same across the trials with the exception of the presence of pigs that had no serological response (NSR) after vaccination Trial 1 (striped pattern).

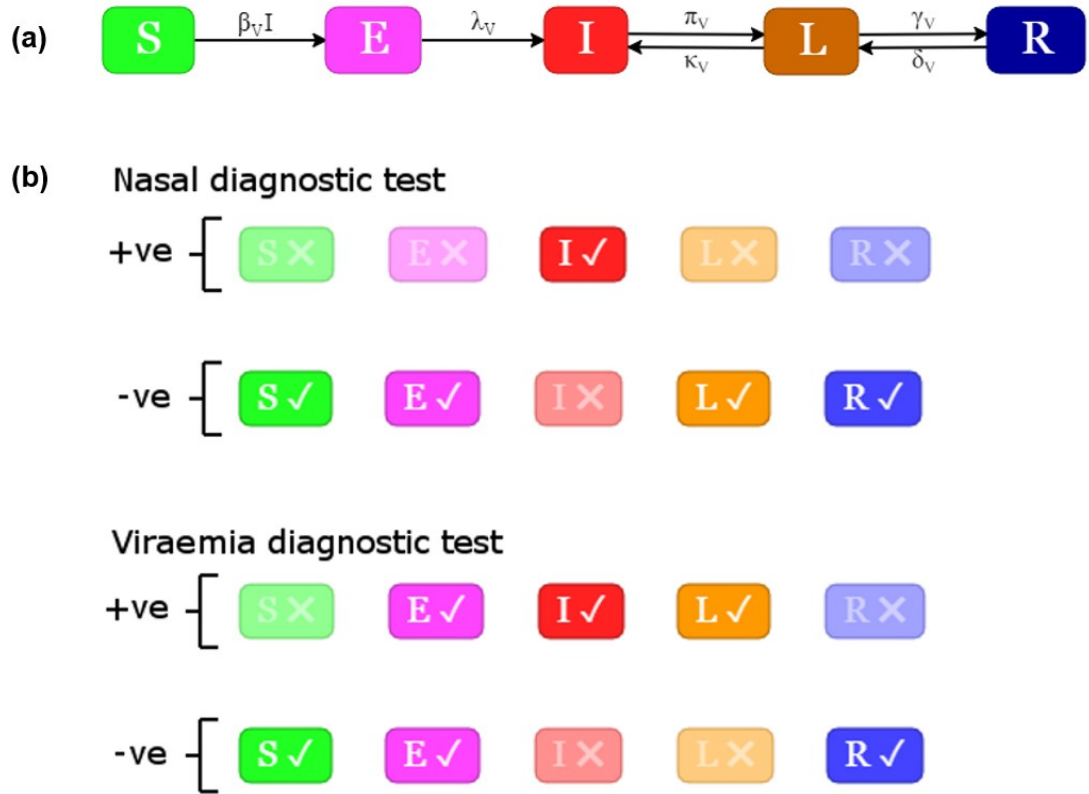


Fig. 2.

A. The compartmental epidemiological model. Individuals in the corresponding compartments are susceptible (not yet infected) S, exposed (infected but not yet infectious) E, infectious I, latent (infected but not infectious) L, and recovered R (which here means not detectable by the two qPCR tests), respectively. The parameters β_V , λ_V , π_V , γ_V , κ_V and δ_V are transition rate parameters, where V denotes the fact that these parameters depend on the vaccination status of individuals. Note, β_V is multiplied by "I", which is the time-dependent total number of infected individuals in the same contact group.

B. The observation model. This model identifies possible infection states (S, E, I, L, or R) in the compartmental epidemiological model at each sampling time based on the individual's corresponding binary diagnostic test results (negative / positive) for (a) nasal test (here a positive result is consistent with an individual in the I classification) and (b) a viremia test (here a positive result is consistent with E, I and L). Considering a possible realisation for the transition events ξ , the observation model takes the value $\pi(y|\xi)=1$ if putative compartmental state (as determined by ξ) can be assigned to each individual at the time when each of the diagnostic test measurements are made, otherwise $\pi(y|\xi)=0$. The observations model, therefore, restrict MCMC to only those states ξ consistent with the data y .

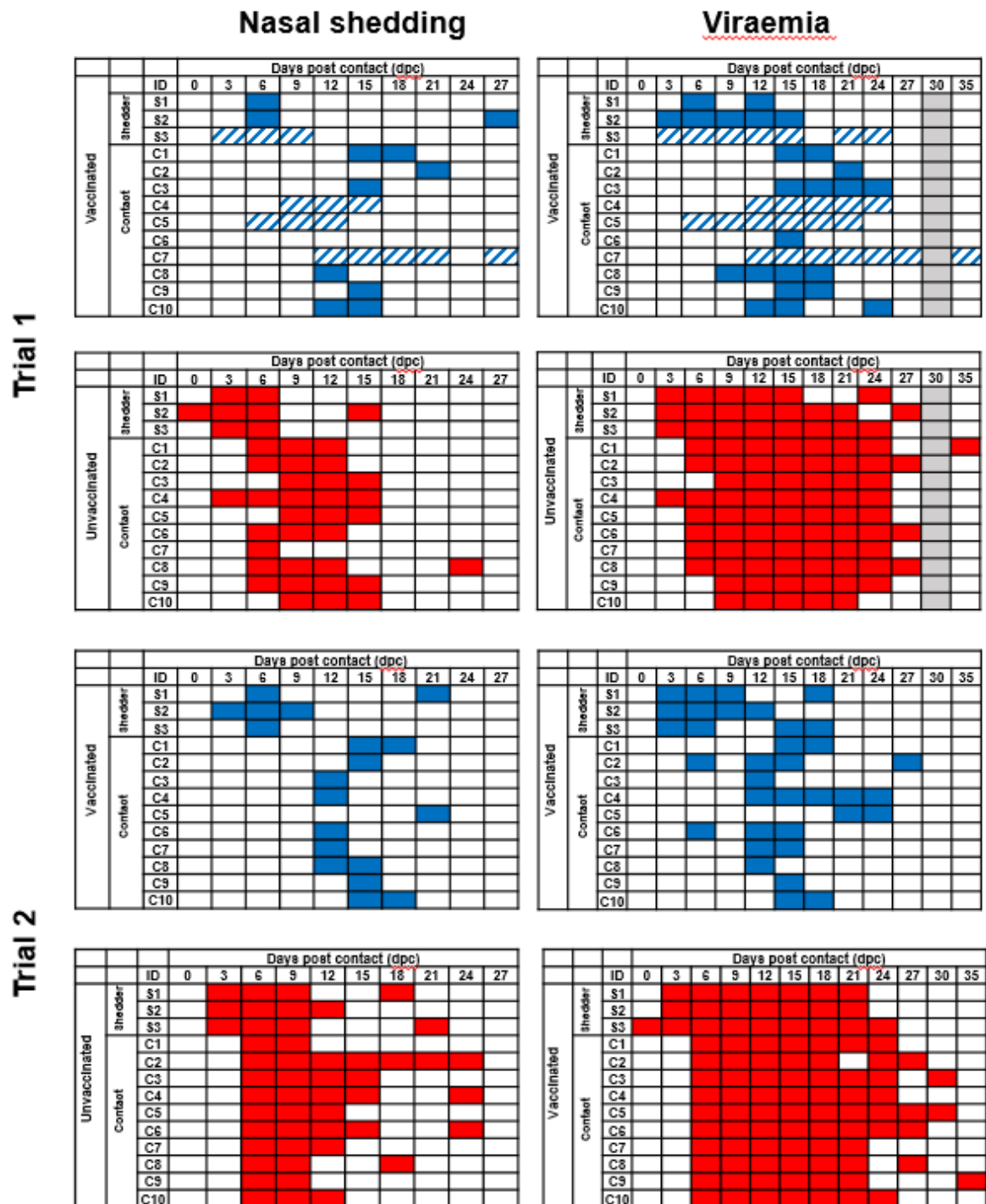


Fig. 3. Individual infection diagnostics. Schematic diagram summarising the individual pig results from Trial 1 and Trial 2. The colour of the boxes for each sampling time (dpc, days post contact) represents whether the virus titre level was above or below the limit of detection (LOD). White, viral titre < LOD; Blue box, vaccinated pig > LOD; red box, unvaccinated pig > LOD; striped box, no serological response to vaccination (NSR) > LOD; Grey box, no sample taken at this time point. LOD was 2.5 Log TICD₅₀.g⁻¹ and 0.8 Log TICD₅₀.ml⁻¹ for nasal secretions and serum respectively.

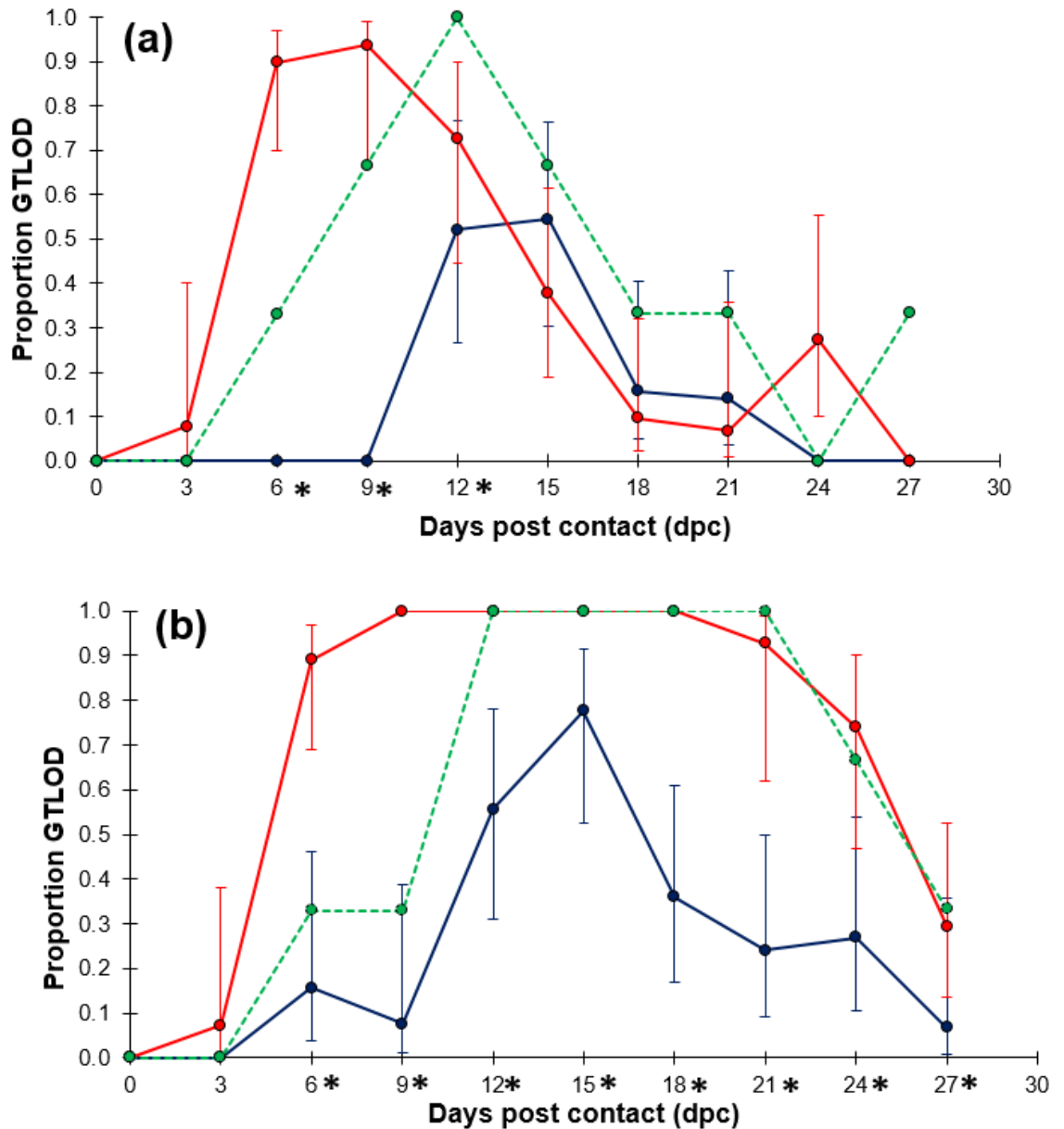


Fig. 4. Virus titre profiles for Nasal shedding (A) and Viraemia (B). Least square means (95%CI) for contact pigs in the vaccinated contact group (blue, solid line) and unvaccinated contact group (red, solid line) over the two consecutive trials. Pigs with no serological response (NSR) (green, dashed line) are included in the graphical output but due to low sample size confidence intervals were not available from the GLMM are shown but the estimates are not based on a statistical model. Asterisks indicate statistical significant differences (Fisher's exact test, $p < 0.05$) between contact pigs in the vaccinated and unvaccinated contact groups on a given day post contact (dpc) Least square means were generated from the binary GLMM (see methods; Supplementary Appendix A).

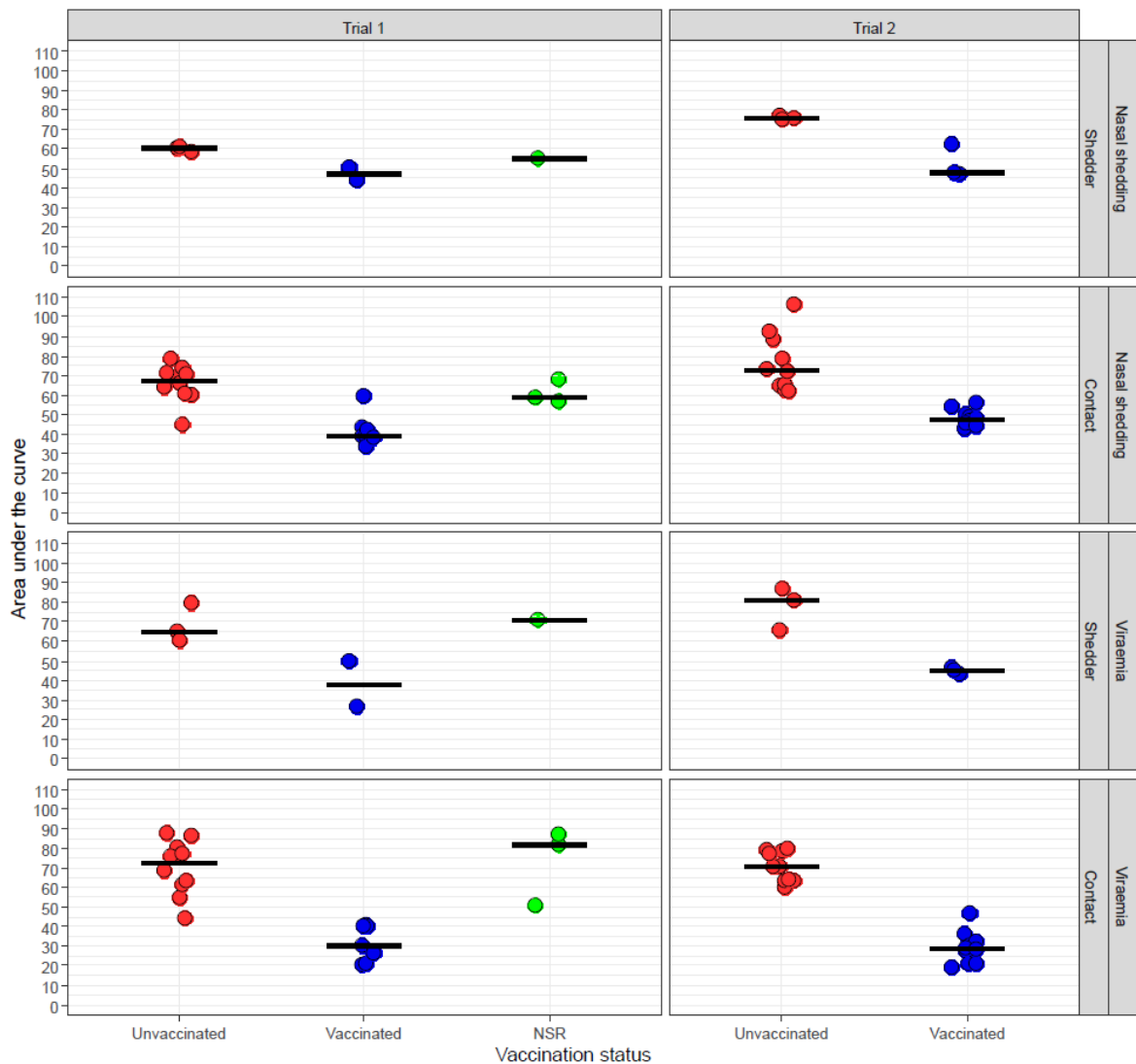


Fig. 5. Viral load. Individual data plot Area Under the Curve (AUC) for contact and shedder pigs in the vaccinated (blue) and unvaccinated (red) groups from nasal shedding (Concentration - Log TCID₅₀.g⁻¹) and viraemia (Concentration - Log TCID₅₀.ml⁻¹) samples collected throughout the study (n=52 pigs). Pigs that had no serological response (NSR) to the vaccination (green) were only present in Trial 1. Black line, representing the median AUC is shown for all groups except the NSR shedders where n=1.

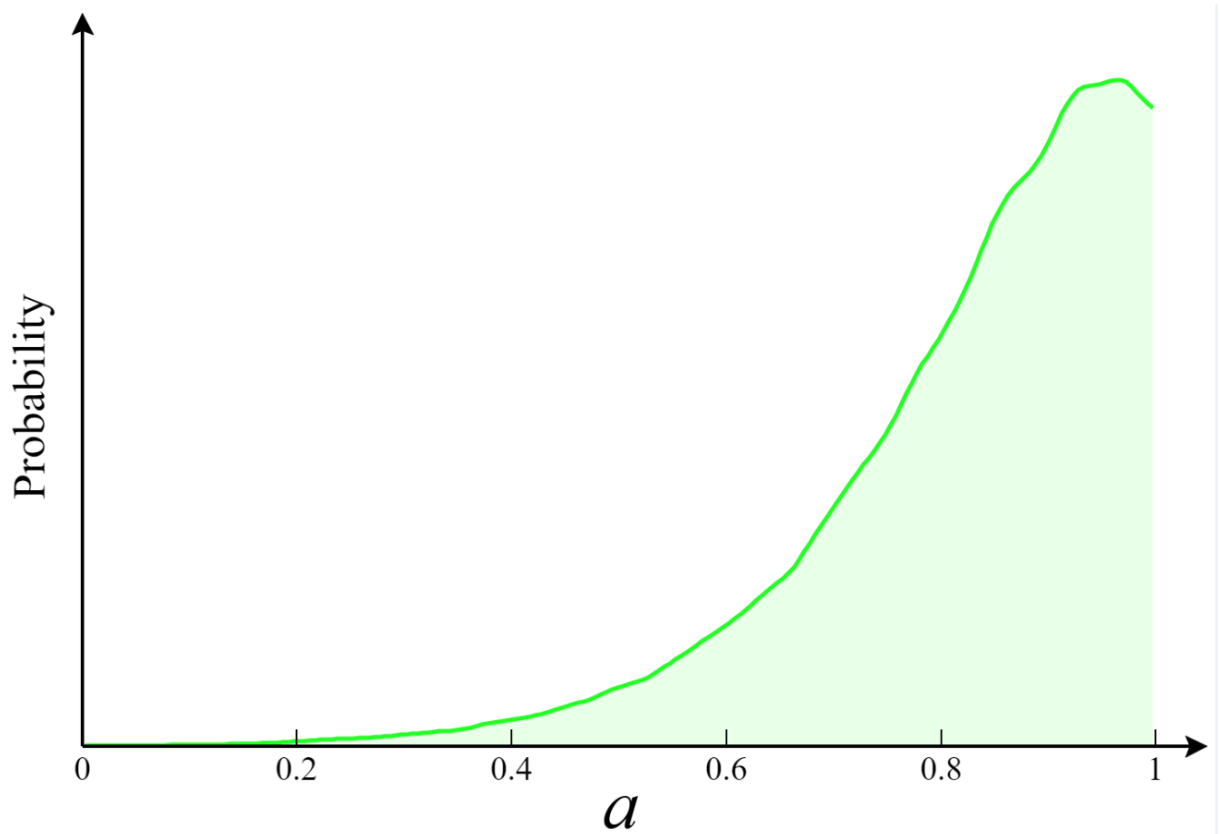


Fig. 6. Probability distribution for pigs with no serological response (NSR after vaccination). Model selection parameter “ a ” (0 implies NSRs behave like vaccinated individuals and 1 implies NSRs behave like unvaccinated individuals).

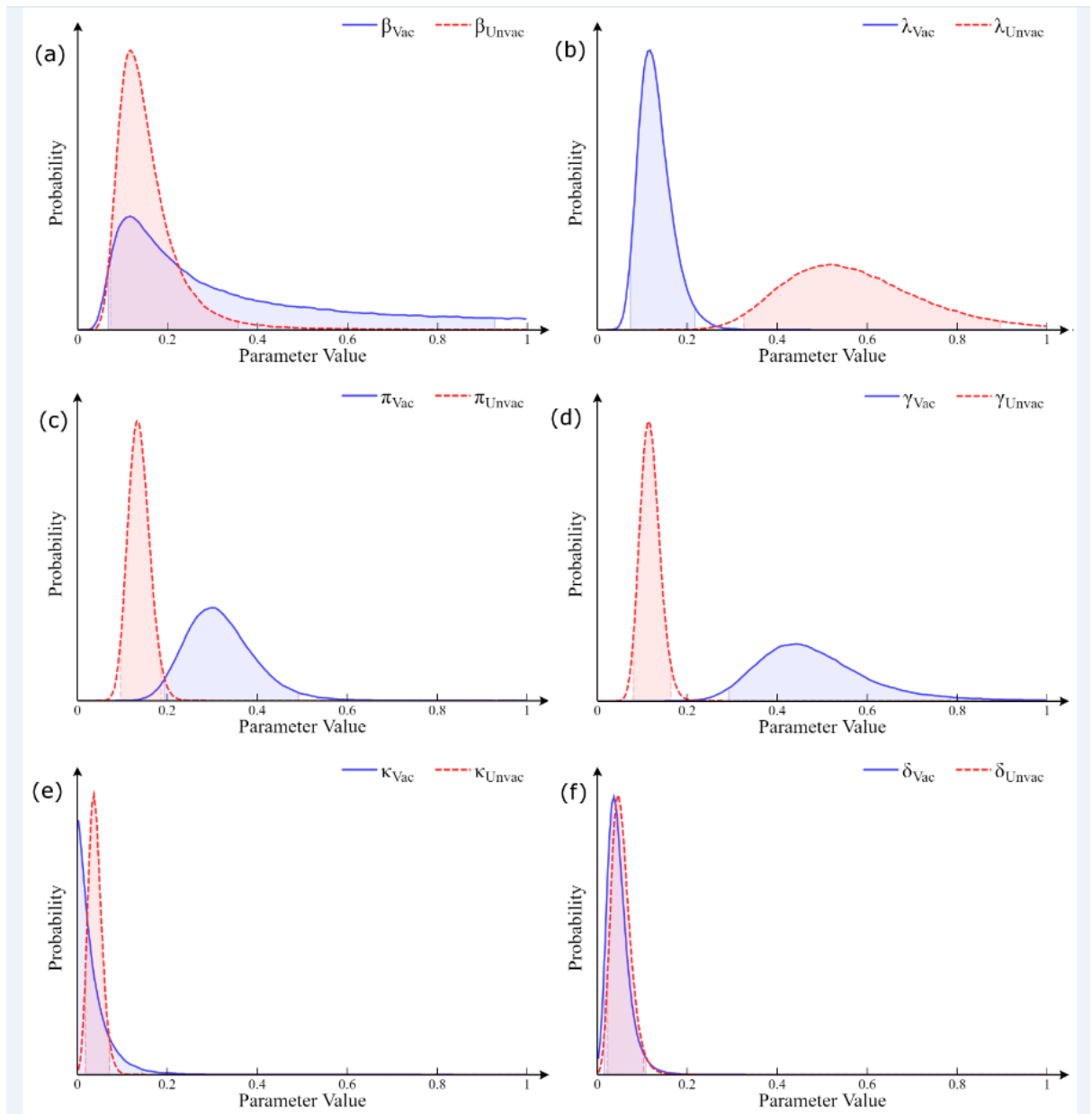


Fig. 7. Posterior distributions for the parameter estimates of the epidemiological model. Posterior distributions stratified by group vaccination status for (a) the transmission rate β , (b) the incubation rate λ , *i.e.* transition rate from exposed to infectious state (c) transition rate from infectious to latent state π , (d) the recovery rate γ , (e) the transition rate from recovered to latent state κ and (f) the transition rate from recovered to latent state. The blue and red curves represent the results for vaccinated (Vac) and unvaccinated (Unvac) individuals, respectively. Pigs that had no serological response (NSR) to the vaccination are assumed to behave the same as unvaccinated.

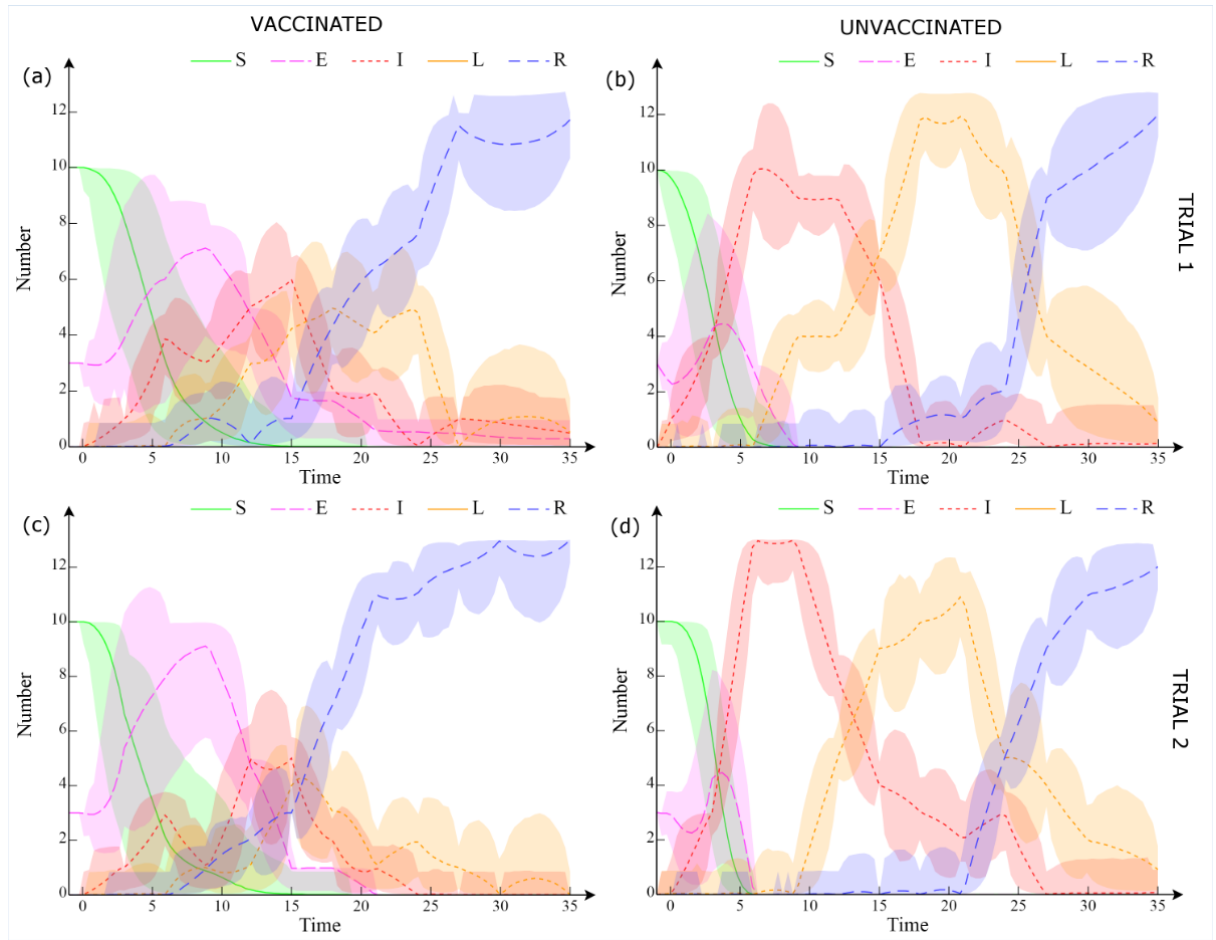


Fig. 8. Populations estimates. Posterior estimates for the time trends for the number of contact pigs in each infection state (compartment of the epidemiological model). Solid lines show the mean and shaded areas represent the corresponding 95% credible intervals) for (a) vaccinated contact group in Trial 1 (with pigs that had no serological response (NSR) to the vaccination considered as unvaccinated), (b) unvaccinated contact group in Trial 1, (c) vaccinated contact group in Trial 2, and (d) unvaccinated contact group in Trial 2.

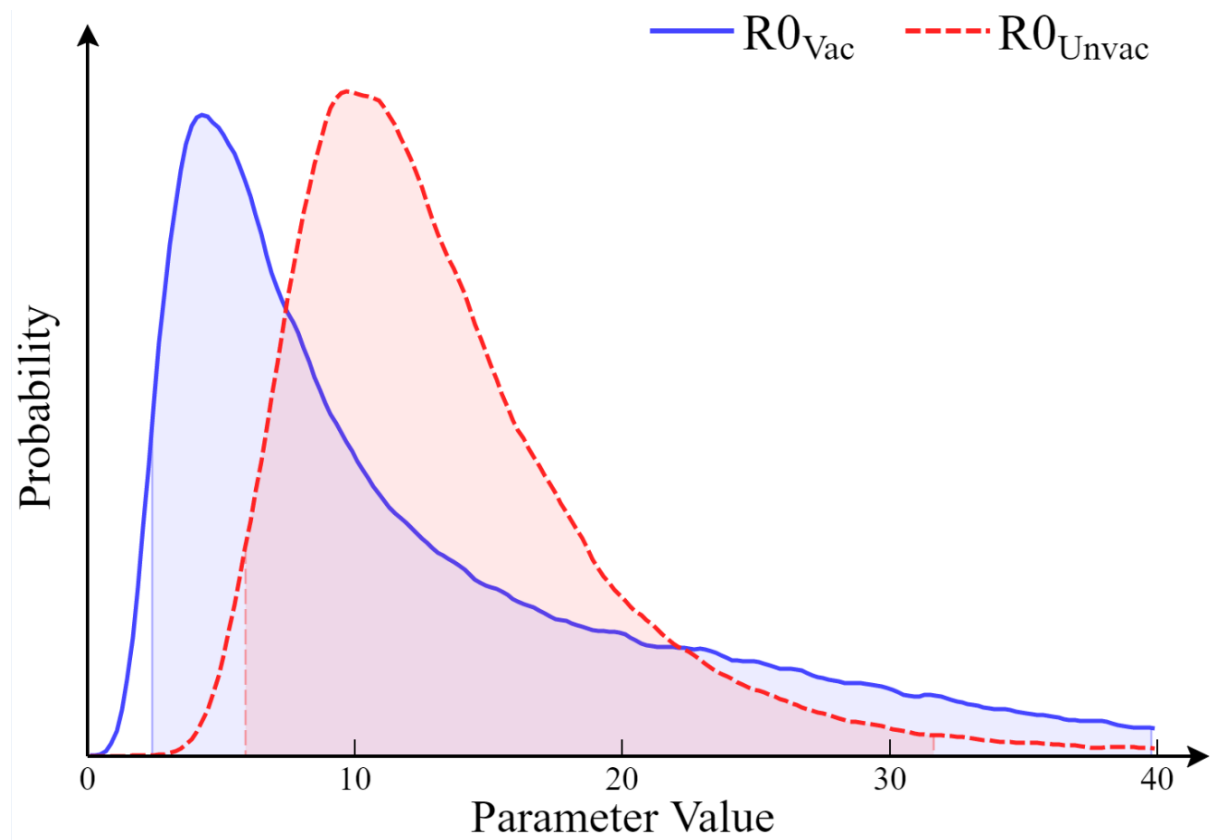


Fig. 9. Reproductive ratio R_0 . The posterior density plots of R_0 for the vaccinated contact group (blue) and the unvaccinated contact group (red). The shaded area is the 95% credible interval.