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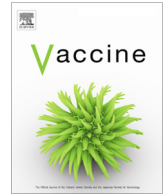
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# Evaluation of early single dose vaccination on swine influenza A virus transmission in piglets: From experimental data to mechanistic modelling

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## ABSTRACT

Swine influenza A virus (swIAV) is a major pathogen affecting pigs with a huge economic impact and potentially zoonotic. Epidemiological studies in endemically infected farms permitted to identify critical factors favoring on-farm persistence, among which maternally-derived antibodies (MDAs). Vaccination is commonly practiced in breeding herds and might be used for immunization of growing pigs at weaning. Although interference between MDAs and vaccination was reported in young piglets, its impact on swIAV transmission was not yet quantified. To this aim, this study reports on a transmission experiment in piglets with or without MDAs, vaccinated with a single dose injection at four weeks of age, and challenged 17 days post-vaccination. To transpose small-scale experiments to real-life situation, estimated parameters were used in a simulation tool to assess their influence at the herd level.

Based on a thorough follow-up of the infection chain during the experiment, the transmission of the swIAV challenge strain was highly dependent on the MDA status of the pigs when vaccinated.

MDA-positive vaccinated animals showed a direct transmission rate 3.6-fold higher than the one obtained in vaccinated animals without MDAs, estimated to 1.2. Vaccination nevertheless reduced significantly the contribution of airborne transmission when compared with previous estimates obtained in unvaccinated animals.

The integration of parameter estimates in a large-scale simulation model, representing a typical farrow-to-finish pig herd, evidenced an extended persistence of viral spread when vaccination of sows and single dose vaccination of piglets was hypothesized. When extinction was quasi-systematic at year 5 post-introduction in the absence of sow vaccination but with single dose early vaccination of piglets, the extinction probability fell down to 33% when batch-to-batch vaccination was implemented both in breeding herd and weaned piglets.

These results shed light on a potential adverse effect of single dose vaccination in MDA-positive piglets, which might lead to longer persistence of the swIAV at the herd level.

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## 1. Introduction

Swine influenza virus (swIAV) is a major pathogen affecting pigs and is recognized as a pivotal viral cofactor in the porcine respiratory disease complex (PRDC) [1–3]. Although responsible for mild symptomatic expressions with relatively short febrile period when pigs are infected solely with swIAV [4,5], some co-infections may dramatically exacerbate clinical expressions with

acute respiratory outbreaks in growing pigs and potential reproductive disorders in sows [6,7]. The economic losses due to swIAV were evaluated from US\$3.23 to US\$10.31 per pig produced in weaning-to-finish pig herds in the USA [8]. Several epidemiological studies revealed a specific feature of viral dynamics in pig farms consisting in recursive outbreaks on farms, hitting quasi-systematically all successive batches [9,10]. This phenomenon, known as recurrent or endemic influenza infections, along with the observed co-circulation of different viral subtypes on farms, represents a serious threat to the swine industry regarding long-term disturbances and the possible emergence of reassortant viruses [11–13]. As swine influenza viruses are potentially zoonotic, such ende-

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mic infections and new emerging reassortants may have potential public health consequences. Understanding the factors favoring the persistence and spread of the virus within herds is therefore essential to design adequate and feasible mitigation measures.

In that view, epidemiological studies allowed identifying risk factors associated to endemic situations in the field [14–16]. Environmental conditions, such as the temperature in the different sectors, airflows between compartments or the number of neighboring herds, were highlighted as potential factors favoring the persistence of the virus at the herd level [17,18]. Husbandry practices such as cross-fostering, mingling piglets of different batches, the number of pigs per pen or the flow of animals between sectors also played a significant role in the spreading pattern at the population level [9,14,15]. In addition to these farm-level characteristics, host-level factors were pointed out as affecting the transmission process, one of the most being undoubtedly the maternally-derived antibodies (MDA) [19,20]. Those antibodies, passively acquired by piglets through the colostrum during the early lactation after birth, were for a long time deemed to preserve the newborn from clinical disease after infection. Experimental transmission trials were carried out to estimate the impact of MDAs on swIAV transmission [21,22]. Parameter estimates were then used to feed a mechanistic model of transmission in farrow-to-finish pig farm [23]. Results strongly suggested that MDAs play a dual role at the individual and batch levels with potential involvement in the persistence of swIAV on farms by extending the infectious process at the population level in the nursery compartment.

Another puzzling issue for influenza infection in pigs relies on the effect - or the lack of effect - of vaccination in field conditions. Here again, experiments were carried out to estimate the transmission parameters in vaccinated animals, showing that decreasing the reproduction number below the critical threshold 1 would be difficult except in case of vaccination with fully homologous strains [24]. Moreover, vaccination of sows, commonly practiced in the field [25], may yield to counterproductive results through the induction of MDA in their progeny, favoring on-herd persistence of the virus. Vaccination strategies in influenza modelling frameworks confirmed the difficulty in reducing the persistence of the virus in the population on farms by immunization [26–28]. In several countries, vaccination based on partial vaccination protocols (1 injection without any booster) in young growing pigs has been experimented in field conditions empirically to try to circumvent the permanent circulation of swIAV in nursery piglets [5,29]. Results are highly variable between farms and the impact of this early single-dose vaccination on swIAV transmission among piglets with or without MDA has not been quantified to date. Based on these first observations, reflections towards the interplay between MDAs and vaccination with respect to swIAV transmission and persistence on farms were initiated.

This study aimed at evaluating the impact of swIAV single dose vaccination on virus transmission in weaned piglets, depending on their MDA statuses at the time of vaccination. An experimental transmission trial was first set up to estimate the transmission parameters for swIAV. The inferred parameters were then used to feed a simulation model allowing to transpose the experimental setting to farm level situation.

## 2. Materials and methods

### 2.1. Experimental design

#### 2.1.1. Animals, vaccination and infectious challenge designs

The experiment trial was approved by the French national committee for ethics in animal experimentation ANSES/ENVA/UEPC

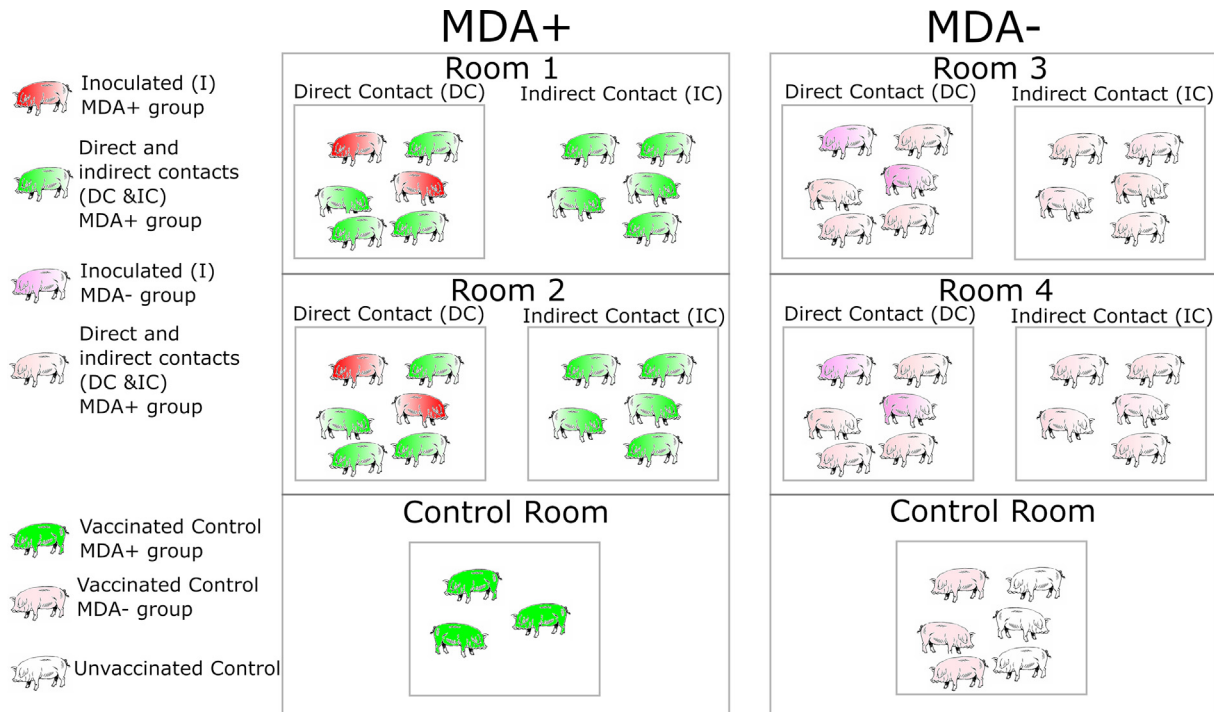
(approval n°13/12/18–1) and authorized by the French Ministry of Research (APAFIS n°2018110716271875 (#17449)).

Six specific pathogen free (SPF) sows from the air-filtrated pig herd of ANSES, Ploufragan, France, and fifty-three piglets born to these sows were involved in the experiment. One week before insemination (about seven months prior to the starting date of trial), three sows were isolated and intra-muscularly vaccinated twice with two mL RespiPorc®Flu3 (CEVA Santé Animale, Libourne, France) at three-week interval. RespiPorc®Flu3 is an adjuvanted inactivated vaccine including three antigens: A/Bakum/IDT1769/2003 (H3N2), A/Haselünne/IDT2617/2003 (H1<sub>av</sub>N1, H1 clade 1C.2.2) and A/Bakum/1832/2000 (H1<sub>hu</sub>N2, H1 clade 1B.1.2.1). A third boost injection was performed 14 days before parturition to ensure high levels of maternal antibodies being transferred to the 25 piglets offspring, who were further included in the MDA-positive group (MDA+, left panel of Fig. 1). Blood samples were taken from sows for serological analyses. Samples obtained prior to vaccination confirmed their SPF status, with no seroconversion towards swIAV, whereas those repeated during the vaccination scheme, i.e. before the second vaccination, during gestation and just before parturition, confirmed anti-swIAV antibodies were produced following vaccination (data not shown). The other three sows were kept unvaccinated and gave birth to a total of 28 piglets forming the MDA-negative group (MDA–, right panel of Fig. 1). At three weeks of age, just after weaning, three MDA+ and six MDA– animals were kept as controls and isolated in separate rooms. One week later, all MDA+ and MDA– piglets except three MDA– control pigs were intra-muscularly vaccinated with a single dose injection of RespiPorc®Flu3 vaccine (CEVA Santé Animale, Libourne, France; 2 mL per pig). For each group, vaccinated piglets were randomly assigned into two independent rooms (rooms 1 and 2 for MDA+ groups, and rooms 3 and 4 for MDA– groups; Fig. 1). Each room contained two pens: six direct contact (DC) pigs were housed in pen 1 and five indirect contact (IC) animals in pen 2. On day 17 post-vaccination, based on early serological analyses performed at one week of age, the two pigs with the highest MDA levels were selected from the pens containing six animals, and gathered in a specific room to be each inoculated intra-tracheally with 10<sup>6</sup> TCID<sub>50</sub> (50% tissue culture infectious dose) of Eurasian avian-like swIAV strain A/Sw/Cotes d'Armor/0388/09 (H1<sub>av</sub>N1, H1 clade 1C.2.1) in a volume of five mL. Thereafter, the day of inoculation will be referred as D0, corresponding to the beginning of the challenge phase of the protocol, whereas the day of vaccination will be denoted as D-17. At D1, inoculated animals came back to their original pen to start the transmission of the inoculated strain by direct contact with their four penmates, and by indirect contact with the five pigs in the neighbouring pen. To assess the transmission dynamics, a thorough follow up of the animals was carried out from D1 to the end of the trial at D28, i.e., four weeks post-challenge. Piglets were euthanized after anesthesia (Zoletil®, Virbac, Carros, France, 10 mg/kg), followed by bleeding and then necropsied. Post-mortem examination of lungs was carried out and tissue samples were collected where pneumonia gross lesions were observed, and stored at –80 °C for further analysis [29].

#### 2.1.2. Clinical observations and sampling scheme

Clinical observations were performed daily to assess the occurrence of symptomatic expressions post-inoculation. Individual rectal temperatures and weights were recorded daily and weekly, respectively, throughout the trial.

Blood samples were taken from piglets to monitor their serological profiles. Piglets were first sampled at one week of age to ensure the presence of antibodies in MDA+ group (D-41), then weekly from the day of vaccination (D-17) until the end of the trial (7



**Fig. 1.** Description of the experimental design. MDA+ animals were born to sows vaccinated with RespiPorc<sup>®</sup>Flu3. All but three MDA– control pigs were vaccinated on day 17 prior to challenge (D-17). Inoculations were performed on day 0 (D0), with a follow up of the transmission chains to direct and indirect contacts (DC, IC).

blood samples taken in each pig at D-17, D-10, D-3, D7, D14, D21 and D28).

Virus shedding from inoculated and contact piglets was assessed through individual nasal swabs (MW951 sent, Virocult<sup>®</sup>, Corsham, UK). They were taken three days prior to inoculation (D-3), then, at a daily frequency during the two first weeks of contact (from D1 to D14) and every two days during the two following weeks (at D16, D18, D21, D23 and D25).

### 2.2. swIAV genome detection

RNA were isolated from nasal swab supernatants and from lung tissues samples using NucleoSpin 8 RNA kit or NucleoSpin RNA kit (Macherey Nagel, Hoerd, France), respectively, and submitted to a duplex M gene/ $\beta$ -actin gene RT-qPCR (Cador et al., 2016). Briefly, five  $\mu$ L of RNA were tested using the GoTaq<sup>®</sup> Probe 1-Step RT-qPCR System (Promega, Charbonnières-les-bains, France) with 0.4  $\mu$ M and 0.8  $\mu$ M of M gene forward and reverse primers, respectively, 0.6  $\mu$ M of  $\beta$ -actin gene primers and 0.25  $\mu$ M of TaqMan<sup>®</sup> probes, in a final volume of 25  $\mu$ L. Reverse transcription was performed for 30 min at 45 °C, and after an initial activation step of 2 min at 95 °C, PCRs were run for 40 cycles of 15 s at 95 °C and 1 min at 60 °C on a Mx3005P QPCR System (Agilent Technologies, Les Ulis, France). Virological results were analyzed based on the individual shedding characteristics (Ct values), evaluated through the computation of areas under the curves (AUC, flux R library). Comparisons between groups were made using a non parametric statistical test (Kruskal-Wallis test).

### 2.3. Serological analyses

Anti-swIAV antibodies were detected in sera using an indirect ELISA (PrioCHECK<sup>™</sup> Swine Influenza Ab Serum Plate Kit, ThermoFisher Scientific, Lelystad, The Netherlands) according to manufacturer's instructions. The mean relative index per cent (IRPC) was calculated for each group of piglets, i.e., inoculated, direct contact,

indirect contact, control, with or without MDA, at each sampling time. The mean levels of antibodies obtained for vaccinated and/or challenged and/or contact groups were compared to those of the unvaccinated MDA– control group.

### 2.4. Parameter estimation from the transmission experiment

The framework described by Cador et al. was used to assess the transmission dynamics according to the MDA status of the animals [21]. Briefly, the transmission chain was analysed using a SEIR (Susceptible – Exposed – Infectious – Removed) model. Bayesian inference was performed using Monte Carlo Markov Chains to estimate three parameters:

- two transmission rates were considered, corresponding to direct contact between individuals at the pen level, denoted  $\beta$ , and airborne transmission at the room level, denoted  $\beta_a$ ;
- one parameter representing the relative susceptibility factor for the MDA+ group compared to MDA– one, denoted  $\epsilon$ .

With this notation, the force of infection exerted on susceptible individuals was given by  $\lambda(t) = \epsilon(\beta_a \pi_r + \beta_p \pi_p)$ , with  $\epsilon = 1$  in MDA– groups, and  $\epsilon \geq 0$  estimated for MDA+ groups.  $\pi_p$  and  $\pi_r$  represented the prevalences of infectious pigs, based on data from swIAV genome detection, at pen and room levels, respectively.

Adapted from the work of Weesendorp et al. [30], the estimation was based on the maximization of the likelihood, assuming an interval for the latency duration  $[e_1; e_2]$  varying from 0.5 to 1.5 days. The likelihood was expressed as:

$$L = \prod_{k \in \text{contact-infected}} e^{-\int_0^{e_1,k} \sigma_k \lambda_k(t) dt} * \left( 1 - e^{-\int_{e_1,k}^{e_2,k} \sigma_k \lambda_k(t) dt} \right),$$

where the first term corresponded to the probability for individual  $k$  to escape from infection up to time  $e_1,k$ , given the force of infection  $\lambda_k(t)$  exerted by infectious individuals sharing the same room. Intu-

itively, the second term corresponded to the probability of being infected on time interval  $[e_{1,k}, e_{2,k}]$ . Optimization was performed using Nelder-Mead algorithm (function mle2 from the R package bbmle) and confidence intervals were obtained using likelihood profiles (confint function).

2.5. Modelling framework

The dynamic model representing swIAV transmission dynamics within a farrow-to-finish pig farm was previously described in two companion papers [23,26]. Briefly, a typical farrow-to-finish pig farm using a 7-batch-rearing system was represented. The herd was subdivided into gestation, farrowing and fattening sectors; the breeding herd evolved in the gestation and farrowing sector, while growing pigs in farrowing and fattening sectors. An all-in-all-out management was considered with fixed periods in each sector. Sow renewal through introduction of gilts was stochastic: at the end of each cycle a proportion of sows are removed from the system and replaced by susceptible gilts before the start of the next cycle (insemination). The number of culled sows is defined to reach a total replacement rate of 38.5% per year [31]. Swine Influenza A infection being generally not lethal, mortality was not included in the model and litter size was fixed to 12 for all sows, corresponding to the average number of weaned pigs per litter.

The population dynamics was coupled with an epidemiological MSIRS (Maternally Immune-Susceptible-Infectious-Recovered-Susceptible) model. Piglets born to immune sows were considered in a M state due to passive transfer of MDA during early lactation. They were in R state after implementation of post-infectious immunity. Durations of passive and active immunity, i.e., M and R states, respectively, were modeled using Erlang distributions [32,33]. In absence of vaccination, the model consisted in 24 possible transitions between 16 health states: seven for passive and active immunity waning ( $M_1$  to  $M_7$  and  $S$ ), seven representing the possible infection of MDA+ animals ( $M_i, i \in \llbracket 1, 7 \rrbracket$  to  $I$ ), one for infection of naïve animals, and one for recovery ( $I$  to  $R$ ). We refer to Cador et al. for a full description of the model implementation and assumptions [23,26]. The model was event-driven, imple-

mented using Gillespie direct algorithm. Individual transitions between epidemiological states were represented based on stochastic occurrence time, computed using transition rates described in the table in supplementary material.

The present study being dedicated to the evaluation of the impact of vaccination in MDA+ animals, a specific class was added to represent vaccinated animals. Three vaccination schemes were considered: (i) vaccination of piglet at weaning age (28 days) only, (ii) mass vaccination of breeding sows every three months combined with vaccination of piglets at weaning age and (iii) batch-to-batch vaccination of sows combined with vaccination of piglets at weaning age. Vaccinated animals enter the V stage, corresponding to vaccine-induced immunity, which duration was modeled using Erlang distribution, as other immune states. Although successful in reducing clinical expression, especially in breeding sows, vaccine-induced immunity might only provide partial protection towards infection. Therefore a transition from V to  $I_v$  was considered with a lower transmission rate susceptibility. As transmission process in vaccinated pigs could depend on their MDA status at the time of vaccination, the force of infection by direct contacts in nursery rooms was defined as

$$\lambda_r(t) = \pi_r^+ \varepsilon \beta_v + (1 - \pi_r^+) \beta_v,$$

where  $\pi_r^+$  represented the proportion of infected pigs that were vaccinated in the presence of MDA.

The simulation procedure was similar as the one exposed in Cador et al. [23]. Briefly, the herd was managed according to a 7-batch-rearing system, with 24 sows per batch and 3-week intervals between batches [23,26]. The process was initialized with the sequential introduction the breeding batches every 21 days, filling the different sectors of the herd during the first cycle. One infectious gilt was introduced during the first replacement process of the second year of simulation. The transmission process was observed up to four years post-introduction. Hundred simulations were performed to analyze the on-farm persistence of the virus for the different vaccination schemes.

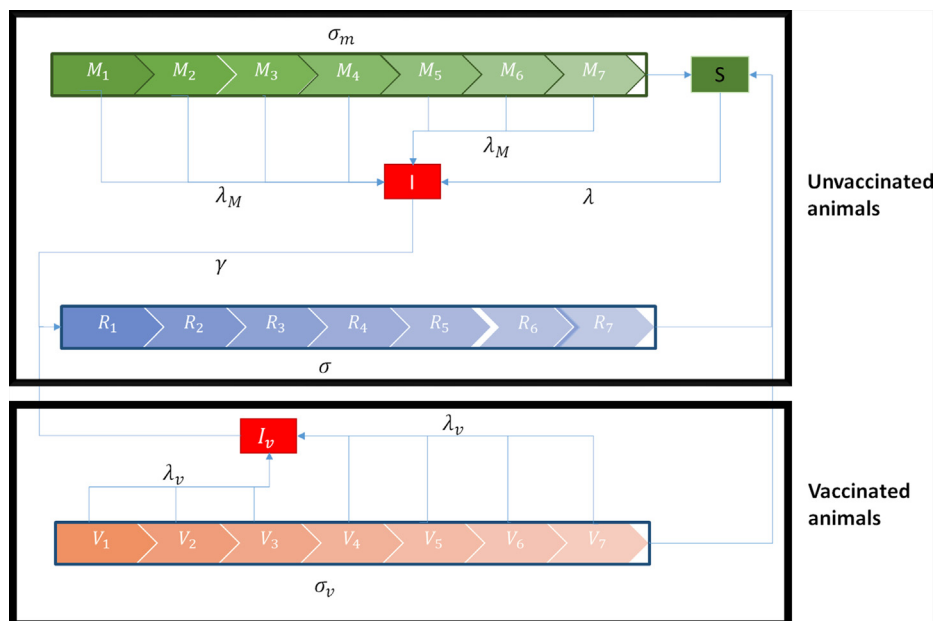


Fig. 2. Description of the epidemiological model. The model, adapted from [21], was implemented using Gillespie algorithm with transitions described in supplementary materials.

### 3. Results

#### 3.1. Clinical follow up and post-mortem examination

Five MDA– individuals, among which 2/4 inoculated and 3/10 indirect contact animals showed sporadic hyperthermia on seven daily records, either at D1 or D2 for the formers, or from D4 for the others. By contrast, 3/4 inoculated, 4/8 direct contact and 4/10 indirect contact pigs, i.e., 11 animals, exhibited 18 temperature records above 40 °C from D1 to D11 in the MDA+ group. No respiratory disorder was observed in the MDA– group, whereas sneezing and sporadic coughing were observed for nine consecutive days in MDA+ group from D3 to D11. SwIAV inoculation had no impact on the growth and food intake in contact pigs. In inoculated animals, a slight decrease in daily weight gain was observed following inoculation but it was rapidly compensated thereafter.

At post-mortem examination, 1/4 pig from the inoculated MDA+ group and 2/10 pigs from the indirect contact MDA+ group showed limited pneumonia lesions, with low scores of 2/28. No lung lesion was observed in MDA– piglets.

#### 3.2. Virus shedding and virus detection in lung samples

As revealed by M-gene RT-qPCR on nasal swab supernatants, the four inoculated pigs from the MDA– group started to excrete viral particles from one to four days post-inoculation (Fig. 3). For one of them, virus shedding was observed for one day only, but others shed the virus during four to five consecutive days. Except one out of eight, all pigs in direct contact group started shedding the virus three to seven days after having being placed into contact with inoculated pigs, i.e., from D4 to D8, and for three to seven days. A longer delay was observed before excretion by indirect contact pigs, with M-gene positive animals being detected six to 13 days after contact, i.e., from D7 to D14, and their viral excretion time ranged from two to six days.

Within the MDA+ group, the inoculated pigs started excreting from two to five days post-inoculation (Fig. 3). All direct contacts became infected and shed the virus from day three or four after contact, and for seven to nine days consecutively, thus more rapidly and for a longer period than direct contacts within MDA– group. The same observation was made for MDA+ indirect contacts, as they shed the virus from five to seven days post-contact, for six to nine days.

The swIAV genome was not detected in nasal swabs taken in any control groups (data not shown).

Moreover, the swIAV genome was not detected in the lung tissue samples collected from the three MDA+ pigs (one inoculated and two indirect contacts) where pulmonary gross lesions were observed at necropsy.

#### 3.3. Serological responses

Variations in levels of anti-swIAV antibody in sera of piglets were followed in the time-course of the experiment. In the MDA– group, all I, DC and IC piglets were seronegative before vaccination at D-17 (Fig. 4A). Two weeks after vaccination (D-3), IRPC values for those piglets were still not different from those from the non-vaccinated group (Ctrl\_NV). Inoculated pigs were detected seropositive from D7 with a mean IRPC of 37%, a level that was maintained until the end of the experiment (D28). In direct and indirect contacts, seroconversion was detected from D14 and D21, respectively. The greatest mean antibody levels (around 50% IRPC) measured in contact groups were higher than that measured in the inoculated group. In the vaccinated control group (Ctrl), no pigs seroconverted as IRPC values remained negative, as observed

for the unvaccinated control group (Ctrl\_NV) all along the experiment.

All piglets born to vaccinated sows (MDA+ group) were detected seropositive at one week of age (D-41), as all IRPC values were clearly above the mean one obtained for Ctrl\_NV, with mean IRPC values ranging from 90% to 50% (Fig. 4B). Anti-swIAV antibodies were still detected at the time of vaccination (D-17) in all of them, except a few animals included within the contacts, i.e., those with the lowest IRPC values at D-41 (data not shown). Thus, average IRPC values obtained at D-17 for inoculated and contact pigs were 69% and 40%, respectively, in line with initial batching as the animals to be inoculated were deliberately selected among those with the highest IRPC values at birth. After vaccination, MDA levels continued to decline in all groups. Moreover, no humoral immune response was induced post-infection in inoculated animals as the decrease in swIAV antibodies was still similar to that recorded in control pigs in the later phase of the experiment. In direct and indirect contact groups, a slight increase in IRPC values was observed at D14/D21, indicating that post-infection seroconversion occurred in some piglets with lower MDA levels.

#### 3.4. Parameter estimation

Transmission parameters estimates are provided in Table 1. In the absence of MDA, the direct transmission rate was estimated to 1.2 pigs per day [0.9–1.9]. The airborne transmission rate was estimated to 0.08 [0; 0.3] in vaccinated MDA– group. Finally, the transmission was found 3.6 [1.6; 8.5] fold higher in vaccinated MDA+ animals as compared to vaccinated MDA– piglets.

MDA– status of vaccinated animals was also found to affect the duration of the shedding period. Indeed, when individual nasal swabs from MDA– piglets were found swIAV M-gene positive for a median period of 5 days on average, this period was estimated up to 7 days in MDA+ group (Fig. 5A.). Moreover, the analysis of virological data evidenced that MDA+ piglets shed significantly higher quantity of virus during their shedding period as compared to MDA– piglets (Fig. 5B., Kruskal Wallis p-value < 0.001).

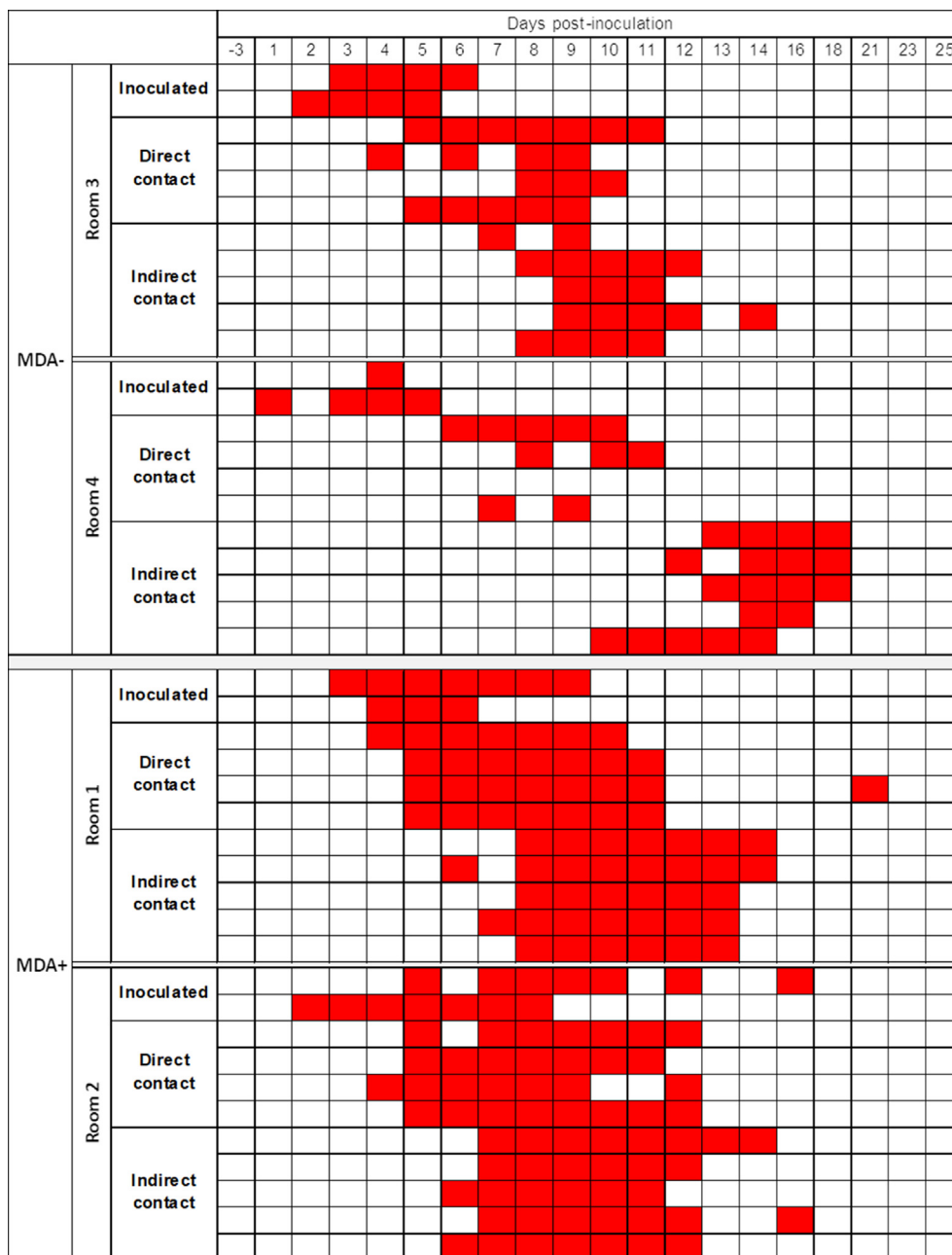
#### 3.5. Model outcomes

Feeding the simulation model with obtained estimates revealed that vaccination of MDA+ piglets would significantly extend the persistence of the virus in a farrow-to-finish pig farm. In the absence of MDA, i.e., absence of sow vaccination, but single dose vaccination of piglets, extinction was observed quasi-systematically after 4 years post-introduction (Fig. 5). Sow vaccination induced delivery of MDA to their piglets, which, combined with single dose vaccination of piglets at weaning age, resulted in high persistence probabilities varying between 33 and 67% after 5 years post-introduction, depending on the vaccination scheme applied to breeding animals, mass or batch-to-batch vaccination (Fig. 6).

### 4. Discussion

A few modelling studies focused on swIAV dynamics in growing pigs, with two main objectives, (i) estimating transmission parameters [21,34–36]; and (ii) analyzing the conditions for virus sustainability using simulation approaches [23,26,28,37]. Our study comes across these two objectives through a two-step procedure.

Formerly, experimental designs were developed to infer transmission parameters either in MDA+ [21,22] or in vaccinated animals [24]. Thus, results obtained in earlier studies highlighted a partial protection conferred by MDAs, reducing the transmission



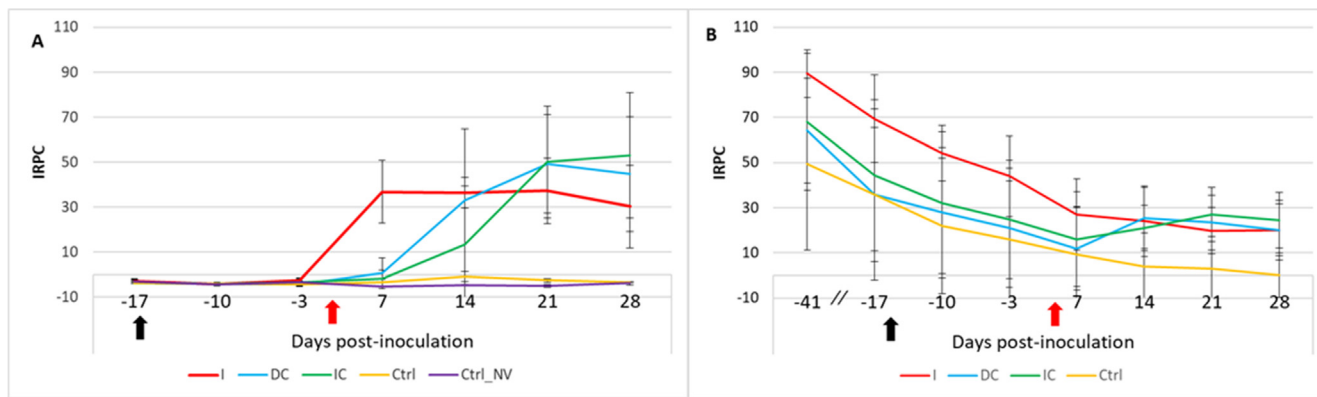
**Fig. 3.** swIAV shedding in piglets previously vaccinated in the absence (MDA-) or presence (MDA+) of maternally-derived antibodies. Piglets were either swIAV inoculated at D0, or placed in direct or indirect contact to inoculated animals at D1. One line represents a piglet; red boxes show the sampling dates when the swIAV genome was detected in nasal swab supernatant by M-gene RT-qPCR; white boxes indicate that the swIAV M-gene was not detected. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

by a factor 0.39 when compared to MDA- animals [21]. On another note, vaccination was also shown to significantly reduce the transmission, with reproduction numbers varying from 10 in unvaccinated animals to 1 in vaccinated pigs [24]. In this latter study, piglets were vaccinated with an inactivated vaccine, with two injections two weeks apart. However, despite this full vaccination scheme, recommended by vaccine manufacturer, the reproduction number did not fall below the critical threshold 1, showing that the spread remains active in the absence of total homology between the vaccine antigen and the challenge strain (transmission was abolished only when using an autogenous vaccine).

In any case, the interplay between MDA and vaccine efficacy on transmission remained unraveled. Thus, we first developed an orig-

inal experimental design to assess the transmission pattern of a wild swIAV strain within a population of pigs vaccinated with a single dose of an inactivated commercial vaccine at four weeks of age, in the presence or not of MDAs.

In MDA- piglets, a partial protection was obtained with only a single dose injection of the vaccine. The post-vaccination humoral responses obtained in MDA- pigs after this single injection were probably too low to be measured using the ELISA, however these pigs were likely primed as they very quickly exhibited high levels of antibodies in sera after challenge. A partial protection regarding clinical expressions was observed, with only mild and sporadic symptoms in a few individuals. In the absence of MDA, single-dose vaccination decreased the virus transmission by 50% when



**Fig. 4.** Variations in anti-swIAV antibody levels in sera of piglets from MDA– (without maternally-derived antibodies) (A) and MDA+ (with maternally-derived antibodies) (B) groups in the time course of the experiment. Vaccine was injected at D-17 (black arrow) to all animals except those of the MDA– [Ctrl\_NV] group; H1<sub>av</sub>N1 swIAV was inoculated to the inoculated (I) groups at day D0 (red arrow); direct (DC) and indirect (IC) contact pigs were placed into contact at D1. Anti-swIAV antibodies were detected by indirect ELISA; IRPC = Relative Index Per Cent. Results are given as mean IRPC ± standard deviations for the I, DC, IC and control [Ctrl] groups of vaccinated MDA– and MDA+ pigs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**

Parameter estimation depending on MDA– status in single-dose vaccinated piglets.  $\beta$  and  $\beta_{air}$  represent the direct and airborne transmission rates, respectively;  $\epsilon$  corresponds to the relative impact of MDA on the transmission of swIAV (worsening factor if  $\epsilon > 1$ ).

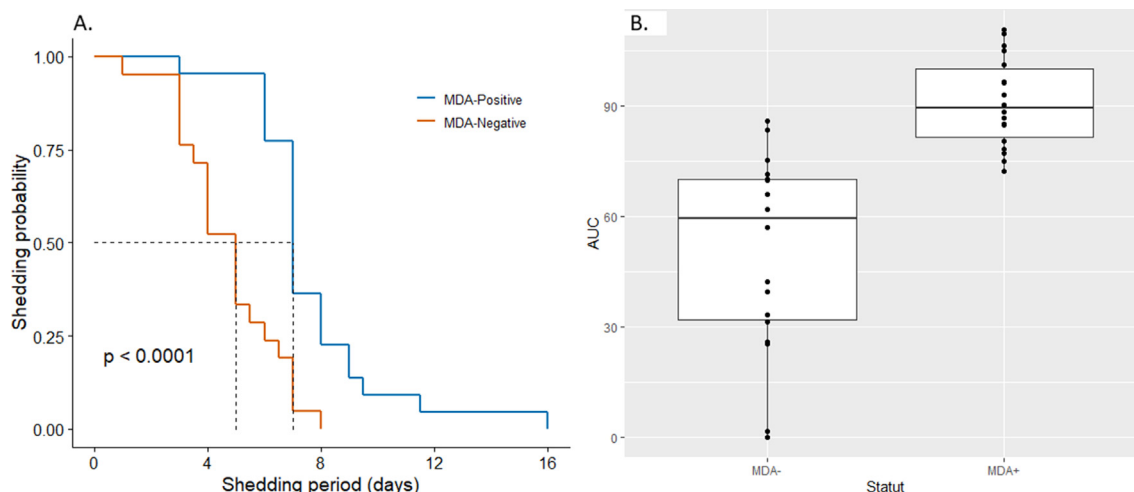
Group	Parameters		
	$\beta$	$\beta_{air}$	$\epsilon$
MDA–	1.2 [0.7; 1.9]	0.08 [0; 0.3]	–
MDA+			3.6 [1.6; 8.5]

compared to the results obtained with a similar experimental setting in unvaccinated pigs [21].

In contrast, single dose vaccination of MDA+ animals failed to confer a similar level of partial protection in terms of transmission. Clinically, the frequency of symptomatic animals was slightly higher but remained sparse. However, an impairment of vaccine-induced humoral immunity was clearly observed, as was the case after swIAV challenge in MDA+ piglets [19,38]. Some vaccinated MDA+ animals nevertheless developed humoral immunity after infection. The level of the response was inversely correlated with the level of MDA, the lower the maternal immunity, the higher the immune response post-challenge. In our experimental settings,

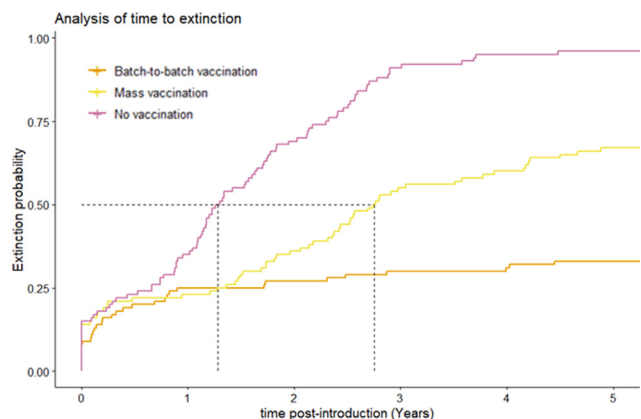
the transmission of the virus among vaccinated MDA+ pigs was estimated 3.6 times higher than in vaccinated MDA– group. Infected single-dose vaccinated MDA+ individuals also exhibited longer infectious period. Combining these results, one infected individual, vaccinated in the presence of MDA, would theoretically be able to infect about 30 individuals in a homogenously mixed population. However, the structure and management on farms also play a role on the infection dynamics as described in risk factor analyses [14,15]. Therefore, transposition of small-scale experiments to real life situations requires the development of simulation tools capturing the complexity of swine production systems [39].

Fed with the parameter estimates obtained from transmission trial, modelling approaches have been designed to understand the persistence of swIAV on farms and evaluate the impact of control strategies [23,27,28,37,40]. Infections of MDA+ piglets have been shown to extend the transmission dynamics at batch level, favoring the spread to animals from other sectors of the farms. Indeed, the transmission between physically independent farms, through airborne route [17,41], or due to breaches in biosecurity procedures [14], were reported as risk factors for swIAV persistence. Reducing these transmission pathways, e.g. through isolation of replacement gilts [28,42] and/or limiting airflow between



**Fig. 5.** A. Kaplan-Meier survival curves for the shedding duration in swIAV infected contact pigs according to their MDA status. B. Distributions of individual shedding-level data (area under the curve of Ct values) according to animal's MDA status.





**Fig. 6.** Probability of extinction of swIAV infection in a farrow-to-finish pig herd where all piglets were considered to be vaccinated at weaning age, depending on the vaccination scheme (mass or batch-to-batch or no vaccination) performed in sows.

sectors [23,27], may help reducing the infection burden. A common trait in simulation studies relies on the assessment of the impact of vaccination strategies, all of which were found ineffective to stop the spread of swIAV. Reynolds *et al.* nevertheless ended up with the theoretical option of vaccination of growing pigs using a strict homologous vaccine-strain (as would be the case with an autogenous vaccine), providing complete protection towards infection, after MDA waning as the unique solution to eliminate the virus in post-weaning and fattening period [27].

Here, we integrated the parameters estimated from our transmission trial in a model developed earlier [23], to evaluate the impact of the interplay between MDAs and the inactivated vaccine on the course of infection of a swIAV strain, in a typical farrow-to-finish pig herd. Vaccination of the breeding herd is a common practice in swIAV infected farms, with positive outcomes in terms of reduction of clinical expressions in sows (especially hyperthermia and respiratory disorders), and allowing transfer of passive immunity to newborns via colostrum intake [25]. However, the level of passively acquired immunity depends on the immune status of sows, which is in turn dependent on vaccination scheme applied in the sow herd. On the one hand, sows can be vaccinated following a batch-based approach, i.e., with two injections before their first gestation with in addition a booster vaccination before each parturition to ensure a homogenous immune level at farrowing. On the other hand, mass vaccination can be performed by simultaneously immunizing all breeding sows in the herd whatever their physiological status, leading to heterogenous immunity levels at farrowing times. These two vaccination protocols were shown to reduce effectively the transmission in the breeding herd [26]. A recent study showed that MDAs induced by sow immunizations could be beneficial for piglets up to weaning age [43]. However, the outcomes from a simulation model in farrow-to-finish pig farms strongly suggested that MDAs only postpone infection to older age groups and contribute to sustain in-farm viral dynamics by increasing the duration of swIAV epidemics in the nursery compartment [26]. Our results showed that single dose vaccination in piglets may reinforce this negative impact of MDAs, if performed before antibody waning. When extinction was quasi-systematic at year 5 post-introduction in the absence of sow vaccination, the extinction probability fell down to 67 to 33% when combining piglet vaccination with batch-to-batch and mass vaccination of breeding herd, respectively.

Such an extension of the persistence of the virus within the herd is a puzzling issue for multiple reasons. The longer the persistence, the higher the risk for emergence of new antigenic variants

through virus mutations [44]; this risk is even increased by the possible co-circulation of multiple strains on herds, favoring reassortments in co-infected animals [11,45]. Furthermore, the zoonotic potential of influenza viruses cannot be neglected and the risk of cross-species transmission of novel viral strains is omnipresent [46,47]. Therefore, there is a need to identify which strategy would be less detrimental for the farm in terms of clinical consequences but which would also limit on a short term the permanent circulation of swIAV in farrow-to-finish farms. In a context of frequent exposure to swIAV as in densely pig populated area, the absence of clinical protection of the breeding herd seems difficult to cope with, but the vaccination of piglets too early after weaning may lead to adverse effects in terms of swIAV circulation and for sure would not mimic the positive effect of export of batches of weaned piglets as shown previously [26].

A recent study showed that farms vaccinating against swIAV had higher economic losses than unvaccinated herds [48]. As underlined by the authors, this result may be due to several factors that were not accounted for in the study, such as the herd biosecurity level or the co-circulation of co-infecting pathogens. Our study proposes an additional hypothesis relying on the vaccination schedule in growing pigs. Indeed, according to our results, the presence of MDA at vaccination time could enhance the transmission of a wild swIAV strain, possibly increasing the on-farm persistence of the virus. The infectiousness of swIAV is so high that the vaccine coverage when sows only are vaccinated is inefficient to block the transmission at the population level. Therefore, should vaccination be practiced in growing pigs, its schedule should account for the decline of MDAs. The manufacturer recommendations preconize vaccination of piglets at the age of 56 days. This should be considered as a lower age-limit since MDAs were found to persist up to 10 to 12 weeks of age [21]. With transmission rates as high as those estimated in our experimental conditions, single dose vaccination of only a few MDA+ pigs may have dramatic consequences on the transmission dynamics. By contrast, a full vaccination (two injections three weeks apart) after MDA waning (10 weeks of age) with a vaccine adapted to circulating strains would provide a solid protection to the whole fattening sector of the farm until slaughter age. The vaccination coverage of the population would also be considerably enlarged with possible progressive side effect on the circulation in the nursery compartment. However, further studies are required to evaluate this strategy in various conditions as permanent infectious process in the nursery might be self-maintained in some farms, without contribution of the fattening sector and such strategy would probably be less efficient under these conditions.

#### Data availability

Data will be made available on request.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Description of the transitions defined in the epidemiological model with related values [21]. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2023.04.018>.

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