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Maternally-derived neutralizing antibodies reduce vaccine efficacy against porcine reproductive and respiratory syndrome virus infection

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ABSTRACT

Modified live virus (MLV) vaccines are commonly used to reduce the impact of porcine reproductive and respiratory syndrome (PRRS) but limited efficacy is achieved in field conditions. Here, we evaluated the impact of maternally-derived neutralizing antibodies (MDNAs) on vaccine efficacy after PRRS virus (PRRSV) challenge. Piglets with low (A−) or high (A+) MDNA levels derived from a commercial pig herd were moved to experimental facilities to be vaccinated (V+) or not (V−) with a PRRSV-1 MLV vaccine at 3 weeks of age (woa). Because of unexpectedly low vaccine detection in A−V+ piglets post-vaccination (pv), all V+ piglets received a second vaccination at 4 woa. Five weeks (W5) pv, piglets were inoculated with a PRRSV-1 field strain to evaluate vaccine protection, and were mingled 24 h later with non-inoculated piglets of similar immune status to assess viral transmission. Vaccine strain was detected at W2 pv in 69% and 6% of A−V+ and A+V+ piglets, and at W5 pv in 50% and 25% of A−V+ and A+V+ piglets, respectively. At W5 pv, 94% of A−V+ and 44% of A+V+ piglets seroconverted, with a significant IFNg response induction in the A−V+ group only. After challenge, compared to the V− inoculated group, viremia was 100-fold lower at 10 days post-infection in A−V+ whereas viremia was not significantly reduced in A+V+ piglets. A lower transmission rate was estimated for the A−V+ group: 0.15 [0.07–0.29] versus 0.44 [0.18–1.76] and 0.32 [0.14–0.68] for the A+V+ and V− groups, respectively. Investigations about the low vaccine strain detection after the first vaccination suggested a relationship between IFNa levels and vaccine strain detection in A−V+ piglets. We showed that MDNAs impair vaccine efficacy against PRRSV both in inoculated and contact piglets, probably by reducing vaccine replication. IFNa may also interfere with PRRSV vaccination. These new data could help improving vaccination protocols.

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1. Introduction

Porcine reproductive and respiratory syndrome (PRRS), caused by a small RNA virus, member of the *Arteriviridae* family [1], is one of the most costly diseases in swine production world-wide [2,3]. In Western Europe, PRRS virus 1 (PRRSV-1) is the main circulating PRRSV species. PRRSV infection is characterized by reproductive failure in sows and by respiratory disorders, growth retardation and increased mortality in growing pigs. PRRSV predisposes pigs to secondary infections associated with the porcine respiratory disease complex [4]. To limit the impact of PRRS, modified live virus (MLV) vaccines based on cell culture attenuated PRRSV strains are routinely used in gilts, sows and growing pigs, but control of PRRS in the field is still a challenge. Only partial protection is achieved, mainly limiting the clinical signs and lesions [5–7]. However, in experimental conditions, these vaccines provide good protection against PRRSV challenge in piglets, controlling the viremia in infected pigs and decreasing transmission to contact pigs [8,9]. Unlike to experimental conditions, in field conditions, vaccinated piglets are generally born to PRRSV infected, exposed or vaccinated sows since they are commonly vaccinated against PRRSV to prevent PRRSV circulation in farrowing units and improve farrowing

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rates [10]. Consequently, high levels of maternally-derived antibodies (MDAs) against PRRSV are frequently detected in piglets vaccinated at weaning [11]. Among MDAs, maternally-derived neutralizing antibodies (MDNAs) can protect suckling piglets against PRRSV infection during their first weeks of life and prevent viremia in weaned piglets [12,13]. However, we recently demonstrated a negative impact of MDNAs on PRRSV vaccination in piglets vaccinated at 3 weeks of age (woa) with a PRRSV-1 MLV vaccine [14]. In this study, vaccine strain replication was impaired and both PRRSV antibody and IFNg-secreting cell production were inhibited for 4 weeks post-vaccination (pv) in piglets with high levels of MDNAs. This interference of MDNAs with post-vaccination immune response suggested weak protection against PRRSV infection of piglets vaccinated in presence of high MDNA levels that could explain the lower vaccine efficacy observed in the field. Previous studies reported that vaccination in piglets with high MDA levels had no impact on vaccine efficacy but neutralizing antibodies (NAs) were not considered [15]. In the present study, piglets were vaccinated in the presence of low or high MDNA levels and further challenged with a wild PRRSV-1 to assess the impact of MDNAs on the efficacy of PRRSV-1 MLV vaccination.

2. Material and methods

2.1. Animal selection and experimental design

The experiment was performed using 56 (Large White * Landrace) * Pietrain piglets selected in a conventional farrow-to-finish herd free from PRRSV circulation in growing pigs and sows but that maintains PRRS-1 MLV mass vaccination of sows with Porcilis PRRS (MSD, Beaucouzé, France) in order to keep a certain level of immunity in case of PRRSV reintroduction in the herd. Before starting the study, the absence of PRRSV circulation was individually checked by ELISA in pigs sampled at the end of the fattening period and by PCR in weaned piglets using sample pools of 5 animals. Piglets used in this study were born from 4 sows with high PRRSV NA titres and from 5 sows with low PRRSV NA titers. At 1 woa, blood was collected from piglets in order to assign them according to their PRRSV-specific MDNA level: **A+** (high level, mean NA titre 201 ± 73) and **A**– (low level, mean NA titre 10 ± 5). At 3 woa, study piglets were weaned, transferred to the Anses biosafety level 3 animal facilities in Ploufragan and randomly distributed according to A+/A– status, weight and gender (Fig. 1; 8 piglets per group). The absence of PRRSV infection in piglets at 3 woa was checked by RT-PCR using sample pools of 5 animals. The decay of MDA was then individually evaluated at 3 woa afterwards (A+: mean NA titer 10 ± 5; A–: no-longer detectable NA). Piglets were vaccinated (**V+**) or not (**V**–) twice at 3 and 4 woa using one dose of Porcilis PRRS vaccine (MSD, Beaucouzé, France) by intramuscular injection in the neck using a syringe with a needle 0.6 * 25 mm (vaccine lot No. A208DB01). At 5 weeks post-first vaccination (W5 pv), half of the piglets were inoculated with $5{\cdot}10^5$ $TCID_{50}$/pig of the PRRSV-1 Finistere strain (PRRS-FR-2005-29-24-1) by nasal inoculation using a syringe without needle (2.5 mL per nostril). The day after, non-inoculated contact (**C**) piglets of the same immune status were mingled with inoculated (**I**) piglets in the same pens (2 C and 2 I piglets by pen). Eight unvaccinated and non-inoculated piglets (4 A+ and 4 A–) were assigned to control groups. Due to the waning of MDNA, at the time of PRRSV inoculation the A+ piglets from the V– and control groups had undetectable PRRS antibody levels (VNT and ELISA tests, data not shown). This identical immune status between A+ and A– allowed us to mingle the results of the A+ and A– piglets from V– and control groups for the post-inoculation period.

Blood samples were collected from the jugular vein once a week for 5 weeks pv to individually detect viremia and monitor humoral and cellular immune responses. After challenge, clinical signs were recorded daily and blood was collected twice a week until animals were euthanized at day 42 post-inoculation (D42 pi) to individually quantify the Finistere strain viremia and to evaluate the immune response. All experiments were authorized by the French

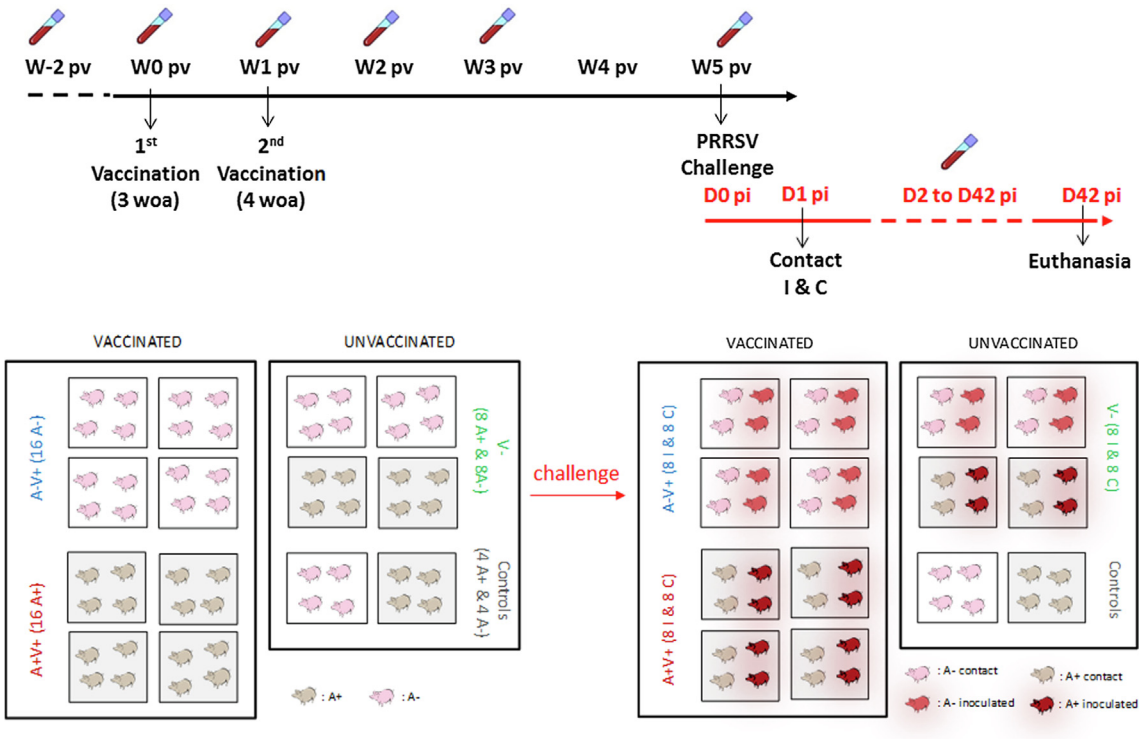

Fig. 1. Experimental scheme and timeline.

Ministry for Research (project No. APAFIS#3694-2016012009236 491_v5) and approved by the national ethics committee number 16.

2.2 Virus neutralization test

PRRSV-specific NAs were quantified in serum on MARC145 cells targeting the vaccine strain, as previously described [14].

2.3. RT-PCR

Before challenge, the vaccine strain genome was detected using the Adiavet™ PRRS real-time RT-PCR kit (Adiagene, Saint-Brieuc, France). After challenge, specific detection of the Finistere strain genome was assessed by qRT-PCR, as described by Rose et al. [8].

2.4. ELISPOT

PRRSV-specific IFNg-secreting cells (IFNg-SCs) were quantified as previously described [14], using 16 h PRRSV stimulation of 4×10^5 PBMCs with a multiplicity of infection of 0.2 for either the vaccine strain or the Finistere strain. The number of spots per well was counted using an ImmunoSpot S5 UV Analyzer (CTL, Shaker Heights, OH, USA).

2.5. ELISA

Antibodies against PRRSV were detected in serum using PRRS X3 Ab ELISA tests (IDEXX laboratories, Liebefeld, Switzerland). Sample-to-positive (S/P) ratios with values equal to or greater than 0.4 were considered positive.

Porcine IFNa was quantified in serum using an in-house ELISA test, as previously described [16].

2.6. Statistical analysis

All the data and calculated areas under the curve (AUCs) were compared between groups using the Kruskal–Wallis test ($p < 0.05$). Post hoc pairwise comparisons were then performed using the Holm test to adjust the p-values of these comparisons according to the number of tests conducted ($p < 0.05$). The relationship between the blood genomic viral load and the number of IFNg-SCs for all the inoculated animals during the post-challenge follow-up period was assessed with a Spearman correlation test ($p < 0.05$).

The estimation of the transmission parameters was based on a SEIR model, where each individual was considered according to the virological results as susceptible (uninfected), exposed (infected without virus excretion), infectious (infected with virus excretion) or removed (protected without a role in the infectious process). The duration of the latency period and the transmission rate of the virus were estimated by Bayesian inference using the Metropolis-Hastings algorithm, as previously used [8]. Convergence was assessed by visual inspection and diagnostic tests (Gelman-Rubin, autocorrelation, Heidelberger).

3. Results

3.1. MDNAs reduce PRRS immunization in vaccinated piglets

At W1 pv, the vaccine strain was detected in only 2 out of 16 A−V+ piglets (Fig. 2A). A second vaccination was thus performed at W1 pv in the same conditions as the first. At W2 pv, 11 out of 16 A−V+ piglets (69%) were viremic, whereas only 1 out 16 (6%) were so in the A+V+ piglets. The difference between A+V+ and A−V+ was maintained until W5 pv, with two-fold fewer viremic piglets in A+V+ compared to A−V+ piglets (Fig. 2A). A significant IFNg response was observed at W3 pv and W5 pv for A−V+ piglets, whereas for the A+V+ group, the number of IFNg-SCs was only significantly increased at W3 pv (Fig. 2B). Regarding the seroconversion, all A−V+ piglets except one showed a detectable PRRSV antibody level in serum at W5 pv (Fig. 2D), while at the same time, only 7 out of 16 A+V+ piglets were seropositive (Fig. 2C).

3.2. MDNAs reduce PRRS vaccine efficacy in challenged piglets

After PRRSV challenge, very mild clinical signs were observed. Unvaccinated inoculated (V-I) piglets showed a significant raise of rectal temperature at 3, 8 and 9 days post-challenge compared to unchallenged control piglets but no statistical difference was observed in growth performance (Supplementary Fig. 1). In the vaccinated inoculated pigs, whatever their MDNA status (A+V+I and A−V+I), no significant difference was observed with the V-I group for rectal temperature nor growth performance.

A lower Finistere strain viral load was detected for A−V+I piglets compared to V−I animals (AUC = 21.8 log10 equivalent $TCID_{50}$/mL * week ± 7.8 and 53.0 log10 equivalent $TCID_{50}$/mL * week ± 4.7 respectively; $p = 0.003$), with 100-fold lower values at D10 pi ($p = 0.019$) (Fig. 3A). No difference was observed between the A+V+I and V−I groups, or between vaccinated groups. Mean viremia duration was shortened from 21 ± 6 days for V−I or 21 ± 7 days for A+V+I to 16 ± 9 days for A−V+I animals, but not significantly.

Vaccination induced an early IFNg response against the Finistere strain at D7 pi in inoculated piglets for both the A−V+ and A+V+ groups (Fig. 3B). Even though more IFNg-SCs were detected in A−V+I compared to A+V+I piglets, the difference was not significant. Interestingly, considering all inoculated pigs during the D7-D15 pi period, a negative correlation could be established between the number of IFNg-SC and the Finistere strain genomic load in serum ($r = -0.55$; $p < 0.05$). NAs were detected from D30 pi in inoculated pigs. At D42 pi, 6 out of 8 A−V+I piglets had quantifiable NAs titres (>10) compared to 3 out of 8 for A+V+I and 1 out of 8 in V-I animals (Supplementary Fig. 2).

3.3. MDNAs impair vaccine reduction of PRRSV transmission

A reduction in viremia was observed in A−V+ C contact pigs compared to V-C piglets (AUC = 30.3 log10 equivalent $TCID_{50}$/mL * week ± 15.5 and 56.9 log10 equivalent $TCID_{50}$/mL * week ± 10.3 respectively; $p = 0.002$), but not in A+V+C piglets (Fig. 3C). At D7 pi, the Finistere strain was not yet detected in A−V+C piglets, whereas the first positive piglets were identified at D4 pi in the other contact groups (Fig. 3D). The mean viremia duration for A−V+C was shortened to 6 ± 3 days compared to 12 ± 6 and 19 ± 6 days for the A+V+C and V−C groups, respectively. The Finistere strain was detected in all unvaccinated piglets. In vaccinated groups, all the contact animals became infected, except one A+V+C pig (Fig. 3D).

The A+V+C piglet that remained uninfected had seroconverted post-vaccination. Considering this outlier animal as protected against PRRSV infection with no role in the transmission process, the estimates for the transmission rate of the A+V+ group was comparable to those of the V− group (0.44 [0.18; 1.76] and 0.32 [0.14; 0.68] for A+V+ and V− respectively; Table 1). For the A−V+ group, the estimated transmission rate was reduced to 0.15 [0.07; 0.29].

3.4. High IFNa level was present at the time of the first vaccination

In order to understand the unexpectedly low vaccine viremia detected in A− piglets after the first vaccination, the IFNa level

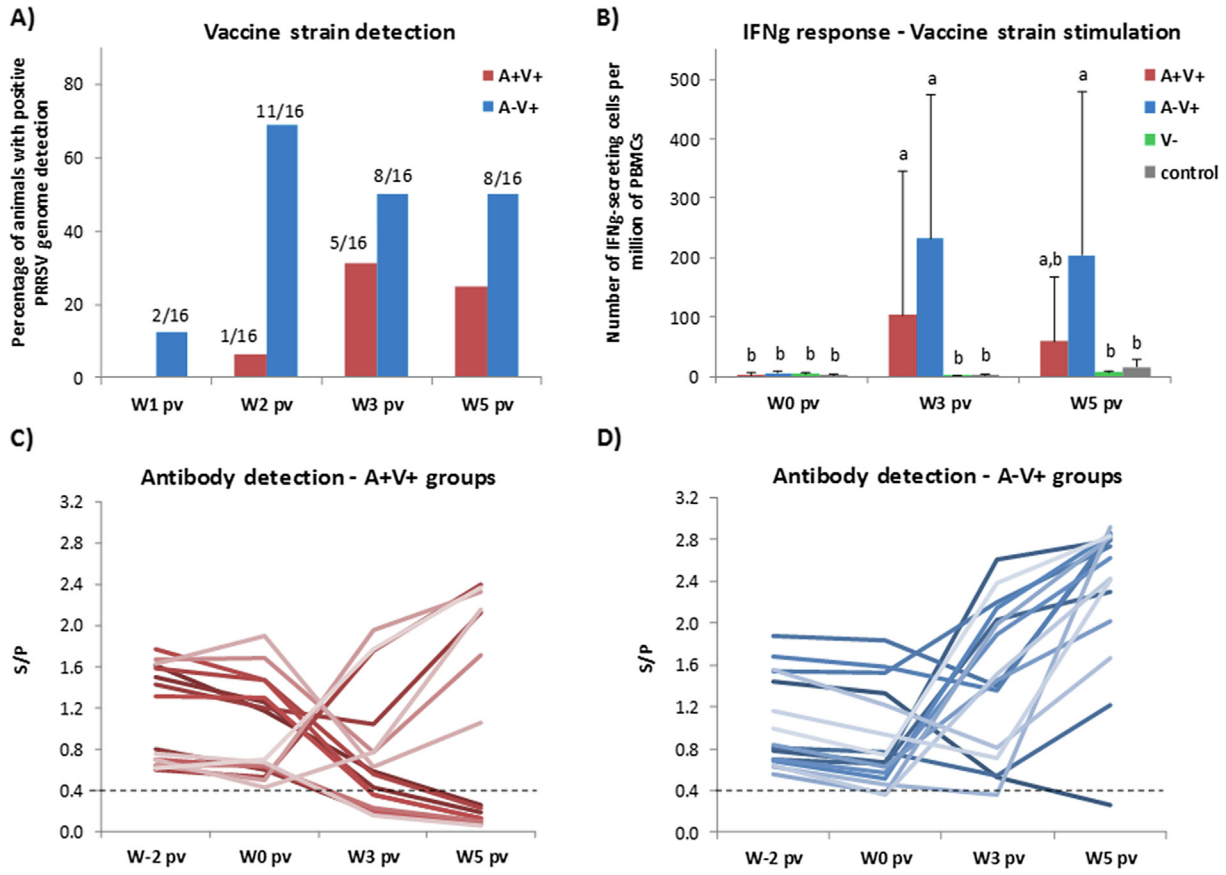

Fig. 2. Post-vaccination data. (A) Percentage of vaccinated animals with positive PRRSV vaccine strain genome detection by RT-PCR in serum during the post-vaccination period. Positive numbers of piglets out of the 16 A+ or A- vaccinated piglets are indicated. (B) Count of IFNg secreting cells (IFNg-SCs) responding to *in vitro* vaccine strain stimulation among PBMCs purified from blood of piglets before challenge. Data are reported as the mean (±SD) of results obtained from A+V+I, A−V+I, V−I and control groups (n = 8 in each group). Different letters (a, b) indicate that the groups are significantly different from each other with $p < 0.05$. (C) Detection of PRRSV antibodies by ELISA in serum of each A+ vaccinated piglet before challenge (n = 16), or (D) in serum of each A-vaccinated piglet before challenge (n = 16). Post-vaccination times were related to the first vaccination week as W0 pv. A second vaccination was performed at W1 pv.

was assessed in serum samples collected at W0 pv during the present experiment (Experiment B) and compared with those collected at the same time during our previous study [14] (Experiment A) where the vaccine viremia was detected in most of the A− animals after vaccination. The results showed significantly higher levels of IFNa for piglets in Experiment B than for those in Experiment A (Fig. 4A). A relationship could be thus hypothesized between high IFNa levels detected at the time of vaccination and low subsequent vaccine strain detection (Fig. 4B).

4. Discussion

Vaccination programs hardly eradicate PRRSV circulation in farms whereas PRRS MLV showed a good efficacy in piglets to control PRRSV transmission in experimental conditions [5,8]. In a previous study, we demonstrated an interference of MDNAs on post-vaccine immune responses induction during 4 weeks post-vaccination, suggesting a negative impact of MDNAs on vaccine efficacy in piglets [14]. To test this hypothesis, PRRSV-1 MLV vaccinated piglets with low or high levels of MDNAs were challenged with a field PRRSV-1 strain.

Despite the animals had to be vaccinated twice in the present study, vaccine viremia was detected in only one A+V+ piglet whereas it was detected in 11 A−V+ piglets at W2 pv, which was in accordance with our previous data [14] and confirmed the interference of MDNAs with vaccine viremia. This experiment also indicated that in piglets with low levels of MDNAs, vaccination induced significant humoral and cellular post-vaccine immune response, which also corroborates our previous results [14].

In the present study, we observed a lower interference of MDNAs on post-vaccine immune responses compared to the previous one reported by Fablet [14] where a complete impairment was observed during 4 weeks pv. This reduced interference could be ascribed to the need to vaccinate the piglets twice and the ensuing decrease of MDNAs levels at the time of the second vaccination. At the time of first vaccination (3 woa), the decrease of MDNA level was already high but the mean NA titre in the present study (10 ± 5) was comparable to the one in Fablet's study (20 ± 15). Despite the slightly reduced post-vaccine differences between A+V+ and A−V+ we observed in the present study, the post-challenge results demonstrated for the first time that the PRRSV vaccination was effective only in piglets with low levels of MDNAs. The low virulence of the PRRSV-1 strain used to challenge the animals limited the evaluation of the vaccine efficacy to virological parameters only. We previously demonstrated that the Porcilis PRRS vaccine can protect SPF piglets (no MDAs) from a challenge with the same low virulent strain, decreasing the viremia in inoculated piglets and considerably reducing the transmission of the Finistere strain to contact piglets [8]. In the present study, the challenge strain viral load was significantly reduced only in A−V+ inoculated and contact piglets compared to unvaccinated piglets. In a similar unpublished study, we also showed that PRRSV challenge strain viremia was significantly reduced for A−V+ inoculated

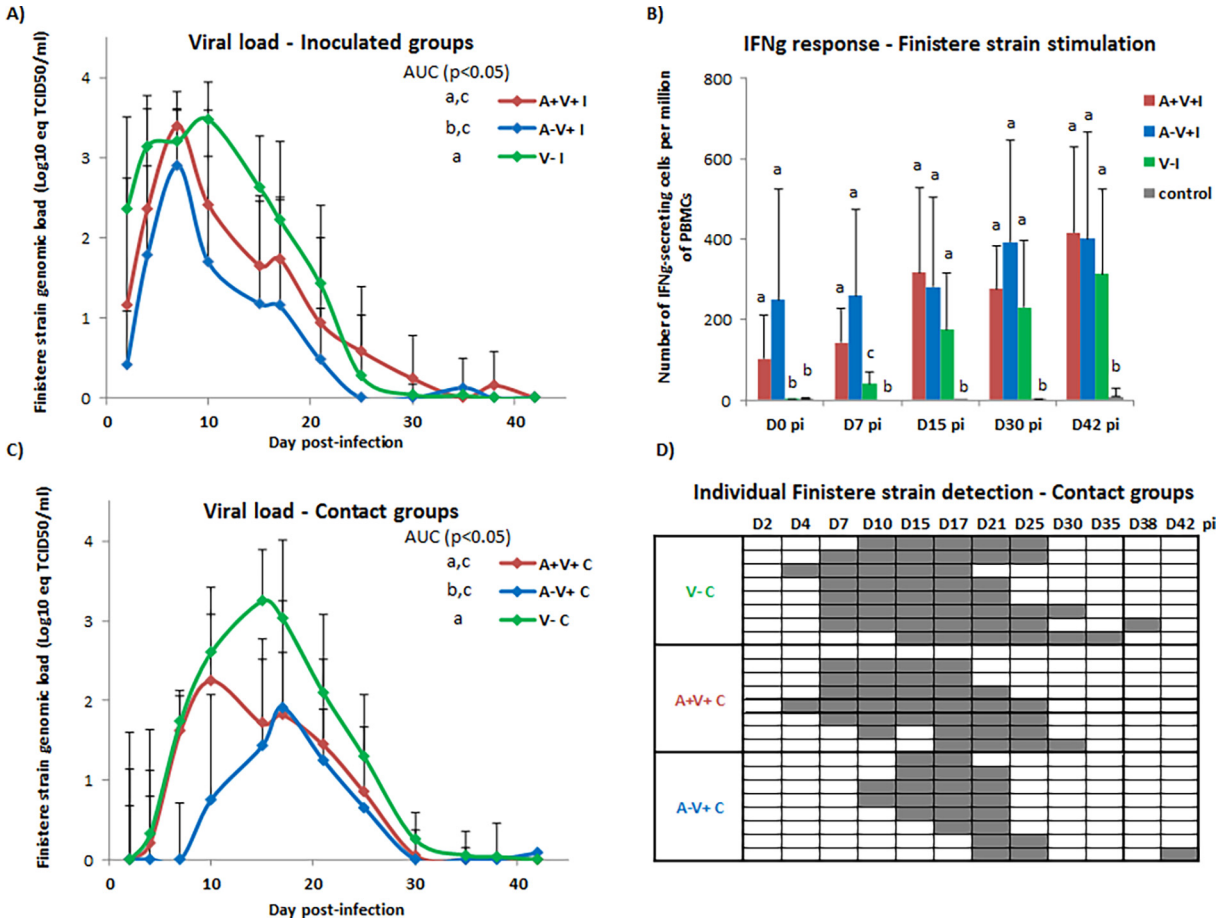

Fig. 3. Post-inoculation data. (A) Quantification of PRRSV Finistere strain genome load by qRT-PCR in serum collected from inoculated piglets, or (C) in contact piglets. Different letters (a, b, c) indicate that the groups are significantly different from each other with $p < 0.05$. (B) Count of IFNg secreting cells (IFNg-SCs) responding to an *in vitro* Finistere strain stimulation among PBMCs purified from blood of inoculated piglets. All data are reported as the mean (±SD) of results obtained from piglets in each group (n = 8 in each group). Different letters (a, b, c) indicate that the groups are significantly different from each other, with $p < 0.05$. (D) Serum detection of PRRSV Finistere strain genome by qRT-PCR for each contact piglet. Grey areas: positive detection; White areas: negative detection.

Table 1
Transmission parameters estimation.

	Transmission rate		Latency duration[#]	
	Median	95% CI[*]	Median	95% CI[*]
A-V+	0.15	[0.07; 0.29]	1.94	[1.19; 2.93]
A+V+[□]	0.44	[0.18; 1.76]	2.00	[1.38; 2.92]
V−	0.32	[0.14; 0.68]	0.99	[0.14; 1.84]

[□] A+V+ group considering the uninfected pig as protected.
[*] 95% credibility interval.
[#] Days of latency duration.

piglets compared to A+V+ animals during the first 3 weeks pi (Supplementary Fig. 3). In this study, vaccine viremia was also only detected in 1 out of 12 A−V+ piglet at W2 post-vaccination but a second vaccination was not performed before challenge, indicating that the negative impact of MDNAs on PRRS vaccine efficacy also occurred in the absence of vaccine viremia and with usual single vaccination protocols implemented in the field.

Using a closely-related approach, Jeong et al. evaluated the efficacy of a PRRSV-2 MLV vaccine in 1-day-old piglets with maternally-derived antibodies (MDAs) but no impact of MDAs was observed [17]. In Jeong's study, all the piglets were MDA+ at vaccination (no MDA- group included) and thus this precluded to clearly evaluating the impact of MDAs on the vaccine efficacy. As the same, Balasch et al. recently showed that vaccination of 1-day-old piglets using a new PRRSV-1 MLV in presence of MDAs induced a partial protection against a challenge at 67 days pv [18]. In Balasch's study, NAs were quantified in piglets at the vaccination time but here also the absence of comparison with vaccinated piglets without MDNAs limits the conclusions of the study.

The interference of MDAs on vaccine efficacy was previously demonstrated for many other swine diseases. Using a killed vaccine against porcine circovirus type 2, a similar study comparing vaccine efficacy in piglets with low or high MDA levels showed a significant reduction of percentage of PCR positive animals in A−V+ group compared to A+V+ group at 22 days post-challenge [19]. Using a MLV against classical swine fever, 83% of piglets with high MDNA levels at vaccination died from a challenge at 10 weeks of age, whereas all piglets with low MDNA levels, survived from the challenge [20].

In addition, considering the contact animals, our results also demonstrated that MDNAs decreased the PRRS vaccine efficacy on virus transmission. In the present study, PRRS vaccination in A−V+ piglets reduced the transmission rate of the Finistere strain two-fold compared to the rate estimated for the V- group. The magnitude of the effect is substantially lower than what was found in our previous study with a ten-fold reduced transmission rate in vaccinated SPF pigs, with non MDA [8]. One possible explanation for this discrepancy may be the intradermal route (ID) used for vaccination in our previous work. This immunization route was previously shown to induce an enhanced cell-mediated immune

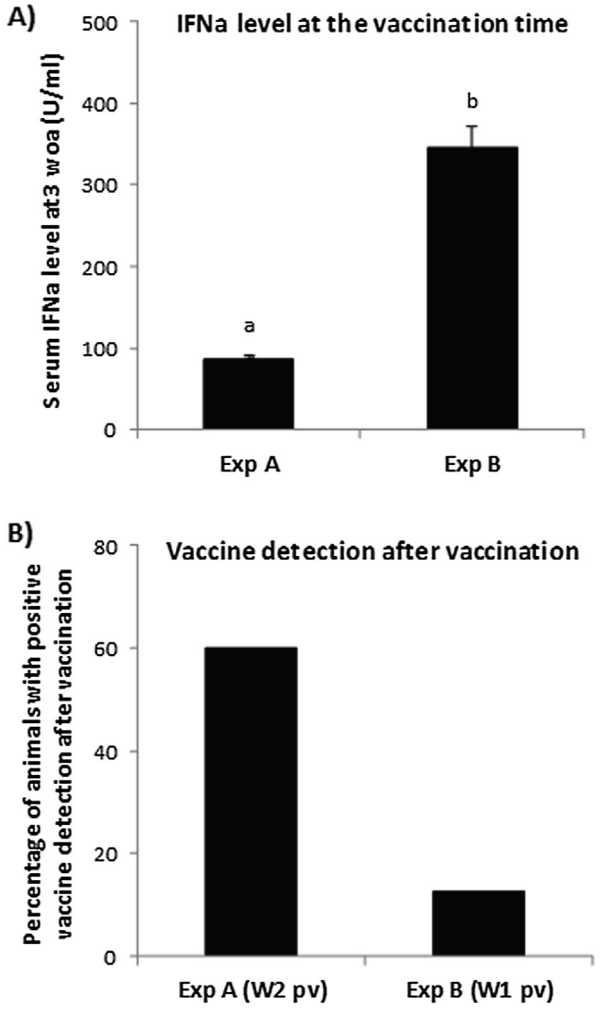

Fig. 4. Relation between IFNa levels at the time of the first vaccination and vaccine detection in serum of A−V+ piglets of different experiments. (A) IFNa level was quantified by ELISA in serum collected from piglets at 3 weeks of age (woa) (at the age of the first vaccination) in Experiment A previously published by Fablet et al, 2016 (Exp A; n = 30) or in the present study : Experiment B (Exp B; n = 16). (B) Percentage of animals with positive vaccine strain genome detection in serum by RT-PCR at one week post-vaccination (W1 pv) in Experiment B or at W2 pv in Experiment A. All data are reported as the mean (±SEM) of results obtained from piglets in each group. Different letters (a, b) indicate that the groups are significantly different from each other with $p < 0.05$. Post-vaccination times were related to the first vaccination week as W0 pv.

response after PRRSV exposure and a lower respiratory clinical score compared to IM vaccination [5]. The better immune response, especially at the mucosal sites, elicited after ID immunization, may have limited viral replication in the respiratory tract, thus reducing virus excretion and transmission.

In Fablet et al. study [14], vaccine viremia was detected in 60% of A−V+ piglets at W2 pv whereas only in 12.5% of A−V+ piglets of the present study after the first vaccination. To explore these unexpected results, we attempted to investigate the origin of this low vaccine replication displayed in A−V+ piglets after vaccination at 3 woa. The vaccine strain was titrated, but no problem was detected. As IFNa was recently shown to strongly inhibit the replication of a genotype 2 PRRS MLV [21], we hypothesized this cytokine could be responsible for the inhibition of vaccine replication in our A−V+ piglets. Indeed, the levels of IFNa detected in piglets from the present study at 3 woa were much higher than those from our previous study [14] and in the same range as the concentration shown to be able to completely inhibit PRRS MLV replication [22].

To bring some preliminary conclusions, it seems that IFNa concentrations likely to interfere with PRRS MLV replication could be achieved in piglets under field conditions. In the absence of suitable samples (not of the right type or not collected at the right time) for further exploration, we were not able to identify the cause of these increased IFNa levels. However, this cytokine is known to be produced in response to many viral infections such as swine influenza [23] or porcine respiratory coronavirus [24,25] that could infect piglets at weaning when they are frequently vaccinated against PRRSV. As a result, this potential viral interference with the PRRS MLV vaccine may be another possible explanation for the limited PRRS MLV vaccine efficacy observed in the field.

5. Conclusions

Our results confirm that MDNAs impair PRRS vaccine strain replication and post-vaccination immune response. Furthermore, we demonstrated for the first time that this impaired immune response results in decreased vaccine efficacy based on virological parameters evaluation after a low virulent PRRSV challenge. The use of a more virulent strain for the challenge of the animals may bring further clinical arguments regarding the reduced vaccine efficacy in presence of high levels of MDNAs. Unexpectedly, we also showed that other factors such as IFNa may interfere with PRRS MLV vaccination. Further research is needed to explore a possible viral interference phenomenon toward PRRS MLV vaccines. At the end, the low efficacy of PRRS vaccination observed in the field could thus result from endogenous (MDAs) as well as exogenous (viral infection) factors.

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Author contributions

PR analyzed the samples, interpreted the results and drafted the manuscript. CF participated in the design of the experiment, coordinated the study at the farm, and performed the statistical analyses. MA developed the mathematical model and participated in the data analyses. VN and AL identified the study farm. FP coordinated the animal experiments. NR participated in the design of the study. OB designed the study, supervised the analyses, and interpreted the data. All co-authors revised the manuscript and approved the final submitted version.

Declaration of Competing Interest

The authors state that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found,in the online version, at https://doi.org/10.1016/j.vaccine.2019.06.045.

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