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# Efficacy and safety of simultaneous vaccination with two modified live virus vaccines against porcine reproductive and respiratory syndrome virus types 1 and 2 in pigs

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ABSTRACT

The objective of the study was to compare responses of pigs vaccinated with a PRRS MLV vaccine against PRRSV-1 or PRRSV-2 with the responses of pigs vaccinated simultaneously with both vaccines. Furthermore, the efficacy of the two PRRSV MLV vaccination strategies was assessed following challenge. The experimental design included four groups of 4-weeks old SPF-pigs. On day 0 (DPV0), groups 1–3 (N = 18 per group) were vaccinated with modified live virus vaccines (MLV) containing PRRSV-1 virus (VAC-T1), PRRSV-2 virus (VAC-T2) or both (VAC-T1T2). One group was left unvaccinated (N = 12). On DPV 62, the pigs from groups 1–4 were mingled in new groups and challenged (DPC 0) with PRRSV-1, subtype 1, PRRSV-1, subtype 2 or PRRSV-2. On DPC 13/14 all pigs were necropsied. Samples were collected after vaccination and challenge. PRRSV was detected in all vaccinated pigs and the majority of the pigs were positive until DPV 28, but few of the pigs were still viremic 62 days after vaccination. Virus was detected in nasal swabs until DPV 7–14. No overt clinical signs were observed after challenge. PRRSV-2 vaccination resulted in a clear reduction in viral load in serum after PRRSV-2 challenge, whereas there was limited effect on the viral load in serum following challenge with the PRRSV-1 strains. Vaccination against PRRSV-1 had less impact on viremia following challenge. The protective effects of simultaneous vaccination with PRRSV Type 1 and 2 MLV vaccines and single PRRS MLV vaccination were comparable. None of the vaccines decreased the viral load in the lungs at necropsy. In conclusion, simultaneous vaccination with MLV vaccines containing PRRSV-1 and PRRSV-2 elicited responses comparable to single vaccination and the commercial PRRSV vaccines protected only partially against challenge with heterologous strains. Thus, simultaneous administration of the two vaccines is an option in herds with both PRRSV types.



## 1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most devastating infections in most swine producing countries globally. In the US, the annual losses due to PRRS reach $644 million annually [1] and the losses after an acute outbreak has been estimated to be between 59 and 379 Euro/sow in Holland [2]. Therefore, huge efforts are put into the elimination and control of the PRRS virus (PRRSV). Due to horizontal transmission of PRRSV [3], the risk of PRRSV infection is high in swine dense areas and therefore the strategy employed in most farms in Denmark and other parts of Europe is to establish a PRRS stable sow herd where sows are PRRSV antibody positive and PRRS virus negative and wean PRRSV free pigs. PRRSV vaccines are commonly used to immunize young breeding animals before introduction to the sow herd.

Both Modified Live Virus (MLV) vaccines and killed vaccines are available, but the efficacy of killed PRRS vaccines in stimulating protective immunity is questionable [4] and therefore MLV vaccines are used in most herds. Several studies have shown good

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**Table 1**
Experimental design. The pigs were allocated to three vaccination groups vaccinated with MLV vaccines containing PRRSV-1 (VAC-T1), PRRSV-2 (VAC-T2) or both (VAC-T1T2). Each of the vaccination groups were split into three different groups prior to challenge. The NON-VAC group was kept as unvaccinated control group. *Four pigs died prior to challenge and one pig was excluded due to lack of seroconversion after vaccination resulting in a lower number of pigs in some of the challenge groups (4 or 5 pigs per group instead of 6 pigs).

| Group | No. pigs | PRRSV vaccination | PRRSV challenge |
|---|---|---|---|
| VAC-T1 | 18* | Porcilis®PRRS VET | 5* pigs PRRSV-1 subtype 1<br>5* pigs PRRSV-2<br>5* pigs PRRSV-1 subtype 2 |
| VAC-T2 | 18 | Ingelvac®PRRS VET | 6 pigs PRRSV-1 subtype 1<br>6 pigs PRRSV-2<br>6 pigs PRRSV-1 subtype 2 |
| VAC-T1T2 | 18* | Porcilis®PRRS VET +<br>Ingelvac®PRRS VET | 6 pigs PRRSV-1 subtype 1<br>6 pigs PRRSV-2<br>4* pigs PRRSV-1 subtype 2 |
| NON-VAC | 12 | No vaccination | 4 pigs PRRSV-1 subtype 1<br>4 pigs PRRSV-2<br>4 pigs PRRSV-1 subtype 2 |

efficiency of MLV against challenge with related strains [5]. Some studies also found partial protection against challenge with more divergent strains, whereas others found a poor cross-protection of vaccines containing more divergent strains [reviewed in 5]. These apparent differences in outcome of different experiments are probably due to the different experimental designs, different vaccines used, different challenge strains, different breeds, age of the animals, challenge dose etc. Nevertheless, it is generally accepted that the degree of protection elicited by PRRSV vaccines are related to the level of genetic and antigenic similarity between the challenge and vaccine strain, even though that the level of genetic and antigenic similarity is not necessarily predictive of protection [6].

Both PRRSV-1, subtype 1 and PRRSV-2 are circulating and causing disease in some European countries [7,8]. In contrast, PRRSV-1 strains belonging to subtypes 2, 3, and 4 have never been detected in Western Europe [23]. In Denmark, it is common that pigs are simultaneously vaccinated with two different PRRS MLV vaccines containing PRRSV-1 and PRRSV-2. There is limited published data on the impact on duration of viremia, immune responses and efficacy after administration of two PRRS MLV vaccines containing PRRSV-1 and PRRSV-2 at the same time [9].

The objective of the study was therefore to compare the safety and efficacy of single PRRS MLV vaccinated pigs with responses in pigs simultaneous vaccinated with PRRSV Type 1 and 2 vaccines.

## 2. Materials and methods

### 2.1. Experimental design

In total, 66 four-week-old PRRSV-negative pigs were included in the study. The pigs were purchased from a specific pathogen-free herd and tested free of a range of pathogens including PRRSV, swine influenza A virus, *Actinobacillus pleuropneumoniae* (AP) and *Mycoplasma hyopneumoniae* by serology prior to the study. The pigs also tested negative by real-time PCR for Porcine circovirus type 2 (PCV2) virus at arrival. The pigs were housed at the experimental animal facilities at the National Veterinary Institute under appropriate biosecurity conditions. On arrival, the pigs were randomly allocated into four groups housed in separate rooms.

One week after arrival (0 days post vaccination, DPV 0), the pigs in groups 1–3 (N = 16) were vaccinated with either Porcilis® PRRS VET (MSD Animal Health, Denmark) containing PRRSV-1 (VAC-T1), Ingelvac® PRRS VET (Boehringer Ingelheim Animal Health, Denmark) containing PRRSV-2 (VAC-T2) or both vaccines simultaneously (VAC-T1T2) (Table 1). Porcilis® PRRS VET was administrated with 2 mL at the left side of the neck and Ingelvac® PRRS VET administrated with 2 mL at the right side of the neck.

Nine weeks after vaccination (DPV 62), all pigs were moved to new separated groups according to the PRRSV strain they were planned to be challenged with (Table 1). The challenge was done with either PRRSV-1, subtype 1 (strain 18794 [10]), PRRSV-2 (strain 19407b) or PRRSV-1 subtype 2 (strain ILI6 [10]) according to Table 1. The PRRS-19407B had been isolated in January 1997 from the lungs of a stillborn pig. This pig originated from a swine herd with a sudden high occurrence of stillborn pigs and increased piglet mortality in the nursing period, consistent with an acute outbreak of PRRS. The following day (0 days post challenge (DPC 0), corresponding to DPV 63), all pigs were inoculated intranasally by placing the pigs on their buttocks perpendicular to the floor and expanding the neck fully. The inoculum was slowly dripped into the nostrils (2 mL/nostril) of the pigs taking approximately 3–5 min/pig.

The PRRSV-1, subtype 1 inoculum contained $5 \times 10^5$ culture infective dose ($TCID_{50}$)/mL) of PRRSV (passage 6, PAM, 1 mL virus suspension in 3 mL MEM). The PRRSV-2 inoculum contained $5 \times 10^5$ $TCID_{50}$/mL of PRRSV (passage 3, Marc-145, 1 mL virus suspension in 3 mL MEM) and the PRRSV-1, subtype 2 inoculum contained $3.7 \times 10^5$ $TCID_{50}$/mL.

The study was carried out in accordance and permission grated by the Danish legislation on animal experiments (LBK nr 1306 – 23/11/2007; permission number 2014–15–0201–00091) and EU regulations on the use of laboratory animals for research.

### 2.2. Sampling

Blood samples were collected on days 2, 6, 14, 21, 28, 35, 42, 49, 56 and 62 DPV from vaccinated pigs and on day 62 for non-vaccinated control pigs (NON-VAC). Blood samples were also collected on days 1, 3, 5, 9 and 13 after challenge (DPC). Serum was separated from the blood and stored at −80 °C until test. Nasal swabs were collected on DPV 1, 2, 5, 14, and 21 and DPC 1, 3, 4, 5, and 9. The swabs were collected in 1 mL PBS and stored at −80 °C until test.

### 2.3. Clinical observation

A clinical score was assessed daily based on general health condition (normal, mild lethargic, lethargic or apathetic), respiration (normal, increased respiration, respiratory distress, severe respiratory distress), and appetite (normal, slow eating, not eating). Rectal

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