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Efficacy of a Spanish modified live virus vaccine against homologous porcine reproductive and respiratory syndrome virus infection

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Abstract

The objective of this study was to determine the protective properties of the Spanish MLV vaccine *Amervac-PRRS*® against porcine reproductive and respiratory syndrome virus (PRRSV) infection with the homologous wild-type strain 5710. The experimental design comprised eighteen 3-week old pigs randomly divided into three groups. Animals from group A received one dose of MLV vaccine *Amervac-PRRS*® on days –42 and –21. Animals from groups B and C served as infected and uninfected controls, respectively. On day 0, pigs from groups A and B were intranasally challenged with homologous strain 5710 and 18 days after challenge all pigs were euthanized. The results reported in this study show that, although the titre of neutralizing antibodies elicited by vaccination was modest, MLV vaccine *Amervac-PRRS*® provided protection against homologous PRRSV infection and drastically reduced post-challenge viremia and completely block PRRSV dissemination to peripheral tissues.

Additional key words: homologous challenge, immunization, PRRSV protection, swine.

Resumen

Eficacia de una vacuna viva modificada española frente a la infección homóloga con el virus del síndrome reproductor y respiratorio porcino

El objetivo de este estudio fue la determinación de las propiedades protectoras de la vacuna viva modificada española *Amervac-PRRS*® frente a la infección con la cepa homóloga 5710 del virus del síndrome reproductor y respiratorio porcino (PRRSV). El estudio experimental incluyó dieciocho cerdos de tres semanas de edad divididos aleatoriamente en tres grupos. Los animales del grupo A recibieron una dosis de la vacuna viva modificada *Amervac-PRRS*® en los días –42 y –21. Los animales de los grupos B y C sirvieron como controles infectados y no infectados, respectivamente. En el día 0 los cerdos de los grupos A y B fueron desafiados por vía intranasal con la cepa homóloga 5710 y 18 días después del desafío todos los cerdos fueron eutanasiados. Los resultados aportados en este estudio muestran que, pese a que el título de anticuerpos neutralizantes inducido por la vacunación fue modesto, la vacuna viva modificada *Amervac-PRRS*® proporcionó protección homóloga frente a la infección por el PRRSV y permitió reducir drásticamente la viremia posterior al desafío y bloquear completamente la diseminación del PRRSV a tejidos periféricos.

Palabras clave adicionales: desafío homólogo, inmunización, porcino, protección frente a PRRSV.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most relevant swine diseases, and is characterized by reproductive failure in sows and respiratory distress in pigs of all ages (Christianson and Joo, 1994; Rossow, 1998). The causative agent, PRRS virus (PRRSV), is a small, enveloped RNA virus classified within the *Arteriviridae* family (Cavanagh, 1997) and divided into European and American subtypes based on the high genetic, antigenic and pathogenic heterogeneity among different viral isolates (Meng, 2000).

Nowadays, PRRSV has spread worldwide in major swine-producing areas, becoming a severe swine health

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problem responsible for enormous economic losses. In order to reduce the high impact of PRRSV in the swine industry, different strategies have been designed from sanitary to medical prophylaxis (Dee *et al.*, 1998). However, utilization of mere sanitary practices has not provided successful results owing to PRRSV ability to persist for weeks or months in asymptomatic hosts, even in presence of PRRSV specific antibodies (Allende *et al.*, 2000). Consequently, vaccination strategies involving inactivated and modified live virus (MLV) vaccines have been developed to control the disease in fattening pigs and breeding females. The high safety of inactivated virus is the main advantage of this kind of vaccines and the characteristic that permitted their licence for use in pregnant females. However, the low efficacy of these vaccines against PRRSV infection has been confirmed (Osorio *et al.*, 1998; Scortti *et al.*, 1999). To overcome the problem of efficacy, several MLV vaccines have been developed. In this scenario, MLV's ability to replicate *in vivo* could improve vaccine efficacy, inducing longer and more intense immune response. However, MLV vaccines are not directed to use in pregnant females and are approved to prevent respiratory distress caused by PRRSV infection in young piglets, fattening pigs and non-pregnant females. MLV vaccines have shown to confer protection against clinical manifestations of the disease, though they are not capable of preventing from PRRSV infection. Most of the efficacy studies about current MLV vaccines against PRRSV have been carried out using marketed vaccines derived from the American PRRSV strain VR-2332 (Gorcyca *et al.*, 1995; Roof *et al.*, 1999; Desrosiers, 2000; Nodelijk *et al.*, 2001). Studies based on non-commercial attenuated American PRRSV isolates have been also performed showing the evolvement of a complete homologous protection that could even avoid PRRSV viremia and peripheral dissemination (Lager *et al.*, 1997, 1999; Mengeling *et al.*, 2003). However these studies can not be directly extrapolated to European type viruses due to marked differences between both types of PRRSV (Wensvoort *et al.*, 1992; Murtaugh *et al.*, 1995; Meng, 2000). Little is known about the degree of homologous protection provided by European MLV vaccines currently on the market. Therefore, the evolution of the immune response and the degree of protection induced by these vaccines should be carefully evaluated in order to determine if they could provide sterilizing homologous immunity.

The design of this study was aimed at determining the protective properties of the Spanish MLV vaccine *Amervac-PRRS*® (Hipra Laboratories, Spain), against infection with the homologous wild-type PRRSV strain 5710.

Material and Methods

Animals and facilities

Eighteen 3-weeks-old pigs without PRRSV serum antibody titres measured by ELISA test (*CIVTEST-suisPRRS*®, Hipra Laboratories, Spain) were included in this experiment. The pigs were randomly divided into 3 groups, assigned to one treatment group and housed in isolated pens with a concrete floor and automatic watering system.

Vaccines

The commercial PRRSV MLV vaccine *Amervac-PRRS*® was used for active immunization of pigs following manufacturer's instructions. This MLV vaccine is based on a European-type strain (5710), belonging to Lelystad-like cluster, isolated in Spain in 1992 (Suárez *et al.*, 1994).

Virus and cell cultures

Challenge was performed with the Spanish PRRSV strain 5710. This PRRSV strain was propagated on swine alveolar macrophage (SAM) cultures prepared as previously described (Prieto *et al.*, 1997).

Evaluation of the presence of PRRSV in clinical samples collected before challenge was accomplished by sample cultivation on MARC-145, a MA-104 cell clone highly permissive to PRRSV (Kim *et al.*, 1993), and on SAM. Samples collected after challenge were evaluated on SAM cultures prepared and maintained as previously described (Prieto *et al.*, 1997).

Experimental design and sample collection

The study comprised three experimental groups. Six pigs (numbered 1 to 6) members of Group A were vaccinated twice by intramuscular route on days –42 and –21 with the MLV vaccine *Amervac PRRS*®. Six pigs of group B (numbered 7 to 12) and six pigs of group

C (numbered 13 to 18) were not vaccinated and served as infected and uninfected controls, respectively.

Three weeks after the second immunization (day 0) pigs from groups A and B were challenged by intranasal inoculation of 10^5 tissue culture infectious dose 50 ($TCID_{50}$) of PRRSV strain 5710 prepared on primary cultures of SAM, and pigs from group C were intranasally inoculated with an uninfected SAM culture lysate.

All pigs were examined daily for clinical signs and rectal temperatures were measured from 10 days before challenge until the end of the experiment. Furthermore, food intake, average daily gain (ADG) and food conversion efficiency index (FCE) were recorded.

Blood samples were collected in serum-clot vacuum tubes on each vaccination day and then 2, 7 and 14 days post-vaccination, at challenge and 2, 5, 8, 12 and 18 days post-challenge (p.c.). Serum was obtained from all blood samples and stored at –80ºC until its utilization for virus isolation and PRRSV antibody detection.

Eighteen days after challenge (day +18) all pigs were euthanized and tissue samples were collected, namely lung, tonsils, kidney, spleen, liver and submandibular, superficial iliac and retropharyngeal lymph nodes. All these tissues were stored at –80ºC until they were processed for virus isolation.

Virus isolation

The aliquots of sera obtained from the blood samples were thawed at room temperature, diluted 1:4 in Dulbecco's Modified Eagle's Medium (DMEM) and passed through a filter with 0.45 µm pores. One gram of each tissue sample was homogenized with 9 ml of DMEM, centrifuged at 1,200 g for 20 min at 4ºC and supernatant was passed through a filter with 0.45 µm pores.

All samples were added in quadruplicate (100 µl/well) onto SAM or MARC-145 cell monolayers seeded in 96-well plates and incubated for 90 min at 37ºC to facilitate adsorption. The cultures were washed twice with DMEM and fresh DMEM containing 10% fetal bovine serum (FBS) was added. The culture plates were incubated at 37ºC in humidified atmosphere containing 5% CO_2. Monolayers were examined for cytopathic effect (CPE) on days 4, 5 and 6 post-inoculation.

As a positive control, PRRSV strain 5710 was added to give a final concentration of 10^4, 10^3, 10^2 $TCID_{50}$ ml^{-1} (i.e. 10^3, 10^2 and 10 $TCID_{50}$/well). Only batches of SAM with sensitivity to infection of at least 50% of the wells in which 10 $TCID_{50}$ had been introduced were used. Virus-free DMEM was used as a negative control.

Serological examinations

Serum samples were examined for PRRSV-specific antibodies using a commercial ELISA test (*CIVTEST-suisPRRS®,* Hipra Laboratories, Spain). Serum samples collected on vaccination days (days –42 and –21), at challenge (day 0) and at the end of the experiment (day +18) were tested using a neutralization assay performed onto MARC-145 cultures and following indications previously described (Yoon *et al.*, 1994).

Results

Clinical examination

Experimental immunization of the animals from group A did not produce any local reaction or side effect. After challenge, animals from group A remained healthy and did not show detriment to productive parameters (ADG, food consumption or FCE). However, animals from group B had fever from day +2 (i.e. 2 days p.c.) to day +6 (i.e. 6 days p.c.) and clinical signs such as anorexia or lethargy between days +2 and +7 (i.e. 2 and 7 days p.c.). All animals belonging to group C remained in normal health status during the experiment.

Detection of PRRSV in samples collected from pigs

Before challenge, vaccine virus was isolated on SAM from serum samples collected from pigs belonging to group A from 7 days after the first vaccination to 14 days after the second one. However, post-vaccinal viremia was detected earlier when virus isolation was attempted on MARC-145 cell cultures, from 3 days after the first vaccination to 14 days after the second one.

After challenge, PRRSV was only isolated from one serum sample collected from group A on day +2 (i.e. 2 days p.c.) and from two sera on day +8 (i.e. 8 days p.c.). Conversely, in group B all pigs remained viremic from day +2 to the end of the experiment on day +18 (Table 1).

PRRSV was not detected from any of the tissue samples collected from pigs belonging to group A. Conver-

Table 1. Results of virus isolation on swine alveolar macrophages from serum samples collected after challenge

Group	Pig	Days of experiment					
		0	+2	+5	+8	+12	+18
A	1	–	–	–	–	–	–
	2	–	–	–	+	–	–
	3	–	–	–	–	–	–
	4	–	–	–	+	–	–
	5	–	–	–	–	–	–
	6	–	+	–	–	–	–
	Total	0/6[a]	1/6	0/6	2/6	0/6	0/6
B	7	–	+	+	+	+	+
	8	–	+	+	+	+	+
	9	–	+	+	+	+	+
	10	–	+	+	+	+	+
	11	–	+	+	+	+	+
	12	–	+	+	+	+	+
	Total	0/6	6/6	6/6	6/6	6/6	6/6
C	13	–	–	–	–	–	–
	14	–	–	–	–	–	–
	15	–	–	–	–	–	–
	16	–	–	–	–	–	–
	17	–	–	–	–	–	–
	18	–	–	–	–	–	
	Total	0/6	0/6	0/6	0/6	0/6	0/6

[a] Positive/tested.

sely, PRRSV was isolated from lung, spleen, tonsils and lymph nodes (submandibular, iliac and retropharyngeal lymph nodes) from pigs included in group B (Table 2).

All sera and tissue samples collected from group C were negative for virus isolation.

Serology

After immunization, specific PRRS antibodies were detected by ELISA from group A 21 days after the first vaccination in most of the pigs and at challenge (21 days after the second vaccination) in all of them. Moreover, neutralizing antibodies were detected in two animals from group A on day –21 and in four of them on day 0 (Table 3).

After challenge, all pigs belonging to group B had seroconverted by day +18 (i.e. 18 days p.c.) and the titre of neutralizing antibodies reached a maximum value of 1:32 in one animal from group A and another from group B at the end of the experiment (Table 3).

All serum samples collected from group C throughout the experiment were negative for PRRSV antibodies.

Discussion

This study assessed the protective response provided by the commercially available MLV vaccine *Amervac-PRRS*® against homologous PRRSV wild-type strain under experimental conditions. This vaccine is based on the Spanish PRRSV strain VP046BIS, obtained by attenuation of the wild-type strain 5710 in MA-104 cell cultures. *Amervac-PRRS*® is authorized to prevent respiratory distress caused by PRRSV and vaccination is prescribed from the fourth week of age. In this experiment, pigs were vaccinated twice with *Amervac-PRRS*® and challenged with the homologous wild-type strain 5710 of PRRSV three weeks after the second vaccination. During the whole experimental period, humoral immune response was determined. The criteria to evaluate the efficacy of *Amervac-PRRS*® were to

Table 2. Results of PRRSV isolation on swine alveolar macrophages from tissue samples

Group	Pig	Tissue samples							
		Retroph-Ln	**Subm-Ln**	**S-Iliac-Ln**	**Tonsil**	**Kidney**	**Spleen**	**Liver**	**Lung**
A	1	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–
	Total	0/6[a]	0/6	0/6	0/6	0/6	0/6	0/6	0/6
B	7	+	+	–	+	–	+	–	+
	8	+	+	+	+	–	–	–	+
	9	–	–	–	+	–	–	–	+
	10	+	+	–	–	–	–	–	–
	11	+	+	–	+	–	–	–	–
	12	+	–	–	+	–	–	–	–
	Total	5/6	4/6	1/6	5/6	0/6	1/6	0/6	3/6
C	13	–	–	–	–	–	–	–	–
	14	–	–	–	–	–	–	–	–
	15	–	–	–	–	–	–	–	–
	16	–	–	–	–	–	–	–	–
	17	–	–	–	–	–	–	–	–
	18	–	–	–	–	–	–	–	–
	Total	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

[a] Positive/tested. Retroph-Ln: retropharyngeal lymph node. Subm-Ln: submandibular lymph node. S-Illiac-Ln: superficial iliac lymph node.

assess the degree of clinical and virological protection provided by this vaccine.

The results of this study show that pig immunization with the MLV vaccine *Amervac-PRRS*® did not produce any local reaction or side effect. Though, post-vaccinal viremia was detected from 3 days after the first vaccination to 14 days after the second one. Similar results have been previously reported after immunization with other commercial MLV vaccines against PRRSV such as *RespPRRS*®, *Ingelvac*®*PRRS ATP* and *PorcilisPRRS*® (Nodelijk *et al.*, 2001; Pommier *et al.*, 2003; Johnson *et al.*, 2004; Labarque *et al.*, 2004), showing that further research should be done in order to reduce post-vaccinal viremia and, as a result, to improve current MLV vaccines' safety.

The results obtained after challenge showed that animals previously immunized with MLV vaccine *Amervac-PRRS*® did not develop any clinical sign or increase in body temperature associated to PRRSV infection. Resulting from the good health status of the animals, no detrimental effect on productive parameters (ADG, food consumption, FCE) was detected. These results suggest that this MLV vaccine provided high degree of protection against clinical manifestations of the disease. However, the mild clinical reactions observed in the infected control group and the difficulties previously reported to reproduce respiratory symptoms derived from PRRSV infection under experimental conditions (van Woensel *et al.*, 1998; Labarque, 1999; Labarque *et al.*, 2003, 2004) make necessary to evaluate parameters associated to virological protection.

The results of the present study indicate that MLV vaccine *Amervac-PRRS*® was capable of providing remarkable virological protection against PRRSV infection, reducing dramatically post-challenge viremia and preventing PRRSV dissemination to peripheral tissues. Homologous protection has been previously reported in other studies after administration of MLV vaccines such as *Ingelvac PRRS*® and *Prime Pac*®, causing absence of post-challenge viraemia (Hesse *et al.*, 1996; Foss *et al.*, 2002). Likewise, a complete homologous protection against PRRSV has been reported after immunization

Table 3. Serology results obtained by neutralization assay against homologous PRRSV strain

Group	Pig	Days of experiment			
		–42	–21	0	+18
A	1	–	–	–	1:2
	2	–	1:2[a]	1:2	1:16
	3	–	–	1:8	1:8
	4	–	–	1:4	1:4
	5	–	1:2	1:4	1:32
	6	–	–	1:2	1:8
B	7	–	–	–	1:32
	8	–	–	–	1:8
	9	–	–	–	1:16
	10	–	–	–	1:2
	11	–	–	–	1:2
	12	–	–	–	1:2
C	13	–	–	–	–
	14	–	–	–	–
	15	–	–	–	–
	16	–	–	–	–
	17	–	–	–	–
	18	–	–	–	–

[a] Neutralizing antibody titre.

with non-commercial attenuated American PRRSV isolates (Lager *et al.*, 1999; Mengeling *et al.*, 2003).

Despite clinical and virological protection induced by immunization with MLV vaccines have been previously described, little is known about the components of the immune system that effectively protect against PRRSV. In this respect, recent studies highlighted the relevance of neutralizing antibodies in protection against PRRSV in gilts and piglets (Osorio *et al.*, 2002; López *and* Osorio, 2004; López *et al.*, 2004). In the present experiment, neutralizing PRRSV antibodies were detected in pigs of group A 21 days after the first vaccination. However, no anamnestic response was observed after the second vaccination or challenge exposure and the titre of neutralizing antibodies throughout the study was modest, reaching a maximum value of 1:32 in one serum collected at the end of the experiment on day +18 (i.e. 18 days p.c.). In the same way, a moderate post-vaccinal humoral immune response has been previously described in other experiments, whose neutralizing antibody titres were low throughout the whole experimental period (Osorio *et al.*, 1998; Shibata *et al.*, 2000).

Recent studies have shown that the degree of protection provided by neutralizing antibodies against PRRSV infection in young pigs is dose-dependent, being necessary to reach at challenge a minimum serum titre of 1:8 to block extra-cellular viremia and at least 1:32 to get sterilizing immunity (López *and* Osorio, 2004; López *et al.*, 2004). Conversely, although the titre of neutralizing antibodies attained in the present experiment in group A was ≤ 1:8 at challenge, extra-cellular viremia was not detected in 50% of the animals and peripheral tissue dissemination was completely blocked in all of them. Thus, the titre of neutralizing antibodies achieved does not seem to be totally correlated to the level of protection provided by the MLV vaccine *Amervac-PRRS*®, suggesting that a synergic cellular immune response may have been involved. Actually, although the study of the immunity against PRRSV has been previously focused on humoral immune response, characterization of cellular immunity is being carried out (López *et al.*, 1999; Royaee *et al.*, 2004; Lowe *et al.*, 2005). Subsequently, a global understanding of cellular and humoral immunity against PRRSV would provide a useful knowledge to develop new effective vaccines.

In conclusion, the data reported in this study show that pig immunization with the MLV vaccine *Amervac-PRRS*® provides remarkable clinical and virological protection against homologous PRRSV infection. However, further research needs to be done in order to assess the efficiency of *Amervac-PRRS*® to reduce PRRSV transmission, prevent reproductive failure in pregnant females and provide cross-protection against heterologous PRRSV strains, as well as to determine the molecules or cells involved in protective immunity evolvement.

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