The Veterinary Journal xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Effects of challenge with a virulent genotype II strain of porcine reproductive and respiratory syndrome virus on piglets vaccinated with an attenuated genotype I strain vaccine

M. Roca [a,1], M. Gimeno [b,c,1], S. Bruguera [a], J. Segalés [b,c], I. Díaz [b,c], I.J. Galindo-Cardiel [b,c], E. Martínez [a], L. Darwich [b,c], Y. Fang [d,2], J. Maldonado [a], R. March [a], E. Mateu [b,c,*]

[a] Laboratorios Hipra S.A., Avda La Selva 135, Amer, Girona 17170, Spain
[b] Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, Campus de la Universitat Autònoma de Barcelona, Bellaterra, Barcelona 08193, Spain
[c] Centre de Recerca en Sanitat Animal (CReSA), Institut de Recerca i Tecnologia Agroalimentària, Universitat Autònoma de Barcelona, Bellaterra, Barcelona 08193, Spain
[d] Department of Veterinary and Biomedical Science, South Dakota State University, Brookings, South Dakota 57007, USA

ARTICLE INFO

Article history:
Accepted 18 November 2011
Available online xxxx

Keywords:
Porcine reproductive and respiratory syndrome virus
Vaccination
Heterologous protection

ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is endemic in most parts of Asia, where genotype I and II strains of diverse virulence may coexist. This study evaluated the outcome of infection with a highly virulent Asian genotype II PRRSV isolate in piglets vaccinated with a genotype I vaccine. Twenty-one 3-week-old piglets were divided in three groups: Pigs in group V ($n = 8$) were vaccinated with an attenuated genotype I commercial PRRSV vaccine, while pigs in group U ($n = 8$) and a control group (group C; $n = 5$) were unvaccinated; 6 weeks later, pigs in groups V and U were challenged intranasally with a highly virulent strain of genotype II PRRSV (1×10^5 50% tissue culture infectious doses/mL), while pigs in group C received a placebo. Over a period of 21 days after challenge, vaccinated pigs had significantly lower mortality (0/8 versus 2/8), fewer days of fever, a lower frequency of catarrhal bronchopneumonia, higher weight gains (13.4 versus 6.6 kg) and lower levels of viraemia compared to unvaccinated challenged pigs. Immunisation with a genotype I attenuated PRRSV vaccine provided partial protection against challenge with a highly virulent genotype II strain.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major source of economic losses in pigs, causing reproductive failure in sows and respiratory disease in piglets, in the latter case often complicated by secondary bacterial pneumonia. In 2006, 'high fever syndrome', characterised by haemorrhagic lesions, pyrexia and increased mortality, emerged in pigs in China. This disease was caused by genotype II strains of PRRSV that shared a discontiguous 30 (29 + 1) amino acid deletion in the non-structural protein (NSP) 2 region (Li et al., 2007; Tian et al., 2007; Tong et al., 2007). This deletion was not directly related to the virulence of isolates, but instead was an epidemiological marker (Zhou et al., 2009). The highly virulent PRRSV is thought to be derived from a genotype II strain from China, which probably accumulated a series of mutations and changes over time and is now widespread in Southeast Asia (Wu et al., 2009; An et al., 2010).

PRRSV currently is present in all major pig-producing countries of Asia. There is a predominance of genotype II PRRSV strains in the region, but there appears to be an increasing prevalence of genotype I strains (Amonsin et al., 2009; Lee et al., 2010; Wang et al., 2010; Chen et al., 2011) and both PRRSV genotypes may circulate concurrently. Attenuated PRRSV vaccines derived from genotype I or II strains are licensed in several Asian countries, including Vietnam, the Philippines, Thailand and Cambodia.

Strains of PRRSV with low, medium (genotypes I and II) and high (genotype II) virulence may co-circulate in localities where pigs may have been vaccinated against genotype I, genotype II, both genotypes or neither. The aim of the present study was to determine the effects of challenge with a highly pathogenic genotype II strain of PRRSV on pigs vaccinated with an attenuated genotype I vaccine.

* Corresponding author. Tel.: +34 935811046.
E-mail address: enric.mateu@uab.es (E. Mateu).
[1] These authors contributed equally to this work.
[2] Present address: Department of Bio-microbiology, South Dakota State University, Brookings, South Dakota 57007, USA.

1090-0233/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tvjl.2011.11.019

Materials and methods

Experimental design

Twenty-one 3-week-old piglets were randomly selected at weaning from a farm confirmed by serological testing by ELISAs to be free of PRRSV (Herdcheck2XR, IDEXX Laboratories; Civtest Suis PRRS A/S; Civtest Suis PRRS E/S, Laboratorios Hipra S.A.), Aujeszky's disease virus (Civtest Suis ADVgE, Laboratorios Hipra S.A.) and swine influenza virus (Civtest Suis Influenza, Laboratorios Hipra S.A.). Piglets were seropositive for porcine circovirus 2 (PCV2) by ELISA (Ingezim Circo, Ingenasa), but were not viraemic, as determined by PCR (Olvera et al., 2004).

Animals were ear-tagged and moved to experimental facilities, where they were randomly assigned into three groups: V, vaccinated and challenged ($n = 8$), U, unvaccinated and challenged ($n = 8$) and C (control), unvaccinated and not challenged ($n = 5$). One week later (at 4 weeks of age), pigs in group V were vaccinated IM with 2 mL of a commercial attenuated genotype I vaccine (Amervac PRRS, Laboratorios Hipra S.A.) containing $1 \times 10^{4.6}$ 50% tissue culture infectious doses ($TCID_{50}$) of the vaccine virus. Groups U and C received water as a placebo. Animals were kept at the experimental farm for the next 5 weeks and then were transferred to Biosafety Level 3 facilities at the Centre de Recerca en Sanitat Animal (CReSA) for the challenge phase. Pigs were left to acclimatise for 1 week and then animals in groups V and U were challenged intranasally with the highly virulent genotype II strain HP-PRRS21 (1×10^5 $TCID_{50}$/mL, 1 mL/nostril) or received uninfected cell culture as a negative control (group C).

Animals were monitored for the next 21 days and the experiment was terminated by euthanasia of pigs with an overdose of sodium pentobarbital. All experiments were carried out according to internal guidelines for care and use of experimental animals and the model for the vaccination/challenge procedure was approved by the Commission for Ethics in Animals and Human Experimentation of the Universitat Autònoma de Barcelona (CEEAH) (approval number 665).

Viruses

The highly virulent genotype II PRRSV strain HP-PRRS21 was derived from natural cases of highly virulent PRRS in Asia and was propagated in MARC-145 cells and used at passage 3. Titrations were performed in MARC-145 cells and $TCID_{50}$ was assessed by the immunoperoxidase monolayer assay (IPMA) using monoclonal antibody SDOW17 (SDOW17-B; 1/100 dilution; Rural Technologies). To confirm the identity of the virus, complete *nsp2* and *ORF5* segments were amplified by PCR, sequenced and compared to data in GenBank. The *nsp2* and *ORF5* regions of HP-PRRS21 shared >99% nucleotide identity with the equivalent regions of PRRSV JXA1 strain (GenBank EF112445; Tian et al., 2007). The challenge inoculum was free of *Pestivirus* spp. (Hofmann et al., 1994; Katz et al., 1993) and *Mycoplasma* spp. (Wirth et al., 1994; Uphoff and Drexler, 1999) by PCR. The inoculum was also negative for foot-and-mouth disease virus by serial passage in BHK-21 cells and negative for PCV2 by PCR (Olvera et al., 2004).

Clinical, pathological and microbiological evaluation

From the day of challenge until the termination of the experiment, pigs were examined daily for the development of clinical signs. Rectal temperatures were also recorded from day −2 post-inoculation (PI) until the end of the experiment. Fever was defined as a rise in body temperature >40.0 °C. For each day and pig, a clinical score was calculated according to the criteria shown in Table 1. Animals were also weighed individually at day 0 PI and weekly thereafter.

At the termination of the study, pigs were subjected to a detailed postmortem examination and gross lesions were recorded. Tissues from the lungs (right cranial, middle caudal and accessory lobes), tonsils, lymph nodes (submandibular, mediastinal and sternal), spleen and kidneys were collected and one portion from each site was fixed in neutral buffered formalin, embedded in paraffin, stained with haematoxylin and eosin and examined histologically. Interstitial pneumonia was evaluated on the following scale: 0, absence of lesions; 1, mild; 2, moderate; and 3, severe.

When fibrinous polyserositis was evident at postmortem examination, swabs of exudates were inoculated into brain–heart infusion (BHI) plus nicotinamide adenine dinucleotide (NAD), chocolate, Columbia blood and MacConkey agars and incubated for 3 days at 37 °C in 5% CO_2.

Viraemia and humoral response

Blood samples were taken after vaccination, 5 weeks post-vaccination (immediately before moving animals to CReSA) and at days 0, 3, 7, 10, 14 and 21 PI. Sera were used to determine viraemia by reverse transcriptase (RT)-PCR for *ORF5* and virus titration in MARC-145 cells. For RT-PCR, total RNA was extracted from 150 μL serum using Nucleospin RNA (Macherey–Nagel). Reverse transcription was carried out using 1× RT buffer, 300 ng random hexamers, 0.5 μM deoxynucleotide triphosphates (dNTPs), 5 nM dithiothreitol (DTT), 1 μL RNase inhibitor, 100 U RT Superscript II (Invitrogen) and 2 μL total RNA in a total volume of 20 μL. PCR for *ORF5* was performed in a total volume of 50 μL containing 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 25 pmol each oligonucleotide primer (forward, USF 5′-ATGAGGTGGGCAACTGTTTT-3′; reverse 5′-CTTTTGTGGAGCCGTGCTAT-3′), 2 U *Taq* DNA polymerase (Ecogen) and 2.5 μL cDNA.

For virus titrations, sera (100 μL) were diluted from 10^0 to 10^{-6} in modified Eagle's medium and seeded onto MARC-145 cells (8 wells/dilution). Growth of PRRSV was assessed at 96 h by IPMA using monoclonal antibody SDOW17. Antibodies against PRRSV were determined by ELISA (Herdcheck2XR, IDEXX Laboratories) and by a virus neutralisation test (VNT; Díaz et al., 2005) using the highly virulent HP-PRRS21 strain.

Statistical analysis

Statistical analysis was performed with StatsDirect 2.7.8 using the Kruskal–Wallis test with multiple comparisons (Conover–Inman method) and the χ^2 test. Statistical significance was set at $P < 0.05$.

Results

Clinical signs

Prior to inoculation (days −2 to 0 PI) the average rectal temperature of pigs was 39.6 °C (standard deviation 0.25 °C). Inoculated animals developed fever from day 2 PI onwards, although some animals were pyrexic from day 1 PI (Fig. 1a). Most vaccinated animals exhibited a peak of fever by day 7 PI (mean 40.8 ± 0.4 °C, with 3/8 pigs >41.0 °C), with a subsequent return to normality. By day 12 PI, none of the vaccinated pigs had a rectal temperature >40.0 °C. In unvaccinated pigs, there was an initial rise in rectal temperature at day 2 PI, followed by a decrease at day 4 PI (average 39.6 ± 0.4 °C), then another increase from day 5 to day 9 PI (average 40.8 ± 0.4 °C, with 3/8 pigs >41.0 °C) and 2/8 pigs still had rectal temperatures >40.2 °C at day 14. The proportion of pigs with fever was significantly higher ($P < 0.05$) in unvaccinated animals on at least two occasions (days 11 and 12 PI; 5/8 versus 0/8 at both time points). There were no significant increases in rectal temperatures in negative control pigs.

At day 4 PI, all unvaccinated challenged pigs in group U (8/8) exhibited intense conjunctivitis compared to 1/8 animals in the vaccinated group ($P < 0.05$). Lethargy and/or anorexia were observed from day 4 PI; from days 7–17 PI, all pigs (8/8) in group U exhibited varying degrees of lethargy, while for the same period no more than 2/8 pigs in group V exhibited lethargy at any time point. Laboured breathing was evident from days 6–19 PI in up to 4/8 pigs in group U, but only in 1/8 pigs on days 6 and 7 PI in group V. In the unvaccinated group, 2/8 pigs died at day 19 PI, while no deaths were recorded in vaccinated pigs. Fig. 1b shows the evolution of clinical scores. Animals in group C remained healthy throughout the study.

Prior to challenge, body weights were similar in all groups (group V: 31.1 ± 6.4; group U: 32.7 ± 3.8 kg; group C: 31.3 ± 2.3) (Fig. 2). After challenge, pigs in group V gained 13.4 kg from days 0–21 PI, with an average body weight (ABW) of 46.1 kg at day 21 PI, pigs in group U gained 6.6 kg (ABW 37.2 kg at day 21 PI) and pigs in group C gained 21.2 kg (ABW 52.5 kg at day 21 PI) ($P < 0.05$). Pigs from group V gained 6.8 kg more than pigs from group U ($P < 0.05$). There was no weight gain between days 7 and 14 PI in pigs in group U.

Pathology and microbiology

At postmortem examination, gross lesions in vaccinated animals (group V) were limited to interstitial pneumonia (4/8), fibrinous pleurisy and polyserositis (2/8) and interstitial nephritis (1/8). Unvaccinated and challenged pigs (group U) had interstitial pneumonia (5/8), catarrhal bronchopneumonia (4/8; $P < 0.05$), interstitial nephritis (5/8, $P = 0.06$), fibrinous pleurisy and polyserositis (5/8), oedema of the gall bladder (3/8), fibrinous arthritis (3/8) and necrosis of sternal lymph nodes (3/8). Two pigs showed ascites

Please cite this article in press as: Roca, M., et al. Effects of challenge with a virulent genotype II strain of porcine reproductive and respiratory syndrome virus on piglets vaccinated with an attenuated genotype I strain vaccine. The Veterinary Journal (2012), doi:10.1016/j.tvjl.2011.11.019

Table 1
Clinical scores applied in the examination of pigs.

Clinical signs	Score				
	0	+1	+2	+3	+4
Breathing	Normal	Slight dyspnoea	Clear dyspnoea	Dyspnoea + tachypnoea	Dyspnoea + tachypnoea + cyanosis
Behaviour	Active	Apathetic but responding to stimulation	Apathetic even when stimulated	Prostration	Agonal
Anorexia	Normal eating behaviour	Eats less than normal	Does not eat	–	–

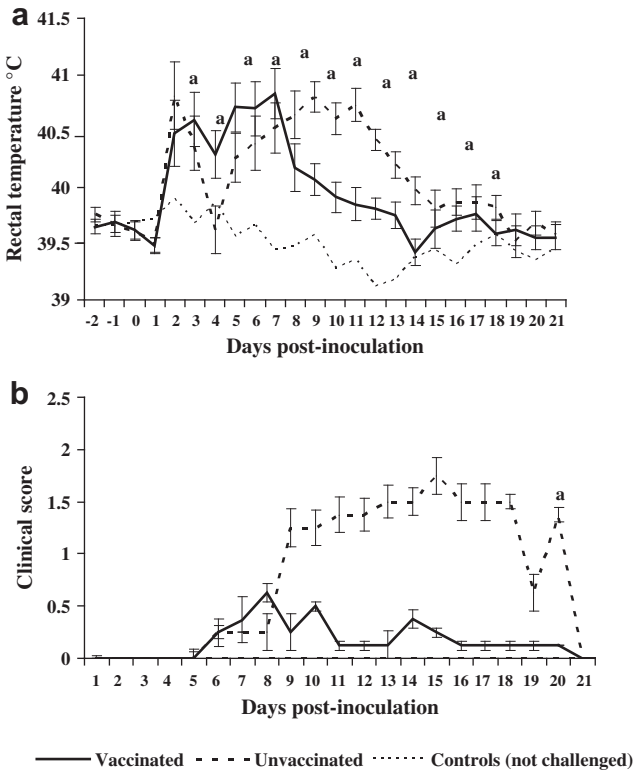

Fig. 1. Changes in rectal temperatures (a) and clinical scores (b) of pigs inoculated with a highly virulent genotype II isolate of porcine reproductive and respiratory syndrome virus (PRRSV). Pigs in group V had been vaccinated previously with an attenuated genotype I PRRSV vaccine. Different letters indicate statistically significant differences among groups ($P < 0.05$) as determined by the Kruskal–Wallis test with multiple comparisons (Conover–Inman method). Bars indicate standard error of the mean.

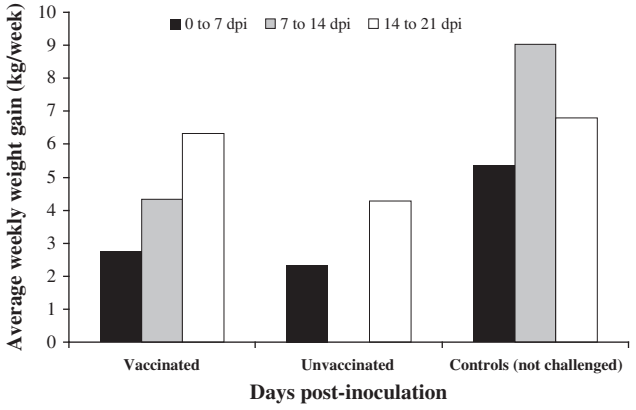

Fig. 2. Weekly weight gains after challenge with a highly virulent genotype II isolate of porcine reproductive and respiratory syndrome virus (PRRSV) in pigs vaccinated with an attenuated genotype I PRRSV vaccine, unvaccinated pigs and unchallenged control pigs. Different letters indicate statistically significant differences ($P < 0.05$) as determined by the Kruskal–Wallis test with multiple comparisons (Conover–Inman method).

or gastric ulcers in each of V and U groups. On histopathological examination, there were no differences in mean score of four lobes for interstitial pneumonia among pigs in groups V and U (1.4 versus 1.6; $P < 0.05$). Histopathological examination of the kidneys confirmed interstitial nephritis. There were no significant lesions in uninfected animals. Microbiological examination of swabs collected from fibrinous exudates did not yield significant pathogens.

Viraemia

At day 3 PI, 6/8 vaccinated pigs (group V) and 7/8 unvaccinated pigs (group U) were viraemic by virus isolation. At day 7 PI, 7/8 vaccinated pigs (group V) and 8/8 unvaccinated pigs (group U) developed viraemia, as shown by virus isolation and PCR. At day 14, only 3/8 vaccinated pigs (group V) were viraemic by virus isolation, while 6/8 unvaccinated pigs (group U) were still viraemic ($P = 0.12$). No virus was detected in serum by virus isolation at day 21 PI (Fig. 3).

There were no differences in viral titres in serum at day 3 PI, with all viraemic animals having viral loads $\geqslant 10^6$ $TCID_{50}$/mL. At day 7 PI, 7/8 vaccinated pigs (group V) that were positive by virus isolation had viral titres in serum from 10^4 to 10^5 $TCID_{50}$/mL, while 8/8 unvaccinated pigs (group U) had $10^{6.3}$ $TCID_{50}$/mL ($P < 0.05$). At 14 days PI, viral loads in viraemic animals were $10^{1.3}$–10^6 $TCID_{50}$/mL.

On day 21 PI, 1/8 pigs in group V and 2/6 surviving pigs in group U were PCR positive but negative on virus isolation (Fig. 3). Unchallenged animals remained negative for PRRSV by PCR and virus isolation.

Humoral immune response

All vaccinated animals were positive for serum antibodies against PRRSV by ELISA at day 0 PI. Challenge did not produce a significant increase in S/P ratios of these previously vaccinated animals. Upon challenge, pigs in group U developed antibodies against PRRSV that were detectable in 4/8 pigs by day 7 PI. All challenged animals were seropositive at day 14 PI. Significant differences in the average S/P ratios were found between vaccinated and unvaccinated pigs for the first 14 days after challenge (Fig. 4), S/P ratios being higher for previously vaccinated pigs. Neutralising antibodies against PRRSV (genotype I or II) were not detected before challenge. At day 21 PI, most of the pigs in groups V and U were negative and none of the animals had neutralisation titres >1:4 against the highly virulent PRRSV.

Discussion

The emergence of highly virulent PRRSV strains in China in 2006 (Tian et al., 2007; Li et al., 2007) has been a cause of much concern

Please cite this article in press as: Roca, M., et al. Effects of challenge with a virulent genotype II strain of porcine reproductive and respiratory syndrome virus on piglets vaccinated with an attenuated genotype I strain vaccine. The Veterinary Journal (2012), doi:10.1016/j.tvjl.2011.11.019

for the pig industry and for animal health authorities worldwide. At present, the co-circulation in Asia of high and medium virulence genotype II strains, along with genotype I strains, and the use of genotype II and genotype I vaccines, creates a unique situation. The present study examined the effects of infection with a highly virulent genotype II PRRSV strain on pigs vaccinated with a genotype I vaccine.

After challenge with virulent PRRSV, unvaccinated animals developed high fever (up to 40.8 °C) and respiratory signs. They had 69% lower average body weight gain from 0 to 21 days PI than unchallenged pigs (+21.2 kg vs. +6.6 kg, respectively). Mortality in unvaccinated pigs was 2/8 (25%). The most prominent lesions at postmortem examination were interstitial pneumonia, interstitial nephritis, oedema of the gall bladder and fibrinous polyserositis. These findings are similar to those observed in pigs infected with highly pathogenic PRRSV in the field (Li et al., 2007; Tian et al., 2007; Tong et al., 2007). In the present study, although these lesions may have derived from bacterial complications, no bacteria were isolated. Furthermore, pigs used for the experiment were screened thoroughly to exclude other common porcine pathogens, including PCV2.

Pigs vaccinated with the PRRSV genotype I vaccine had less severe clinical signs and enhanced weight gains compared to unvaccinated pigs, indicating that the vaccine conferred partial protection against disease due to virulent genotype II PRRSV. No fatalities were recorded among vaccinated animals, lesions (bronchopneumonia and interstitial nephritis) were less frequent in vaccinated pigs compared to their unvaccinated counterparts and the mean body weight of vaccinated pigs was 8.9 kg higher than unvaccinated pigs at 21 days PI. These results suggest that heterologous vaccination is not detrimental when vaccinated pigs are challenged with a virulent PRRSV strain. In a field trial, sows vaccinated with the same genotype I vaccine used in the present study had significantly fewer stillborn piglets than non-vaccinated sows when the farm was infected with a highly pathogenic strain of PRRSV (Quilitis, 2009).

When pigs were vaccinated with an attenuated vaccine based on a highly pathogenic PRRSV strain and challenged with the homologous virulent field strain, protection was dose-dependent (Tian et al., 2009). Thus, pigs vaccinated with 10^3 $TCID_{50}$ of the homologous attenuated strain of PRRSV were partially protected against the development of clinical signs (1/5 animals developed clinical signs and there was no mortality), but rectal temperatures were >40 °C from at least day 5 PI to days 9–10 PI. Protection against development of clinical signs was only achieved with a dose $\geqslant 10^{4.0}$ $TCID_{50}$. In the present study, the dose of the heterologous genotype I vaccine was $10^{4.6}$ $TCID_{50}$ and the result of the vaccination/challenge procedure was similar to that obtained by Tian et al. (2009) using $10^{3.0}$ $TCID_{50}$. This indicates that a certain level of heterologous cross-protection existed and suggests that epitopes potentially relevant for protection are probably shared between strains of different virulence, even within different genotypes. Recognition of such epitopes may be an essential element for the development of universal vaccines against PRRSV even of different genotypes.

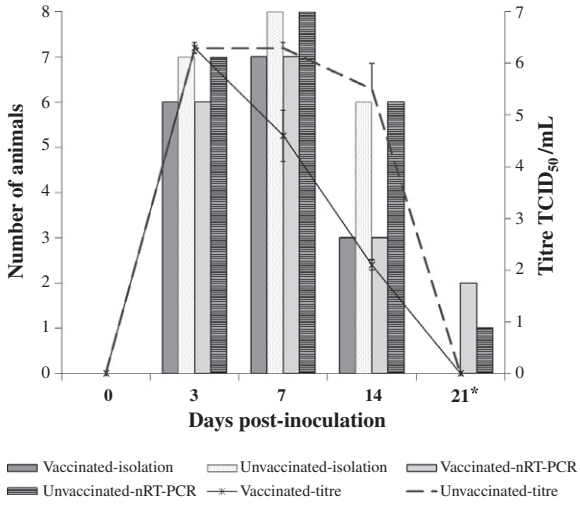

Fig. 3. Viraemia determined by viral titres (titration on MARC-145 cells) and reverse transcriptase (RT)-PCR in vaccinated ($n = 8$) and unvaccinated ($n = 8$) pigs after inoculation with a highly virulent genotype II porcine reproductive and respiratory syndrome virus strain. Error bars show standard deviation of virus load from the positive animals. * At day 21 post-inoculation, two animals in the unvaccinated group died.

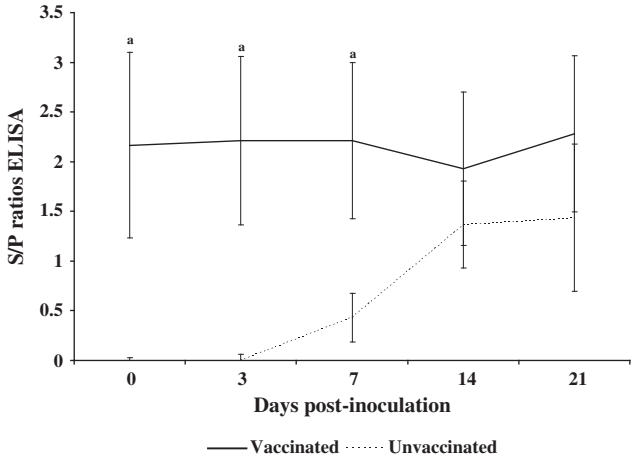

Fig. 4. Humoral immune response as determined by ELISA (S/P ratios) after inoculation with a highly virulent genotype II porcine reproductive and respiratory syndrome virus strain. Bars show standard deviations. Letters indicate statistically significant differences ($P < 0.05$) as determined by the Kruskal–Wallis test.

In our study, 1/8 vaccinated pigs did not have detectable viraemia following challenge with virulent genotype II PRRSV, but there were no statistically significant differences between vaccinated and unvaccinated pigs. Generally, it is assumed that viral loads are associated with PRRSV virulence; for example, the more virulent MN184 and Lena strains produce higher viral loads than other less virulent genotype I or II strains (Johnson et al., 2004; Karniychuk et al., 2010). However, Wu et al. (2009) reported that strains that replicate more efficiently in vitro do not necessarily produce more severe disease. Opriessnig et al. (2007) demonstrated almost complete protection against the development of viraemia in a model of heterologous challenge using only genotype II strains both as immunising and challenge agents.

Since pigs did not develop significant neutralising titres following infection with the challenge strain of PRRSV, cell-mediated immunity is likely to be responsible for the control of infection in vaccinated animals, although cell-mediated immune responses were not measured. Similarly, in a study of heterologous challenge within a given PRRSV genotype, cross-protection was attributed to cell-mediated immunity (Martelli et al., 2009). In contrast, pigs vaccinated with $\geqslant 10^{3.0}$ $TCID_{50}$ of an attenuated highly virulent isolate of genotype II PRRSV developed neutralising antibody titres upon challenge (Tian et al., 2009). This difference probably reflects limited cross-reactivity of neutralising antibodies raised against different genotypes (Plagemann et al., 2002). The extent of cross-protection in genotype I vaccinated pigs infected with virulent genotype II strains does not seems not to be related to the genomic similarity of the strains based on *ORF5* sequencing (Díaz et al., 2006; Prieto et al., 2008).

Please cite this article in press as: Roca, M., et al. Effects of challenge with a virulent genotype II strain of porcine reproductive and respiratory syndrome virus on piglets vaccinated with an attenuated genotype I strain vaccine. The Veterinary Journal (2012), doi:10.1016/j.tvjl.2011.11.019

Conclusions

Immunisation with a genotype I attenuated PRRSV vaccine can afford partial protection against challenge with a highly virulent genotype II strain. This partial protection is sufficient to significantly reduce weight loss and development of clinical signs in vaccinated pigs compared to unvaccinated pigs.

Conflict of interest statement

M. Roca, S. Bruguera, E. Martínez, J. Maldonado and R. March are employees of Laboratorios Hipra S.A. None of the other authors received any funding or honoraria from Laboratorios Hipra S.A., nor had any financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper. This study was funded by Laboratorios Hipra S.A., which provided the animals, the PRRSV strain HP-PRRS21 and the vaccine and carried out the pre-challenge phase of the study. The study was designed by researchers of CReSA in consultation with Laboratorios Hipra S.A. All procedures, data collection, registries, manipulation and analysis of samples and data during the challenge phase were conducted at CReSA by CReSA researchers.

Acknowledgements

We would like to thank Dr Truong van Dung, Director of the National Institute of Veterinary Research in Hanoi (Vietnam) for assisting in the collection of the original samples from which the PRRSV isolate was obtained and for advice regarding animal health and legal considerations for the samples to be exported. We also like to thank Ms Bui Thi My Dung and Mr Thanh Huy from Hipra in Vietnam for their collaboration and efforts in coordination of export of samples from Vietnam. We are also grateful to Joan Casas (Laboratorios Hipra S.A.) and personnel of Biosafety 3 Level facilities of CReSA for assisting with animal handling during the different phases of the study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tvjl.2011.11.019.

References

Amonsin, A., Kedkovid, R., Puranaveja, S., Wongyanin, P., Suradhat, S., Thanawongnuwech, R., 2009. Comparative analysis of complete nucleotide sequence of porcine reproductive and respiratory syndrome virus (PRRSV) isolates in Thailand (US and EU genotypes). Virology Journal 6, 143.

An, T.Q., Tian, Z.J., Xiao, Y., Li, R., Peng, J.M., Wei, T.C., Zhang, Y., Zhou, Y.J., Tong, G.Z., 2010. Origin of highly pathogenic porcine reproductive and respiratory syndrome virus, China. Emerging Infectious Diseases 16, 365–367.

Chen, N., Cao, Z., Yu, X., Deng, X., Zhao, T., Wang, L., Liu, Q., Li, X., Tian, K., 2011. Emergence of novel European genotype porcine reproductive and respiratory syndrome virus in mainland China. Journal of General Virology 92, 880–892.

Díaz, I., Darwich, L., Pappaterra, G., Pujols, J., Mateu, E., 2005. Immune responses of pigs after experimental infection with a European strain of porcine reproductive and respiratory syndrome virus. Journal of General Virology 86, 1943–1951.

Díaz, I., Darwich, L., Pappaterra, G., Pujols, J., Mateu, E., 2006. Different European-type vaccines against porcine reproductive and respiratory syndrome virus have different immunological properties and confer different protection to pigs. Virology 351, 249–259.

Hofmann, M.A., Brechtbühl, K., Stäuber, N., 1994. Rapid characterization of new pestivirus strains by direct sequencing of PCR-amplified cDNA from the 5′ noncoding region. Archives of Virology 139, 217–229.

Johnson, W., Roof, M., Vaughn, E., Christopher-Hennings, J., Johnson, C.R., Murtaugh, M.P., 2004. Pathogenic and humoral immune responses to porcine reproductive and respiratory syndrome virus (PRRSV) are related to viral load in acute infection. Veterinary Immunology and Immunopathology 102, 233–247.

Karniychuk, U.U., Geldhof, M., Vanhee, M., Van Doorsselaere, J., Saveleva, T.A., Nauwynck, H.J., 2010. Pathogenesis and antigenic characterization of a new East European subtype 3 porcine reproductive and respiratory syndrome virus isolate. BMC Veterinary Research 6, 30.

Katz, J.B., Ridpath, J.F., Bolin, S.R., 1993. Presumptive diagnostic differentiation of hog cholera virus from bovine viral diarrhea and border disease viruses by using a cDNA nested-amplification approach. Journal of Clinical Microbiology 31, 565–568.

Lee, C., Kim, H., Kang, B., Yeom, M., Han, S., Moon, H., Park, S., Kim, H., Song, D., Park, B., 2010. Prevalence and phylogenetic analysis of the isolated type I porcine reproductive and respiratory syndrome virus from 2007 to 2008 in Korea. Virus Genes 40, 225–230.

Li, Y., Wang, X., Bo, K., Wang, X., Tang, B., Yang, B., Jiang, W., Jiang, P., 2007. Emergence of a highly pathogenic porcine reproductive and respiratory syndrome virus in the Mid-Eastern region of China. The Veterinary Journal 174, 577–584.

Martelli, P., Gozio, S., Ferrari, L., Rosina, S., De Angelis, E., Quintavalla, C., Bottarelli, E., Borghetti, P., 2009. Efficacy of a modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in pigs naturally exposed to a heterologous European (Italian cluster) field strain: Clinical protection and cell-mediated immunity. Vaccine 27, 3788–3799.

Olvera, A., Sibila, M., Calsamiglia, M., Segalés, J., Domingo, M., 2004. Comparison of porcine circovirus type 2 load in serum quantified by a real time PCR in postweaning multisystemic wasting syndrome and porcine dermatitis and nephropathy syndrome naturally affected pigs. Journal of Virological Methods 117, 75–80.

Opriessnig, T., Baker, R.B., Halbur, P.G., 2007. Use of an experimental model to test the efficacy of planned exposure to live porcine reproductive and respiratory syndrome virus. Clinical and Vaccine Immunology 14, 1572–1577.

Plagemann, P.G., Rowland, R.R., Faaberg, K.S., 2002. The primary neutralization epitope of porcine respiratory and reproductive syndrome virus strain VR-2332 is located in the middle of the GP5 ectodomain. Archives of Virology 147, 2327–2347.

Prieto, C., Alvarez, E., Martínez-Lobo, F.J., Simarro, I., Castro, J.M., 2008. Similarity of European porcine reproductive and respiratory syndrome virus strains to vaccine strain is not necessarily predictive of the degree of protective immunity conferred. The Veterinary Journal 175, 356–363.

Quilitis, M., 2009. Effect of a modified live PRRSV vaccine (AMERVAC®PRRS) in a farm infected by a Chinese-like PRRSV strain. In: Proceedings of the 4th Congress of the Asian Pig Veterinary Society, Tsukuba, Japan, 26–28 October 2009, pp. 276 (Abstract WS2-P-072).

Tian, K., Yu, X., Zhao, T., Feng, Y., Cao, Z., Wang, C., Hu, Y., Chen, X., Hu, D., Tian, X., Liu, D., Zhang, S., Deng, X., Ding, Y., Yang, L., Zhang, Y., Xiao, H., Qiao, M., Wang, B., Hou, L., Wang, X., Yang, X., Kang, L., Sun, M., Jin, P., Wang, S., Kitamura, Y., Yan, J., Gao, G.F., 2007. Emergence of fatal PRRSV variants: Unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. PLoS ONE 2, e526.

Tian, Z.J., An, T.Q., Zhou, Y.J., Peng, J.M., Hu, S.P., Wei, T.C., Jiang, Y.F., Xiao, Y., Tong, G.Z., 2009. An attenuated live vaccine based on highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) protects piglets against HP-PRRS. Veterinary Microbiology 138, 34–40.

Tong, G.Z., Zhou, Y.J., Hao, X.F., Tian, Z.J., An, T.Q., Qiu, H.J., 2007. Highly pathogenic porcine reproductive and respiratory syndrome, China. Emerging Infectious Diseases 13, 1434–1436.

Uphoff, C.C., Drexler, H.G., 1999. Detection of mycoplasma contaminations in cell cultures by PCR analysis. Human Cell 12, 229–236.

Wang, C., Wu, B., Amer, S., Luo, J., Zhang, H., Guo, Y., Dong, G., Zhao, B., He, H., 2010. Phylogenetic analysis and molecular characteristics of seven variant Chinese field isolates of PRRSV. BMC Microbiology 10, 146.

Wirth, M., Berthold, E., Grashoff, M., Pfützner, H., Schubert, U., Hauser, H., 1994. Detection of mycoplasma contaminations by the polymerase chain reaction. Cytotechnology 16, 67–77.

Wu, J., Li, J., Tian, F., Ren, S., Yu, M., Chen, J., Lan, Z., Zhang, X., Yoo, D., Wang, J., 2009. Genetic variation and pathogenicity of highly virulent porcine reproductive and respiratory syndrome virus emerging in China. Archives of Virology 154, 1589–1597.

Zhou, L., Zhang, J., Zeng, J., Yin, S., Li, Y., Zheng, L., Guo, X., Ge, X., Yang, H., 2009. The 30-amino-acid deletion in the Nsp2 of highly pathogenic porcine reproductive and respiratory syndrome virus emerging in China is not related to its virulence. Journal of Virology 83, 5156–5167.

Please cite this article in press as: Roca, M., et al. Effects of challenge with a virulent genotype II strain of porcine reproductive and respiratory syndrome virus on piglets vaccinated with an attenuated genotype I strain vaccine. The Veterinary Journal (2012), doi:10.1016/j.tvjl.2011.11.019