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EUROPEAN SEROTYPE PRRSV VACCINE PROTECTS AGAINST EUROPEAN SEROTYPE CHALLENGE WHEREAS AN AMERICAN SEROTYPE VACCINE DOES NOT

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1. SUMMARY

Pigs were either vaccinated with an American serotype Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) vaccine or with a European serotype vaccine. A control group of was left unvaccinated. At four weeks after vaccination the PRRSV-specific antibody titres were determined and one third of each group was challenged with a Spanish, one third with a German and one third with a Dutch PRRSV wild type strain. The serological responses, measured at 4 weeks after vaccination, confirmed that both vaccines were of a different serotype. It was demonstrated that vaccination with an American serotype vaccine slightly reduced the amount of viraemia after challenge with European PRRSV wild type strains. Only after challenge with the Spanish PRRSV strain a moderate, and statistically significant, reduction in viraemia was observed. This is in contrast to vaccination with a European vaccine strain, where viraemia was completely suppressed after challenge with the German PRRSV isolate and almost completely suppressed after challenge with the Spanish and Dutch isolates.

2. INTRODUCTION

The Porcine Reproductive and Respiratory Syndrome virus, a member of the Arteriviruses, has become endemic in pigs since its discovery in 1991. Although structurally similar, PRRSV isolates can be classified into two distinct serotypes; the North-American serotype (further refered to as the American serotype) and the European serotype (Wens-

Coronaviruses and Arteriviruses, edited by Enjuanes *et al.*
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voort *et al.*, 1992). The serological differences (Wensvoort *et al.*, 1992; Nelson *et al.*, 1993; Drew *et al.*, 1995) are complemented by extensive sequence data (Meng *et al.*, 1994; Mardassi *et al.*, 1995; Murtaugh *et al.*, 1995; Kapur *et al.*, 1996; Suarez *et al.*, 1996) all supporting the distinction between the American and the European serotypes. Now the first vaccines against PRRSV have been brought onto the market it has become quite relevant to investigate the extent of cross-protection between both serotypes.

3. MATERIALS AND METHODS

Two groups, each containing 30 five-weeks old fattening pigs were used for vaccination. One group was vaccinated with Porcilis PRRS, a European based vaccine strain, the other group with a commercial available vaccine based on an American strain. Six sentinel pigs were placed with each group to monitor the spreading of the vaccines. Vaccinations were all done according the manufactures instructions. In addition one group of twelve non-vaccinated pigs was used as a control group. At 4 weeks after vaccination all groups were challenged. One third of each group was challenged with a Spanish (S1), one third with a German (DL3) and one third with a Dutch (I2) PRRSV wild type strain.

The spreading of both vaccine virus strains was determined by the serological examination of the sentinels for the presence of PRRSV specific antibodies at 4 weeks post vaccination. The harvested serum was placed in 2-fold serial dilutions on plates of PRRSV-infected MA104 cells. To prepare the plates MA104 cells were infected with either a European or an American PRRSV isolate and at 24 to 30 hours after infection, fixed with 96% ethanol of -70°C for period 30 min at room temperature. Before the plates were incubated, 2 wash steps with PBS were performed to remove the ethanol. After the sera had been incubated on the plates for 60 min at 37°C, 3 wash steps with PBS were performed and a FITC labelled anti-swine conjugate was added to the plates. After 1 hour of incubation and 3 new washes with PBS, 50 µl of 70% (v/v) glycerol in PBS pH 8.4, was added and the end points determined by fluorescence microscopy. Based on the number of seroconverted sentinels, the reproduction ratio was calculated according the method of Becker.

The efficacy of both vaccines was determined by the amount of viraemia after challenge.

Blood taken from the jugular vein was centrifuged for 10 min. at 1000x*g* and the serum harvested. The serum was incubated in 10-fold serial dilutions on primary porcine macrophages. At 7 days post-inoculation, cells were scored for the presence of CPE and the 50% tissue culture infectious dose was determined. From these data the average titre per group on each sampling day were determined and an estimation was made of the total amount of virus excreted in the blood. The total amount of viraemia was estimated by calculating the area of the average virus titres per day. All date were analyzed with an ANOVA using Fishers least significance test.

4. RESULTS

At challenge, 4 weeks post-vaccination, an average PRRSV-specific antibody titre of 11.7 ($\log_2$) was found in the animals vaccinated with the European serotype and an average titre of 8.6 ($\log_2$) in the pigs vaccinated with the American serotype. However, when an American serotype virus was used in the test system, an average PRRSV specific anti-

Table 1. Average PRRSV specific antibody titres (log_2) per group determined on MA104 cells infected with either a European or American serotype virus

	Average PRRSV specific antibody titres at different weeks after vaccination in the test system		
	European serotype virus		American serotype virus
Vaccine	0	4*	4*
European serotype vaccine	0	11.7	8.3
American serotype vaccine	0	8.6	12.4
Controls	0	0	0

*At challenge. Bold, Statistically significantly better than the competitor vaccine (α = 0.05).

body titre of 8.3 (log_2) was found in the animals vaccinated with the European serotype and 12.4 (log_2) in the pigs vaccinated with the American serotype. PRRSV-specific antibody titres of the vaccinated groups, determined in the homologous system were significantly (α = 0.05) higher than titres found in the heterologous system (Table 1). At challenge the controls were all negative for PRRSV-specific antibodies.

With regard to the number of seroconverted sentinel pigs, significantly more sentinels were seroconverted in the American serotype vaccinated group (5 out of 6) than in the European serotype vaccinated group (1 out of 6). In this experiment is was also demonstrated that the use of either serotype in the test system will lead to a number of false negatives (Table 2).

At the time of challenge no vaccine virus could be detected. The average challenge virus titres in the blood after challenge and the total amount of challenge virus shed in the blood stream are given in Table 3. The total amount of challenge virus shed in the blood, visualised in Figure 1, was strongly reduced by vaccination with the European serotype vaccine and were significantly less than that found in both the controls and in the American serotype-vaccinated pigs. This is in sharp contrast to the results with the American serotype-vaccinated pigs. Only after challenge with the Spanish strain a moderate and statistically significant reduction in viraemia was found. After challenge with the two other European challenge viruses no statistically significant differences were observed between the American serotype-vaccinated animals and the non-vaccinated animals.

Table 2. Number of seroconverted sentinel pigs per group determined by seroconversion at 4 weeks post-vaccination using MA104 cells infected with a either a European or an American serotype virus and the resulting R_0 for each vaccine

	Number of seroconverted sentinels from a total of 6 in the test system		Reproduction ratio vaccine strains* (R_0 [95% interval])
Vaccine	European serotype virus	American serotype virus	
European serotype vaccine	1	1	0.032 [0;0.08]
American serotype vaccine	2	5	≥0.14 [0.09;0.19]

*Reproduction ratios were calculated by the method of Becker.

Table 3. Statistical analysis of the average PRRSV challenge virus titres (log_{10}) found in serum per group and the total virus excretion during the experiment (log_{10} x day)

Vaccine	Challenge virus	Titre (log_{10}) at days post-challenge 3	7	10	14	Total (log_{10} x days)
European serotype	I2	0.2	**0.2**	**0**	0.5	**2.3**
American serotype	I2	1	1.8	1.5	0.5	15.9
Controls	I2	0.6	2.2	2.4	1.1	20.3
European serotype	S1	**0.1**	**0.2**	**0.2**	**0.6**	**3.1**
American serotype	S1	1.2	1.2	1.4	0.3	13.7
Controls	S1	2.1	1.3	2.6	1.9	26.2
European serotype	DL3	0	**0**	**0**	**0**	**0.0**
American serotype	DL3	1.2	2	1.2	0.1	15.9
Controls	DL3	1.5	2.2	2.1	0.5	21.0

Bold: significantly better than the competitor vaccine ($\alpha = 0.05$); underlined: significantly better than the controls ($\alpha = 0.05$).

5. DISCUSSION

Due to the fact that clinical signs of PRRSV are quite variable and hard to reproduce in fattening pigs we chose to assess the efficacy by determining the reduction in viraemia. It is obvious that a reduction in viraemia will proportionally decrease the clinical signs caused by PRRSV. Additionally, the reduction of viraemia is quite indicative for the curtailing of viral spread.

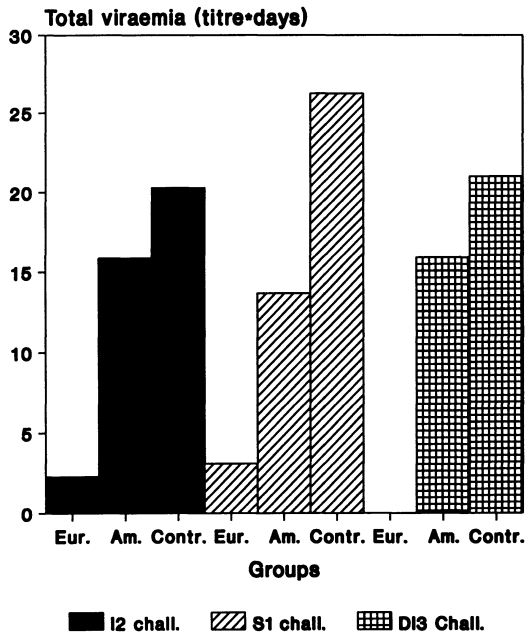

Figure 1. Total amount of viraemia (titre x day) during the experiment after challenge with 3 different European PRRSV strains found in the controls and pigs vaccinated with either an American or a European serotype PRRSV strain.

From serology data and sequence data it was already evident that large differences were present between the European and the American PRRSV strains, suggesting that both types belong to distinct genotypes. The reported serological differences between both PRRSV types were confirmed in this experiment. Sera of pigs vaccinated with the American serotype, incubated on cells infected with a European serotype, had significantly lower end points than the sera from European serotype-vaccinated pigs and vice versa. The necessity to use a test system with a reporter virus of the same serotype was demonstrated by the sentinels included in the test. The relatively low titres of 3 sentinels placed with the American serotype-vaccinated pigs were not detected in the immunofluorescence test using a European reporter virus. Only when a reporter virus of the same serotype was used, these animals were scored as being seropositive. The fact that the American serotype vaccine spreaded significantly more than the European serotype vaccine indicates that the American strain used in this vaccine was less attenuated than the European strain used in this experiment.

With regard to the efficacy it was clearly demonstrated that the reported serological differences and the genomic differences between the American and the European isolates have serious practical consequences with regard to vaccination. Vaccination with an American serotype vaccine will certainly not prevent a European strain from replicating in a vaccinated host. Although not statistically significant in 2 out of 3 challenges, vaccination with an American strain seems to suppress the challenge virus replication to some extent. However, when compared to the European serotype-vaccinated pigs a far more effective suppression and even a total suppression of challenge virus replication in the host could be obtained. Inevitably, after vaccination with an American serotype virus, European wild type viruses are still able to spread substantially more than after using a European serotype vaccine. The demonstrated lack of cross protection shows that both PRRSV types not only differed in their previously reported serological response but that their immunologically distinct. The American and European PRRSV strains therefore, represent two separate PRRSV types with their own unique immunological characteristics. With regard to the practical consequences of this observation, due to the absence of clinical signs in the none vaccinated controls after challenge, it could not be determined if vaccination with an American serotype vaccine might result in a reduction of clinical signs. However, based on the reduction in viraemia, it is clear that a European-serotype vaccine probably will be proportionally more efficacious in the prevention of clinical signs caused by European wild type strains. These results therefore, show that the control of PRRSV in Europe can only be achieved by using European serotype-based vaccines.

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