Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

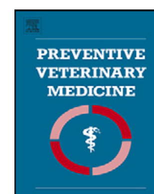
Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Preventive Veterinary Medicine 92 (2009) 31–37

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

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



Frequency of PRRS live vaccine virus (European and North American genotype) in vaccinated and non-vaccinated pigs submitted for respiratory tract diagnostics in North-Western Germany

Elisabeth grosse Beilage [a,*], Heiko Nathues [a], Diana Meemken [a], Timm C. Harder [b], Marcus G. Doherr [c], Inga Grotha [d], Irene Greiser-Wilke [d]

[a] Field Station for Epidemiology, University of Veterinary Medicine Hannover, Buescheler Str. 9, D-49456 Bakum, Germany
[b] Institute of Diagnostic Virology, Friedrich-Loeffler Institute, Suedufer 10, D-17493 Greifswald-Insel Riems, Germany
[c] Dept. Clinical Research & Veterinary Public Health, Vetsuisse Faculty, University of Bern, Bremgartenstr. 109a, CH-3012 Bern, Switzerland
[d] Institute of Virology, Department of Infectious Diseases, University of Veterinary Medicine Hannover, Buenteweg 2, D-30559 Hannover, Germany

ARTICLE INFO

Article history:
Received 16 March 2009
Received in revised form 18 June 2009
Accepted 23 July 2009

Keywords:
PRRS
Live vaccine
Isolates
Spread

ABSTRACT

The frequency of PRRSV corresponding to live vaccines and wild-type was determined in 902 pigs from North-Western Germany submitted for post-mortem examination. Overall, 18.5% of the samples were positive for the EU wild-type virus. EU genotype vaccine virus was detected in 1.3% and the NA genotype vaccine virus in 8.9% of all samples. The detection of the EU vaccine was significantly higher in pigs vaccinated with the corresponding vaccine (OR = 9.4). Pigs vaccinated with NA genotype had significantly higher detection chances for the corresponding vaccine virus when compared to non-vaccinated animals (OR = 3.34) animals, however, NA vaccine was also frequently detected in non-vaccinated pigs. Concluding, the dynamics of NA genotype vaccine and EU wild-type virus corresponds with studies on PRRSV spread in endemically infected herds. The potential of spontaneous spread of the NA genotype vaccine should be considered in the planning of eradication programs.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) virus emerged in Europe in the early 1990s and rapidly spread over the continent, thus becoming one of the major causes of economic losses for swine producers. Simultaneously, the disease emerged in the USA with a similar impact on pig production (Cho and Dee, 2006). Genetic and antigenic comparisons of viruses in Europe and North America showed that PRRS virus segregates into the European (EU) and the North American (NA) genotypes (Allende et al., 1999). A high degree of variability has been demonstrated within both genotypes (Stadejek et al., 2008). Historically, EU and NA genotype viruses have been restricted to the respective continents.

Since 1996, when the first modified live vaccine of the NA genotype (Ingelvac® PRRS MLV, Boehringer Ingelheim, Germany) was introduced in Denmark and Germany, this virus was increasingly being detected in these countries. However, it has also been reported to have appeared in other countries such as Italy, Austria, Poland and Hungary, where the NA genotype vaccine is not authorised (Botner et al., 1997; Storgaard et al., 1999; Martelli, 2002; Indik et al., 2005; Oleksiewicz et al., 2005; Balka et al., 2008; Lillie et al., 2008). Although a basic requirement of live vaccine virus strains is that natural transmission of vaccine virus is minimal or non-existent (Mateu and Diaz, 2008), this widespread appearance of NA vaccine viruses across

* Corresponding author. Tel.: +49 4446 9599115; fax: +49 4446 9599112.
E-mail address: elisabeth.grosse.beilage@tiho-hannover.de (E. grosse Beilage).

0167-5877/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.prevetmed.2009.07.010

Europe raises the suspicion that it is spreading independently from vaccination (Botner et al., 1997; Oleksiewicz et al., 1998).

In 2001, a live EU genotype vaccine from the Lelystad phylogenetic cluster (strain DV; Porcilis® PRRS, Intervet, Boxmeer, The Netherlands) was authorised in all EU member states. To date, only limited information concerning the ability of spontaneous spread of this DV vaccine strain is available (Astrup and Riising, 2002; Voglmayr et al., 2006).

The objectives of this study were (1) to determine the frequency of the EU and the NA genotype PRRS vaccine viruses in a large sample of pigs from North-Western Germany, (2) to assess the association between vaccine isolate detection and vaccination history and (3) to characterise the identified vaccine isolates considering their origin.

2. Materials and methods

2.1. Study design

2.1.1. Origin of the pigs

The pigs in the study predominately (71%) originated from 10 counties in the Western part of Lower Saxony and North-West of North-Rhine Westphalia, Germany. These counties have a pig density ranging between 543 and 1371 pigs per 100 acre of area used for agriculture (Bäurle and Windhorst (2005)). Together they harbour about 8.1 million pigs, which is approximately 30% of the German pig population (Gatzka et al., 2005). A further 15.4% of the pigs were from neighbouring counties with a pig density between 300 and 500 pigs per 100 acre of area used for agriculture. The remaining 13.6% of the pigs were from various regions with lower pig densities.

2.1.2. Selection of pigs and samples

The study was carried out between January and December 2007. During that period 1970 pigs were submitted to the Field Station for Epidemiology for post-mortem examination. Since on-farm necropsies are not allowed in Germany, the Field Station is the only institute offering post-mortem examinations in the Northwest of Lower Saxony. Nine hundred and two of these pigs aged between 1 and 25 weeks of life had a history of respiratory disease and/or emaciation and were included in the study. The 902 pigs belonging to 488 submissions originated from 439 herds and were submitted by 141 different veterinary practices. Almost 21% of the pigs submitted were sent as a single pig from one farm on any one day, 47% from a submission with two pigs and 32% from submissions with three to five pigs per herd.

Lung tissue from these pigs was subsequently frozen at −20 °C and later tested for PRRSV by a *nonaplex* RT-PCR in an accredited diagnostic laboratory (IVD GmbH, Hannover, ISO 17025/2005, AKS-P-20320-EU). Samples positive for PRRSV by *nonaplex* RT-PCR were selected for further examination and genetic typing of PRRSV.

2.1.3. Data collection

The vaccination history of the 902 pigs was obtained from the veterinary surgeons who had submitted the pigs for examination. Telephone interviews were conducted by one of two investigators that followed a standardised questionnaire. The questionnaire consisted of six closed (predefined range of answers or yes/no) questions on PRRS vaccination management/history of the herds of origin. It was hypothesised that these variables were related to the detection of the vaccine and/or wild-type viruses. The questionnaire was pre-tested on three veterinarians by the corresponding author to assess the clarity of questions.

Three binary (positive/negative) diagnostic outcome variables were defined: pig (sample) detection status of (a) PRRS wild-type virus, (b) PRRS EU genotype vaccine virus and (c) PRRS NA genotype vaccine virus.

All potential risk factors (with coding) that were examined for their association with the above listed outcome variables are described in Table 1.

2.2. Detection of PRRSV specific sequences by RT-PCR

2.2.1. Nonaplex RT-PCR

The initial analysis of the samples was performed in an accredited commercially operating diagnostic laboratory using a *nonaplex* RT-PCR. Targeting by a two-tube random-primed RT-PCR protocol six viral and two mycoplasmal

Table 1
Explanatory variables used to identify risk factors for the occurrence of PRRS EU and NA genotype live vaccine virus in pigs from North-Western Germany (2007).

Variable	
Production stage	The age of the pigs, which was usually not available, is described by the production stage. "Suckling pigs" are up to 4 weeks old. "Weaning pigs" usually are 4–10 weeks of age. "Growing pigs" have entered the finishing barns and are 11–15 weeks of age. "Fattening pigs" cover the time from 16 weeks to slaughter. The information was cross-checked with the body weight of each pig.
Piglet vaccination	Pigs could have been vaccinated with an EU genotype modified live vaccine (Porcilis® PRRS), a NA genpotype modified live vaccine (Ingelvac® PRRS MLV) or they were non-vaccinated.
Piglet vaccination time	The week of age when the piglets were vaccinated.
Sow vaccination	Sow herds could have been vaccinated with an EU genotype modified live vaccine (Porcilis® PRRS), a NA genpotype modified live vaccine (Ingelvac® PRRS MLV), with an inactivated vaccine (Ingelvac® PRRS KV or Progressis®) or they were non-vaccinated.
Sow vaccination scheme	Sow herds were vaccinated at a regular interval of 3–5 month or were vaccinated at the 60th day of each gestation and the 6th day after each birth (6/60 scheme).
Sow housing at the same site as the submitted pigs	Sows are housed at the same site where the pigs submitted for necropsy are from (yes/no).

agents: PRRSV (discerning between the EU and NA genotype based on the sequence of ORF7), porcine circovirus type-2, porcine respiratory coronavirus, swine influenza virus, porcine cytomegalovirus, and the mycoplasma species *hypopneumoniae* and *hyorhinis*. It also includes an internal positive control based on detection of transcripts of a porcine single-copy gene. Evaluation is performed using standard agarose gel electrophoresis. Estimates of sensitivity and specificity exceed 90% since for both viral and bacterial targets no unspecific amplificates have been obtained with reference material of closely related pathogens and detection limits measured for PCV-2, PRRSV, and SIV ranged from 50 to 500 DNA plasmid-based copies per assay in combined, multiplex amplifications.

2.2.2. DV PCR

All samples positive for the ORF7 fragment of the EU genotype by *nonaplex* RT-PCR were re-examined by a conventional DV-specific ORF5 RT-PCR. The primers DV-Orf5-FW-221 (5′-GCG GGC GGT ATG TAC TCT G-3′; position 313–332; $ATg_{orf5} = 1$) and DV-Orf5-RV-531 (5′-GTG ACG AGG TTG CCG TCG A-3′; position 531–512; $ATg_{orf5} = 1$) were used for cDNA amplification after random-primed reverse transcription employing the Superscript II polymerase (Invitrogen, Karlsruhe, Germany). PCR was carried out with HotStar *taq* (Qiagen, Hilden, Germany) after denaturation for 15 min at 95 °C and 35 cycles of 20 s at 95 °C, 15 s at 56 °C, 20 s at 72 °C and a final elongation (10 min at 72 °C).

2.2.3. RNA isolation and cDNA synthesis for genetic typing

Samples positive for PRRSV by *nonaplex* RT-PCR were further processed for genetic typing. For reverse transcription, 8 μl of RNA were added to 18 μl of a mixture containing 1× RT buffer and 2.5 mM of each dNTP (Roche, Mannheim, Germany), and heated to 80 °C for 5 min. Immediately thereafter the mixture was transferred to an ice bath. Then 25 μl of a master mix consisting of 0.1 mM DTT, 100 μM random hexamers (Roche, Mannheim, Germany), 2.5 mM $MgCl_2$, and 10,000 U of Superscript III Reverse Transcriptase (Invitrogen, Karlsruhe, Germany) were added. The samples were incubated for 5 min at 25 °C, 60 min at 55 °C, and 5 min at 99 °C. After cooling to 4 °C, the cDNAs were either used directly for PCR or stored at −20 °C.

2.2.4. RT-PCR: primers and assay conditions

The primers Forward 322 (5′-CGG TTG CTI CAT TTC ITG AC-3′) and Reverse 323 (5′-CAC CTT IAG GGC ITA TAT CA-3′) flanking the ORF5 were used for amplification and sequencing. These primers amplify a product of 777 bp from the EU genotype strain Lelystad, and 788 bp from the NA genotype strain VR 2332.

The PCR reactions were performed in a volume of 50 μl. The PCR mix consisted of 1× PCR reaction buffer, 200 μM of each dNTP (Roche, Mannheim, Germany), 2.5 mM $MgCl_2$, 10 pmol of each primer and 1 IU of *taq* polymerase (Invitrogen, Karlsruhe, Germany), and 4 μl of cDNA. Amplification was performed for 39 cycles: denaturation at 94 °C for 45 s, annealing at 50 °C for 45 s, and elongation at 72 °C for 1 min. After this, the samples were incubated for additional 10 min at 72 °C and then cooled to 4 °C until further processing.

In the case of negative reactions for samples considered to be positive as shown by the results of the *nonaplex* RT-PCR, the PCR products were subjected to a second round of amplification using previously described genotype-specific primers (Oleksiewicz et al., 1998). For this PCR, the annealing temperature was set to 60 °C.

2.3. Sequencing

After verifying the success of RT-PCR by electrophoresis in 1.5% agarose gels followed by ethidium bromide staining, the amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Purified products were cycle-sequenced in both directions by MWG (Ebersberg, Germany) using the corresponding PCR primers.

2.4. Genetic typing

Nucleotide sequences were edited and analysed using BioEdit 7.0.9 (Hall, 1999), and aligned using ClustalW (Thompson et al., 1997). The % identities were calculated using the menu alignment, sequence identity matrix as implemented in BioEdit. The sequences of the EU genotype strains Lelystad (M96262) and Porcilis® PRRS-DV (DQ324710) and of the NA genotype strains VR-2332 (AY150564) and Ingelvac® PRRS MLV (AF535152) were included in the phylogenetic analyses. Sequences from the Eastern European subgroups EU-2, EU-3 and EU-4 (Stadejek et al., 2006) were downloaded from GenBank (DQ324677; DQ324686; DQ324696; DQ324694; DQ324671; DQ324682; DQ324667).

2.5. Statistical analysis

Questionnaire and laboratory data were entered into a spreadsheet program (Excel for Office 2007; www.microsoft.com) and further analysed using the statistical packages NCSS 2007 (www.ncss.com) and STATA 10 (www.stata.com).

The population studied consists of pigs submitted for routine diagnostic of respiratory disease and/or emaciation to the Field Station for Epidemiology. Patently, one cannot expect data from one, or even a few, animals from one farm to be representative of herd status. For this reason the present analyses are based on individual pigs as the statistical unit. The occurrence of PRRSV at the individual pig level was combined with data to facilitate statistical extrapolation.

After demonstrating a high agreement between results of the differentiating DV PCR and the sequencing of the detected EU genotype virus isolates (details not shown) it was decided to use the DV PCR results for EU genotype vaccine isolate identification.

In a first analysis step, the survey tab module of STATA 10 was used to derive sample (PCR positivity) prevalence estimates with exact confidence limits for the presence of each of the outcomes in (a) non-vaccinated, (b) EU

genotype vaccinated and (c) NA genotype vaccinated pigs. The analysis was adjusted for survey design structure, i.e. the potential clustering effect of farm of origin when multiple pigs per farm had been submitted for examination during the study period. Wald adjusted *P*-values were derived to assess the association between vaccination status and PRRS virus detection for each outcome.

In order to assess whether, within each outcome group, the prevalence of EU wild-type virus or vaccine isolate, respectively, and NA vaccine isolate detection differed, the observed isolate detection frequencies were compared to those expected under the null hypothesis (no difference). These expected frequencies were adjusted for the fact that, in the sample, the proportion of piglets vaccinated with either vaccine isolate differed substantially (standardisation).

In a final analysis, the robust logistic and Poisson regression routines of STATA 10 with farm as a cluster variable were employed to derive odds ratios (OR; logistic regression model) and incidence risk ratios (IRR, Poisson regression model) with exact confidence intervals for the association between the specific PRRS isolate detection and possible risk factors. The models always contained (a) pig vaccination status (no vaccination, EU genotype vaccination, NA genotype vaccination) and (b) age of the animal when submitted for diagnostics (4 categories). The variables (c) own breeding sows (yes, no) and (d) sow-level vaccination status (no vaccination, EU genotype vaccination, NA genotype vaccination, inactivated EU genotype vaccination) were tested for (i) their statistical significance in the model and, if non-significant, their confounding effect on the factors (a) and (b). Confounding was defined as a change in the parameter estimates of the remaining risk factors in the model of >20%. Factors that were neither significant nor confounders were dropped from the respective models. Due to the low frequency in some outcomes, interactions between risk factors were not included. The alpha level of statistical significance was set to 0.05.

3. Results

3.1. Detection rates and genotypes of PRRSV

Overall, 18.5% (95% CI: 16.7–21.7%) of the examined samples with valid results ($n = 902$) were positive for the PRRS EU wild-type virus by *nonaplex* RT-PCR. The EU genotype vaccine virus was detected in 1.3% (0.76–2.3%) and the NA genotype vaccine virus in 8.9% (6.9–11.4%) of all samples (Table 2).

The production stage-specific detection rates for EU wild-type virus and NA genotype vaccine virus showed the highest values in weaning and growing pigs (Fig. 1).

Detection prevalence of the EU wild-type virus was independent of the vaccination status ($P = 0.3159$). However, the detection of EU genotype vaccine virus was significantly higher in pigs exposed to the corresponding vaccine virus ($P = 0.0302$), and the detection of NA genotype vaccine virus was significantly higher in pigs vaccinated with the NA genotype vaccine ($P = 0.0349$).

Within each vaccination class, the detection prevalence of the PRRS NA genotype vaccine virus was significantly higher than the detection prevalence of the PRRS EU genotype vaccine virus (Table 2, FET *P*-values in last column).

3.2. Regression analysis

The results of the logistic regression models (with OR) and the respective Poisson regression models (with RR)

Table 2
Prevalence with 95% exact confidence intervals (CI) of the detection of (a) PRRS EU wild-type strain, (b) PRRS EU vaccine strain and (c) PRRS NA vaccine strain in non-vaccinated, EU genotype vaccinated and NA genotype vaccinated animals in a sample of 902 pigs collected between January and December 2007 in North-Western Germany.

Piglet vaccination status	Total	Outcome			Wald (Pearson)	FET
		Positive	Prevalence	Exact 95% CI	*P*-value[1]	*P*-value[2]
(a) Detection of PRRS EU wild-type virus					0.3159	
Not vaccinated	509	102	0.200	0.1610–0.2466		
EU genotype live vaccine	262	47	0.179	0.1320–0.2391		
NA genotype live vaccine	131	18	0.137	0.0823–0.2205		
Total	902	167	0.185	0.1567–0.2174		
(b) Detection of PRRS EU vaccine virus					0.0302	
Not vaccinated	509	2	0.004	0.0091–0.0155		
EU genotype live vaccine	262	9	**0.034**	**0.0179–0.0649**		
NA genotype live vaccine	131	1	0.008	0.0011–0.0524		
Total	902	12	0.013	0.0076–0.0232		
(c) Detection of PRRS NA vaccine virus					0.0349	
Not vaccinated	509	34	0.067	0.0456–0.0968		<0.0001
EU genotype live vaccine	262	23	0.088	0.0523–0.1437		0.0054
NA genotype live vaccine	131	23	**0.176**	**0.1114–0.2657**		<0.0001
Total	902	80	0.089	0.0688–0.1137		<0.0001

The prevalence and CI of the groups vaccinated with the corresponding genotype vaccine are marked by bold figures.

[1] STATA 10 Survey design-adjusted Wald *P*-values to compare virus detection prevalences between vaccination groups.

[2] Fishers Exact Test *P*-values to compare observed EU and NA (US) vaccine virus detection frequencies within the three vaccination groups to those expected under the null hypothesis of equality and adjusted for the different vaccination strain proportions (67% of piglets vaccinated with EU strain, 33% vaccinated with NA strain).

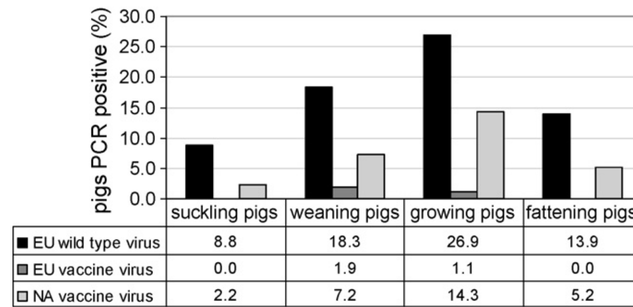


Fig. 1. Production stage-specific detection frequencies (*nonaplex* RT-PCR, DV-PCR) for PRRS EU wild-type virus, PRRSV EU live vaccine virus and PRRSV NA live vaccine virus in a sample of 902 pigs collected between January and December 2007 in North-Western Germany.

strongly agreed; we therefore only presented the OR derived from the logistic regression models (Table 3). With both the vaccination status and animal production class (as a proxy parameter for age) in the model, neither sow vaccination status nor the presence of sows on the farm were statistically significant or acted as influential confounders; those two variables therefore were omitted from the models.

Detection of the PRRS EU wild-type virus did not depend on vaccination status ($P > 0.19$). Weaning pigs had a borderline higher chance (OR = 2.3, $P = 0.10$) of EU wild-type virus detection when compared to suckling pigs, and for growing pigs the difference to suckling pigs was statistically significant (Table 3, Model 1). Fattening pigs showed a slightly increased OR, however, this was not significant.

The detection of the PRRS EU vaccine virus (Table 3, Model 2) was significantly higher in pigs vaccinated with the EU genotype vaccine when compared to non-vaccinated animals (OR = 9.4). Due to the low number of positive pigs ($n = 12$), estimates for the age classes were either invalid or associated with very large confidence intervals, and should be interpreted with caution. The pattern, however, was similar to that seen in Model 1 in that weaning and growing pigs had higher virus detection odds when compared to suckling and fattening pigs.

Animals vaccinated with the NA genotype vaccine had significantly higher detection chances/rates for the PRRS NA genotype vaccine virus when compared to non-vaccinated animals (OR = 3.34, $P = 0.001$). A similar pattern in the association between age class and virus detection as in Models 1 and 2 was seen (Table 3, Model 3).

Table 3
Odds ratio (OR) estimates from a logistic regression model with 95% confidence intervals (CI) and robust *P*-values for the association between vaccination status and animal age and three different outcomes, the detection of PRRS EU wild-type strain (Model 1), PRRS EU vaccine strain (Model 2) and PRRS NA vaccine strain (Model 3) in a sample of 902 pigs collected between January and December 2007 in North-Western Germany.

Factor and level	Odds ratio (OR)	95% CI limits		Robust *P*-value
		Lower	Upper	
Model 1 outcome:	Detection of PRRS EU wild-type virus (167/902)			
Not vaccinated	Baseline			
EU genotype live vaccine	0.884	0.560	1.397	0.598
NA genotype live vaccine	0.663	0.356	1.235	0.195
Suckling pigs	Baseline			
Weaning pigs	2.292	0.856	6.138	0.099
Growing pigs	3.765	1.365	10.383	0.010
Fattening pigs	1.692	0.571	5.012	0.342
Model 2 outcome:	Detection of PRRS EU vaccine virus (12/902)			
Not vaccinated	Baseline			
EU genotype live vaccine	9.394	2.020	43.693	0.004
NA genotype live vaccine	2.157	0.197	23.619	0.529
Suckling pigs	Baseline			
Weaning pigs	7,260,860	1,557,698	3.38*E*+07	<0.001
Growing pigs	4,010,166	n.e.	n.e.	n.e.
Fattening pigs	n.e.	n.e.	n.e.	n.e.
Model 3 outcome:	Detection of PRRS NA vaccine virus (80/902)			
Not vaccinated	Baseline			
EU genotype live vaccine	1.384	0.681	2.811	0.369
NA genotype live vaccine	3.343	1.691	6.612	0.001
Suckling pigs	Baseline			
Weaning pigs	4.247	0.953	18.939	0.058
Growing pigs	9.754	2.184	43.559	0.003
Fattening pigs	2.369	0.463	12.125	0.300

n.e.: p.

3.3. Sequencing

Material from 168 of the 259 samples found positive in the diagnostic laboratory was available for genetic typing by amplifying and sequencing the ORF5. Of these, 104 were of the EU genotype, and 64 of the NA genotype. The ORF5 nucleotide sequence of 11 of the EU genotype isolates had 99.1–100%, identity with the corresponding ORF of the Porcilis® PRRS DV strain. The agreement of the results obtained by sequencing and DV PCR was substantial (kappa index 0.797, 95% CI 0.604, 0.989; 9 samples DV PCR positive and nucleotide identity >98%; 2 samples DV PCR negative and nucleotide identity >98%; 2 samples DV PCR positive and nucleotide identity <98%; 91 samples DV PCR negative and nucleotide identity <98%).

Nucleotide identities of the remaining EU genotype isolates with the Porcilis® PRRS-DV strain were between 85.3 and 91.7%, allowing their classification as EU wild-type virus.

Nucleotide identities of the ORF5 of the NA genotype isolates with the Ingelvac® PRRS MLV vaccine strain were between 96 and 100%.

4. Discussion

The objective of this study was to investigate how often PRRS wild-type virus and/or vaccine virus could be detected in pigs either vaccinated against PRRSV or not. The data was analysed in the light of the vaccination history on farm in order to determine any spontaneous spread of PRRS vaccine virus. However, a full description of the herd factors and practices which might have influenced vaccine spread was not in the scope of this paper. Due to the hierarchical nature of the data (one or several pigs submitted per farm), the expected clustering effect had to be corrected for both the prevalence estimation and the regression analysis. This was achieved by using the respective modules offered by the software package (STATA). Without such adjustment, confidence intervals might have been too narrow and *P*-values too low, leading to effect overestimation. We therefore consider our results to be less biased and rather conservative.

Twelve years after authorisation of Ingelvac® PRRS MLV, NA genotype vaccine virus is widespread in the pig population of North-Western Germany. The average detection rate revealed by *nonaplex* RT-PCR was 8.9% for NA genotype vaccine virus, while the detection rate for EU wild-type virus was only twice as high (18.5%). As expected, highest detection rates (17.6%) for the NA genotype vaccine virus could be observed in pigs vaccinated with the corresponding vaccine. However, the NA genotype vaccine virus was also found in 8.5% of pigs originated from herds where only the sows received the Ingelvac® PRRS MLV and in 5.6% of the pigs from herds with no recent history of vaccination. Given the imperfect sensitivity of the *nonaplex* PCR the true prevalence is assumed to be underestimated.

The spread of vaccine virus within herds has been demonstrated in several studies (Botner et al., 1997, 1999; Kiss et al., 2006). The detection of NA genotype vaccine virus in non-vaccinated piglets may be due to both horizontal and vertical transmission either from sows to their offspring or between weaning and growing pigs. Our results suggest that the latter case is most likely because the statistically significant highest detection rates have been found in growing pigs. This peak in the detection rate in growing pigs was also found for EU wild-type virus.

The highest detection rates for NA genotype vaccine virus in growing pigs were unexpected, due to the fact that piglets were usually vaccinated at 2 or 3 weeks of life. Therefore, the peak in the detection rate was expected in suckling, or at the latest, in weaning pigs. The dynamic of NA genotype vaccine and EU wild-type virus shown by the data in this study corresponds with several studies on PRRS wild-type virus spread in endemically infected herds (Houben et al., 1995; Nodelijk et al., 1997; Larochelle et al., 2003).

The detection of NA genotype vaccine virus in pigs from herds with no recent history of Ingelvac® PRRS MLV vaccination is in accordance with studies performed in Denmark (Botner et al., 1997; Mortensen et al., 2002). However, the Danish studies were performed shortly after the vaccine was released for use in the national pig population, and not in a country were both NA and EU vaccines have been in use for several years, as is the case in this report.

To our knowledge, data on age-dependent detection rates of live PRRS vaccine virus have not been published. The evident similarities in the dynamics of the NA genotype vaccine virus and the EU wild-type virus strongly support the contention that spontaneous transmission of the Ingelvac® PRRS MLV is a common occurrence.

In the present study, the detection of the EU genotype vaccine virus was limited to 12 pigs (1.3%). Nine of these pigs had been vaccinated with the EU genotype vaccine, one pig originated from a herd where sows were vaccinated with the EU genotype vaccine and only two pigs had no history of corresponding vaccination. These findings agree with the outcome of an experimental investigation where only a limited and spontaneous terminating transmission of EU genotype vaccine virus to fully susceptible sentinel pigs could be demonstrated (Astrup and Riising, 2002).

The classification of the positive results obtained by *nonaplex* RT-PCR and DV PCR, respectively were mostly approved by sequencing. The nucleotide identity with the corresponding ORF of the Porcilis® PRRS strain in 12 samples was 99.1 to 100%. For PRRS virus isolates it is generally accepted that if two isolates have a nucleotide identity in the ORF5 gene ≥98% they are closely related (Collins, 1999). Therefore it can be assumed that these isolates are derived from the Porcilis® PRRS strain. The marked differences in sequence identity between isolates categorised either as DV vaccine (≥99.1%) or wild-type (≤91.7%) showed that misclassification of recombinant or mutated vaccine strain isolates probably not have impaired the results of this study.

Sequencing of 64 isolates classified as NA genotype virus isolates by *nonaplex* RT-PCR showed that the virus in 55 samples had a nucleotide identity of 98–100% to the corresponding ORF of Ingelvac® PRRS MLV. As NA genotype PRRS virus was not indigenous in Germany until

introduced with the vaccine, the high nucleotide identities with the Ingelvac® PRRS MLV suggest that they are derivatives of this vaccine. Interestingly, 9 of the isolates had nucleotide identities only between 96.0 and 97.8% with the ORF5 of Ingelvac® PRRS MLV. This reflects the continuous mutations of the viral genome (Allende et al., 2000; Goldberg et al., 2003). It is likely that divergence of the nucleotide sequences of these isolates will continue with time.

In conclusion, the eradication of PRRSV from infected herds requires a more fundamental knowledge of the dynamics of PRRS virus infection and epidemiology (Cho and Dee, 2006). The results of this study show that this is not only needed for the PRRS wild-type virus but also for live vaccine viruses. Obvious differences in the potential of spontaneous spread of different PRRS vaccine viruses should be considered when choosing vaccines to be used in an eradication program.

Acknowledgements

We are grateful to all the veterinarians for their cooperation in the telephone interview. We thank Eva Binder, Verena Gotter, Dr. Kerstin Holthaus and Dr. Lars Moorkamp for their support in the sampling. We would like to thank the IVD GmbH, Hannover, Germany for performing the routine PCRs. Financial support was by Intervet International, Boxmeer, The Netherlands, and is also gratefully acknowledged.

References

Allende, R., Kutish, G.F., Laegreid, W., Lu, Z., Lewis, T.L., Rock, D.L., Friesen, J., Galeota, J.A., Doster, A.R., Osorio, F.A., 2000. Mutations in the genome of porcine reproductive and respiratory syndrome virus responsible for the attenuation phenotype. Arch. Virol. 145, 1149–1161.

Allende, R., Lewis, T.L., Lu, Z., Rock, D.L., Kutish, G.F., Ali, A., Doster, A.R., Osorio, F.A., 1999. North American and European porcine reproductive and respiratory syndrome viruses differ in non-structural protein coding regions. J. Gen. Virol. 80, 307–315.

Astrup, P., Riising, H.J., 2002. Porcilis PRRS: a laboratory assessment of vaccinal virus spread. In: Proc. 17th Int. Pig Vet. Soc. Congr., Ames, IA, USA, p. 380.

Balka, G., Hornyak, A., Balint, A., Kiss, I., Kecskemeti, S., Bakonyi, T., Rusvai, M., 2008. Genetic diversity of porcine reproductive and respiratory syndrome virus strains circulating in Hungarian swine herds. Vet. Microbiol. 127, 128–135.

Bäurle, H., Windhorst, H.W. (Eds.), 2005. Strukturwandlungen in der deutschen Schweinehaltung zwischen den Jahren 1992 und 2003. Vechtaer Druckerei und Verlag.

Botner, A., Nielsen, J., Oleksiewicz, M.B., Storgaard, T., 1999. Heterologous challenge with porcine reproductive and respiratory syndrome (PRRS) vaccine virus: no evidence of reactivation of previous European-type PRRS virus infection. Vet. Microbiol. 68, 187–195.

Botner, A., Strandbygaard, B., Sorensen, K.J., Have, P., Madsen, K.G., Madsen, E.S., Alexandersen, S., 1997. Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified live PRRS vaccine. Vet. Rec. 141, 497–499.

Cho, J.G., Dee, S.A., 2006. Porcine reproductive and respiratory syndrome virus. Theriogenology 66, 655–662.

Collins, J., 1999. Interpreting PRRSV sequencing data. In: A.D. Leman Conf, St. Paul, MN, USA, pp. 1–4.

Gatzka, E.M., Schulz, K., Ingwersen, J. (Eds.), 2005. Schweineproduktion 2005 in Deutschland. Zentralverband der deutschen Schweineproduktion (ZDS), D-53113 Bonn.

Goldberg, T.L., Lowe, J.F., Milburn, S.M., Firkins, L.D., 2003. Quasispecies variation of porcine reproductive and respiratory syndrome virus during natural infection. Virology 317, 197–207.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41.

Houben, S., van Reeth, K., Pensaert, M.B., 1995. Pattern of infection with the porcine reproductive and respiratory syndrome virus on swine farms in Belgium. J. Vet. Med. B 42, 209–215.

Indik, S., Schmoll, F., Sipos, W., Klein, D., 2005. Genetic variability of PRRS virus in Austria: consequences for molecular diagnostics and viral quantification. Vet. Microbiol. 107, 171–178.

Kiss, I., Sami, L., Kecskemeti, S., Hanada, K., 2006. Genetic variation of the prevailing porcine respiratory and reproductive syndrome viruses occurring on a pig farm upon vaccination. Arch. Virol. 151, 2269–2276.

Larochelle, R., Magar, R., D'Allaire, S., 2003. Comparative serologic and virologic study of commercial swine herds with and without post-weaning multisystemic wasting syndrome. Can. J. Vet. Res. 67, 114–120.

Lillie, K., Igelbrink, R., Hoferer, M., Fiebig, K., Nathues, H., Greiser-Wilke, I., Grosse Beilage, E., 2008. Effect of natural exposure to vaccine-derived North American genotype PRRS virus on the serological response in naive pigs. Transbound. Emerg. Dis. 55, 140–143.

Martelli, P., 2002. Experiences with porcine reproductive and respiratory syndrome (PRRS) control programmes in Italy. Pig J. 49, 134–154.

Mateu, E., Diaz, I., 2008. The challenge of PRRS immunology. Vet. J. 177, 345–351.

Mortensen, S., Stryhn, H., Sogaard, R., Boklund, A., Stark, K.D., Christensen, J., Willeberg, P., 2002. Risk factors for infection of sow herds with porcine reproductive and respiratory syndrome (PRRS) virus. Prev. Vet. Med. 53, 83–101.

Nodelijk, G., van Leengoed, L.A., Schoevers, E.J., Kroese, A.H., De Jong, M.C., Wensvoort, G., Verheijden, J.H., 1997. Seroprevalence of porcine reproductive and respiratory syndrome virus in Dutch weaning pigs. Vet. Microbiol. 56, 21–32.

Oleksiewicz, M.B., Botner, A., Madsen, K.G., Storgaard, T., 1998. Sensitive detection and typing of porcine reproductive and respiratory syndrome virus by RT-PCR amplification of whole viral genes. Vet. Microbiol. 64, 7–22.

Oleksiewicz, M.B., Stadejek, T., Mackiewicz, Z., Porowski, M., Pejsak, Z., 2005. Discriminating between serological responses to European-genotype live vaccine and European-genotype field strains of porcine reproductive and respiratory syndrome virus (PRRSV) by peptide ELISA. J. Virol. Methods 129, 134–144.

Stadejek, T., Oleksiewicz, M.B., Potapchuk, D., Podgorska, K., 2006. Porcine reproductive and respiratory syndrome virus strains of exceptional diversity in Eastern Europe support the definition of new genetic subtypes. J. Gen. Virol. 87, 1835–1841.

Stadejek, T., Oleksiewicz, M.B., Scherbakov, A.V., Timina, A.M., Krabbe, J.S., Chabros, K., Potapchuk, D., 2008. Definition of subtypes in the European genotype of porcine reproductive and respiratory syndrome virus: nucleocapsid characteristics and geographical distribution in Europe. Arch. Virol. 153, 1479–1488.

Storgaard, T., Oleksiewicz, M., Botner, A., 1999. Examination of the selective pressures on a live PRRS vaccine virus. Arch. Virol. 144, 2389–2401.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Voglmayr, T., Sipos, W., Schuh, M., Truschner, K., Griessler, A., Mourits, B., Schmoll, F., 2006. PRRS-Eradikation in einem geschlossenen Herdbuchzuchtbetrieb ohne Unterbrechung der Produktion mit Einsatz einer Lebendvirus (MLV-) Vakzine und Schließung der Herde. Tierärztl. Praxis 34(G), 241–248.