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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

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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...
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 Rissing, 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

| Variable                  | Description                                                                 |
|---------------------------|-----------------------------------------------------------------------------|
| Production stage          | The age of the pigs, which was usually not available, is described by the production stage. |
| Piglet vaccination        | Pigs could have been vaccinated with an EU genotype modified live vaccine (Porcilis® PRRS), a NA genotype 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 genotype 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'-CCG GGC GGT ATG TAC TCT G-3'; position 313–332; ATGorf5 = 1) and DV-Orf5-RV-531 (5'-GTG ACG AGG TTG CCG TCG A-3'; position 531–512; ATGorf5 = 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 polymerase (Invitrogen, Karlsruhe, 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), 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 MgCl2, and 10,000 U of Superscript III Reverse Transcriptase (Invitrogen, Karlsruhe, 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 MgCl2, 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'-CCG TTG CTI CAT TTC TTG 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 (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; DQ324694; DQ324697; DQ324682; DQ324667).

### 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; DQ324694; DQ324697; DQ324682; DQ324667).

### 2.5. Statistical analysis

Questionnaire and laboratory data were entered into a spreadsheet program (Excel for Office 2007; www.micros-soft.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 prevalence of the EU wild-type 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) are presented in the following sections.

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 Positive | Prevalence Exact 95% CI | Wald (Pearson) P-value<sup>1</sup> | FET P-value<sup>2</sup> |
|--------------------------|-------|------------------|-------------------------|---------------------------------|------------------------|
| (a) Detection of PRRS EU wild-type virus | | | | | |
| Not vaccinated | 509 | 102 | 0.200 | 0.1610–0.2466 | 0.3159 |
| 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 | | | | | |
| Not vaccinated | 509 | 2 | 0.004 | 0.0091–0.0155 | 0.0302 |
| 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 | | | | | |
| Not vaccinated | 509 | 34 | 0.067 | 0.0456–0.0968 | 0.0349 |
| EU genotype live vaccine | 262 | 23 | 0.088 | 0.0523–0.1437 | <0.0001 |
| NA genotype live vaccine | 131 | 23 | **0.176** | **0.1114–0.2657** | 0.0054 |
| 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.

<sup>1</sup> STATA 10 Survey design-adjusted Wald P-values to compare virus detection prevalences between vaccination groups.

<sup>2</sup> Fisher’s 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).
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 | 3.38E+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.

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