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Comparison of four ELISAs for detection of antibodies against different genetic subtypes of European genotype PRRSV

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Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) strains belong to the two genotypes which are antigenically different. The genetic diversity in the European genotype (EU-PRRSV) is much higher than in the North American genotype (NA-PRRSV) (3). The evidence of the restricted serological cross reactivity between the genetic subtypes exists (1, 3) but it is unknown whether it impacts the sensitivity or specificity of the ELISA diagnostic tests methods. To address this problem 175 sera from farms where different genotypes and genetic subtypes circulated were tested with three commercial and one in house ELISA tests.

Materials and Methods
Serum samples were obtained from 3 Polish, 4 Belarusian and 1 Ukrainian farms, where different genotypes and subtypes circulated as it was determined by PCR and DNA sequencing. In 2 of 3 Polish farms subtype 1 of EU-PRRSV circulated. In one farm the NA-PRRSV strain was present. In Belarusian and Ukrainian farms diverse East European subtypes circulated (3). Additionally, 31 samples from pigs from farms free from PRRSV were used. They included 17 sera from Sweden that earlier were found to react false positive in HerdChek PRRS 2XR (IDEXX) ELISA and negative in IPMA test. The samples were tested with HerdChek 2XR and 3X (IDEXX), Ingezim PRRS DR (Ingenasa) and the in house test produced in our laboratory. The Ingezim test is specific for EU-PRRSV while the remaining tests are specific for both genotypes. Results were compared for each pair of ELISA tests separately using McNemar's test.

Results
All ELISAs detected PRRSV specific antibodies in sera from all farms were EU-PRRSV infection was present. Both IDEXX tests and the in house ELISA detected antibodies to NA-PRRSV in all sera from the Polish farm were this genotype circulated while Ingenasa test detected seroconversion in only 5 of 12 of the samples. In case of samples from one Ukrainian farm infected by EU-PRRSV subtype 2 the Ingezim test appeared to be significantly more sensitive than PIWet and HerdChek X3 tests. The analysis of the specificity proved it to be highest in case of the HerdChek X3 test. The HerdChek 2XR test gave false positive results with most of the Swedish sera as it was found before.

Discussion
Very high genetic diversity of PRRSV poses a serious problem for PCR diagnosis, especially for the detection of the European genotype. However, our preliminary data show that it does not influence serological diagnosis by ELISA. All tests used in the study were sensitive in detection of antibodies induced by different, diverse genetic subtypes of EU-PRRSV. However, the 3X test seemed to be slightly less sensitive than the older 2XR test and the Ingezim DR. The 3X test was the most specific test of all. Further studies are necessary to fully evaluate the impact of the antigenic diversity of the EU-PRRSV on sensitivity and the specificity of the serological methods of PRRS diagnosis.

Acknowledgements
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References
**Introduction**

Vaccination against PRRSV is frequently practised in Germany to control PRRSV. Attenuated live vaccines of the North American (NA) and European (EU) genotype are used. Early after infection antibodies are detected in ELISA, however neutralizing antibodies (nAb) are detected only at low titre and late (>28 days) after infection. Additionally, detection of nAb depends on the virus isolate. Assuming that induction of homologous nAb against vaccine virus after vaccination is a positive quality criterion, a PRRSV-neutralization test (NT) using vaccine virus was established. Data obtained by analysis of field cases will be presented.

**Material and Methods**

NT with 100 CID50 of NA- and EU-vaccine virus, respectively, was performed on Marc145 cells with 2% guinea pig complement (BÖTTCHER et al., 2006). Culture was checked for CPE until day 6.

**Results** are given as titre: <2, 2, 2.8, 4, 5.6, 8, ≥11.2.

Sera were tested by PRRSV-ELISA (Idexx) according to manufacturer’s instruction. A nested RT-PCR was performed according to PESCH et al. (2000). Blood was collected from gilts, sows at 1. and 5. parity and piglets after weaning 4, 8, 12 weeks of age. 10 animals per group were tested. For calculation of geometric mean of NT-titre a titre of ≥ 11.2 was scored as 11.2.

**Results**

1. In herds with an acute outbreak of PRRSV, sera with high EU-NT-titres (≥11,2) were negative for nAbs to NA-PRRSV. Sera with high NA-NT-titres (≥11,2) from herds free from EU-PRRSV and vaccinated with NA-PRRSV were negative in EU-NT. Thus cross-neutralization between genotypes seems unlikely.

2. Comparison of ELISA and NT revealed that 30% of ELISA-negative sera were positive in at least one EU- or NA-NT; in contrast 67% of ELISA-positive sera were positive in NT, too. This difference between tests is explained by longer persistence and late induction of nAb, respectively.

3. As a consequence of an acute PRRSV-outbreak high NT-titres were observed in sows; maternally derived nAb (MDnAb) controlled infection in piglets until 12th weeks of age. PRRSV was detected in piglets with MDnAb by PCR when the geometric mean of the NT-titre dropped below 4.

4. Regarding vaccination 90% of piglets free of MDnAbs at vaccination (14-21 days p.n.) were positive in PCR until 8 weeks of age. A slight increase of NT-titre was observed until the 12th week of age. In contrast, only 10% of piglets with a mean NT-titre of MDnAb of 7 at vaccination were positive in PCR two days p.vacc.

5. Low or no NT-titres were frequently detected month after vaccination.

6. Sows of 5th parity had generally lower titres than sows of 1st parity, unless recent acute outbreaks in the herds were reported. Vaccination every 4 month was not effective in raising NT-titres in these animals. As a consequence offsprings of these sows are earlier susceptible to infection.

**Discussion and Conclusions**

PRRSV NT is a powerful tool in order to determine at least one side of PRRSV immunity; however, it is laborious and expensive. As a consequence NT should be substituted by an appropriate ELISA.

It is questionable if vaccination in the presence of homologous nAb is effective. This is especially important for piglets with MD-nAb. Thus prediction of an optimized time-point of vaccination is necessary. Additionally, gilts should be checked by NT before they are introduced into infected herds.

Extend of cross-neutralization within genotypes especially EU is still not sufficiently analysed. If antigenic heterogeneity of PRRSV isolates due to its high frequency of mutation is a real problem like in Influenzavirus A, Bluetonguevirus, Foot-and-Mouth-Disease Virus etc., serotypes have to be established. NT is the test of choice for establishment of serotypes.

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**References**


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**IL-10 and TGFβ expression in the lung and tonsil of pigs experimentally infected with porcine reproductive and respiratory syndrome virus**

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**Introduction**

Porcine Reproductive and Respiratory Syndrome (PRRS) is characterized by inducing an equivocal immune response (1). Immunomodulatory cytokines, namely IL-10 and TGF β, are able to inhibit the expression of several other cytokines and the onset of an efficient immune response (2), and they are considered as possible key mediators in the impairment of host immune response observed in PRRS (1). Therefore, the main goal of this study was to study the expression of IL-10 and TGF β in the lung and tonsil of pigs experimentally with PRRS virus (PRRSV).

**Materials and Methods**

Thirty-two 5-weeks-old pigs from a high-healthy farm historically seronegative for PRRSV were used for the experimental infection. Twenty eight pigs were randomly distributed in batches of four, inoculated by the intramuscular route with 1 ml of 10³.0 TCID₅₀ PRRSV strain, and killed at 3, 7, 10, 14, 17, 21 and 24 days post-inoculation (dpi). The four remaining pigs were used as controls, inoculated with 1 ml of sterile medium and killed at the end of the study (24 dpi). At the necropsy lung and tonsil samples were collected and fixed with 10 % neutral buffered formalin and Bouin solution, for the histopathological and immunohistochemical studies, respectively. In the immunohistochemical study the following primary antibodies were used: anti-PRRSV (SDOW17/SR30; Rural Technologies Inc.); anti-porcine IL-10 (AF693; R&D Systems Inc.); anti-TGF β (AB101NA; R&D Systems Inc.), as previously described (3). Immunolabelled cells were counted and morphologically identified as macrophages, lymphocytes or neutrophils.

**Results**

A higher expression of IL-10 was observed at 7 dpi in the lung parenchyma of PRRSV-inoculated pigs, whereas TGF β was enhanced at 3 and 10 dpi, and at the end of the study (24 dpi). In addition, the expression of PRRSV antigen peaked at 7dpi, showing a positive correlation with respect to the pulmonary expression of IL-10 and TGF β (r = 0.77 and r = 0.76, respectively).

On the other hand, both IL-10 and TGF β showed an undulant expression in the tonsil of PRRSV-inoculated animals. Interestingly, PRRSV antigen displayed a similar kinetics than the one observed for IL-10 (r = 0.76), but no correlation was observed with respect to the expression of TGF β in the tonsil.

IL-10 was mainly expressed in the cytoplasm of macrophages from the alveolar septa, however, TGF β was chiefly expressed by pulmonary alveolar macrophages (PAMs). In the tonsil, the expression of immunomodulatory cytokines was observed in the cytoplasm of macrophages of the lymphoreticular areas, and secondly by lymphocytes. TGF β was also expressed in the cytoplasm of few neutrophils.

**Discussion**

The correlation and similar kinetics observed between the expression of IL-10 and PRRSV antigen in lung and tonsil suggests that PRRSV replication may induce the expression of this cytokine. On the other hand, the expression of TGF β and PRRSV antigens was correlated only in the lung but displayed a different kinetics. Thus, an indirect pathway to induce TGF β may be activated by PRRSV. Nowadays, the regulatory T cells are considered as possible source of IL-10 and TGF β and their role in the immune response is being studied in PRRS (4).

We have previously reported that septal macrophages were the main cells expressing IL-10 and proinflammatory cytokines (3). Interestingly, PRRSV and TGF β antigens were chiefly expressed by pulmonary alveolar macrophages (PAMs). Thus, TGF β may avoid the lung expression of proinflammatory cytokines and other cytokines by PAMs in PRRSV-infected pigs.

**References**

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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Introduction
A basic requirement of live vaccine virus strains is that natural transmission of vaccine virus is minimal or non-existent (3). The objectives of this study were (a) to determine the frequency of the EU and the NA genotype PRRS vaccine viruses in a large sample of pigs from North-Western Germany, (b) to assess the association between vaccine isolate detection and vaccination history and (c) to characterise the identified vaccine isolates considering their origin.

Material and Methods
During January and December 2007, 1,970 pigs were submitted to the Field Station for Epidemiology for post-mortem examination. 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 originated from 439 herds and were submitted by 141 different veterinary practices. Lung tissue from these pigs was tested for PRRSV by a nonaplex RT-PCR. Those samples positive for PRRSV were selected for further examination and genetic typing. The vaccination history was obtained from the veterinary surgeons who had submitted the pigs. Telephone interviews were conducted that followed a standardised questionnaire consisting 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.

Results
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 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).

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. 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%. 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.

Discussion
The spread of vaccine virus within herds has been demonstrated in several studies (1, 2). However, 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 those countries using this vaccine. Known that an eradication of PRRSV from infected herds would require a fundamental knowledge of the dynamics of PRRS virus infection and epidemiology, 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.

References
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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.