PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) INFECTION IN LITHUANIAN WILD BORIS (SUS SCROFA) POPULATION

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Abstract
Since domestic pigs and wild boars have the same susceptibility to various infections there was major concern to monitor the epidemiological PRRSV situation in feral pigs and to characterize genetically the detected strains. From 659 examined wild boar sera, 43 (6.5 %) were positive to PRRSV antibodies. Antibodies to PRRSV were detected in all age groups, however seroprevalence was significant higher in adults. Wild boars serum samples from 31 locations out of 42 investigated were seropositive for PRRSV. Samples of 13 (8.2%) wild boars tested PRRSV-positive in genotype 1 specific nPCR. No positive results were obtained in genotype 2 specific nPCR. The sequencing and genetic comparison of the selected ORF5 amplicons revealed that these wild boar sequences belonged to two genetic subtypes 3 and 4. The new sequences formed well defined clusters within these subtypes. Interestingly such strains were never found in domestic pigs in Lithuania. Subtype 3 viruses are common in Belarus and subtype 4 was found in two Belarusian and two Latvian farms. This study also has for the first time demonstrated presence of PRRSV in Eastern European wild boars. It has shown that wild boar population can harbour different genetic lineages of PRRSV strains than those found in domestic pigs in Lithuania. The most striking finding is detection of subtype 4 strains in 5 wild boars. Previously this subtype was detected only in Belarus and Latvia. Altogether, these findings are strongly supporting the role of wild boars as a natural reservoir for PRRSV.

Keywords: PRRSV, wild boar, seroprevalence, phylogenetic analyses

Introduction
PRRSV is endemic in most swine-producing countries, and today it is associated with major economic losses. PRRSV strains are divided into two genotypes based on genetic and antigenic characteristics: genotype 1 (formerly European) and genotype 2 (formerly North American) (10). Within the genotype 1, several Eastern European genetic subtypes were defined (11, 12). Antibodies to PRRSV have been found in pigs almost worldwide. The majority pig farms in Lithuania
are also positive for antibodies to PRRSV. Although PRRSV is widespread in domestic swine, very little is known about PRRSV infection in European wild boar. However, comprehensive information on PRRSV infection in wild boars was published only from Germany (7). Wild boars have been found also seropositive to PRRSV in France (1), USA (9), Italy (5), and Croatia (8). Recently PRRSV was identified in hybrid wild boars, also known as special wild pigs in China (14). Thus, most likely, wild boars become infected by domestic swine as a result of seldom direct or indirect contacts. On the other hand, wild boars can act as a reservoir for infectious diseases of domestic pigs and interactions between these two populations can potentially result in the dissemination of these diseases (15). Since domestic pigs and wild boars have the same susceptibility to various infections there was major concern to monitor the epidemiological PRRSV situation in feral pigs and to characterize genetically the detected strains.

Materials and methods

A total of 659 serum samples from wild boars from 42 locations throughout Lithuania were collected during autumn–winter hunting seasons 2008/2009 and 2009/2010. The wild boars sera were analyzed via different producers ELISA test systems: IDEXX PRRS 2XR Ab, IDEXX HERDCHEK* PRRS X3 antibody test kits (Corporate Headquarters IDEXX Laboratories, Inc., USA), Ingezim PRRS Europa (Ingenasa, Madrid, Spain) and others, according to manufacturer’s instructions and in the ISO/IEC 17025:2005 standard accredited laboratory. For genetic characterisation samples of blood sera and lungs of 159 wild boars were collected from 15 hunting grounds situated in 5 regions of Lithuania during autumn-winter hunting seasons from 2007 to 2011. Total RNA was extracted from homogenate of tissue or serum samples using the GeneJET RNA Purification kit (Fermentas). It was used as template in reverse transcription nested PCR specific for ORF5 of genotypes 1 and 2 PRRSV as described previously (11, 12). Gel-purified 606 bp ORF5 PCR products were cycle sequenced using the BigDye Terminator Cycle Sequencing kit (v2.0, Applied Biosystems) and ABI310 genetic analyzer. Sequence alignment was performed using the Clustal W software. A Phylogenetic tree was constructed with MegAlign program from Lasergene program package.

Results

From 659 examined wild boar sera, 43 (6.5 %) were positive to PRRSV antibodies. Investigation of PRRSV antibodies with different ELISA kits did not show difference in detection positive serum samples (p>0.05). The results of serological analysis are summarized in Table 1.
Antibodies to PRRSV were detected in all age groups, however seroprevalence was significant higher in adults (Table 2). Wild boars serum samples from 31 locations out of 42 investigated were seropositive for PRRSV.

**Table 1.** The results of detection PRRSV antibodies in wild boars samples

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of investigated serum samples</th>
<th>Number of positive serum samples</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>286</td>
<td>26</td>
<td>9.1</td>
</tr>
<tr>
<td>2009</td>
<td>274</td>
<td>15</td>
<td>5.5</td>
</tr>
<tr>
<td>2010</td>
<td>99</td>
<td>2</td>
<td>2.02</td>
</tr>
<tr>
<td>Total</td>
<td>659</td>
<td>43</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Samples of 13 (8.2%) wild boars tested PRRSV-positive in genotype 1 specific nPCR. No positive results were obtained in genotype 2 specific nPCR. The sequencing and genetic comparison of the selected amplicons revealed that these wild boar sequences belonged to two genetic subtypes 3 and 4. The new sequences formed well defined clusters within these subtypes (Fig.1). Interestingly such strains were never found in domestic pigs in Lithuania. Subtype 3 viruses are common in Belarus and subtype 4 was found in two Belarusian and two Latvian farms.
**Figure 1.** A Phylogenetic tree based on ORF5 nucleotide sequences. A set of sequences (11) representing the full genetic diversity of genotype 1 PRRSV was used as a reference.

**Conclusions**
In spite of the fact that PRRSV is actively circulating in domestic swine of Lithuania, the seroprevalence in wild boars was only 6.5%. This result indicated very low possibility to contact wild boars and domestic swine, which could present opportunity for PRRSV transmission. Similar results of PRRSV seroprevalence (8.92%) in feral pigs were reported in Croatia (8). However in reports from Italy (5) the prevalence (37.7%) of PRRSV antibodies was quite high and it could be due to PRRSV transmission from domestic pigs to wild boars.
Interestingly, in neighboring countries such as Russia or Poland PRRSV antibodies in feral pigs were not detected (4). This study has for the first time demonstrated presence of PRRSV in Eastern European wild boars. It has shown that wild boar population can harbour different genetic lineages of PRRSV strains than those found in domestic pigs in Lithuania (13). This poses a serious threat for Lithuanian farms where only subtype 2 strains are circulating. Recent studies showed that subtype 3 strains may be highly virulent (3). The most striking finding is detection of subtype 4 strains in 5 wild boars. Previously this subtype was detected only in Belarus and Latvia (11, 13). Altogether, these findings are strongly supporting the role of wild boars as a natural reservoir for PRRSV.

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References


