

Five years seroprevalence study of porcine reproductive and respiratory syndrome virus in Lithuanian pig and wild boar populations

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Abstract

Serological study of porcine reproductive and respiratory syndrome virus (PRRSV) infection in pigs and wild boars was conducted in Lithuania between 2009 and 2013. Antibody level was measured using a commercial ELISA. The 4.32% (95% CI 3.92-4.72) out of 9856 examined porcine sera were positive for the PRRSV antibodies. The antibodies were detected in 11.82% (95% CI 10.28-13.36) of all investigated serum samples of sows and gilts. As much as 8.2% of serologically positive samples (95% CI 6.83-9.57) were determined in the piglets under three months of age. Considerably smaller ($P < 0.05$) seroprevalence was detected in boars (0.62%) and fattening pigs (1.84%). From 1357 examined sera of wild boar, collected between 2009 and 2013 hunting seasons, 5.38% (95% CI 4.52-8.2) of samples were positive for PRRSV antibodies in 23 locations out of 50 investigated. The analysis of seroprevalence in different age groups of wild boars showed that PRRSV antibodies were detected in all age groups; however, it was significantly higher in adults than in juveniles or subadults and reached up to 10.02% (95% CI 7.39-12.65).

Keywords: pigs, wild boars, porcine reproductive and respiratory syndrome virus, antibodies.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) containing 15 kb of RNA, and belonging to the family *Arteriviridae*, which emerged practically during the same period on all the continents more than 25 years ago, is wide spread in pig populations in many countries (5). It causes reproductive failure in sows, and respiratory tract illness in other pigs and piglets of different age. Currently, PRRSV is recognised as one of the most important agents of pig diseases all over the world as it incurs tremendous economic losses practically in all countries where pig-breeding is established (3, 9). Although the disease is highly detrimental and dangerous, PRRSV infection has not been investigated

extensively in Lithuania until now. Since 1997, when clinical symptoms characteristic for PRRSV infection were observed and specific antibodies were determined for the first time (12), serological PRRSV prevalence has been further studied several times in different pig farms (24, 25). In the course of the past 10 years the entire pig breeding technology and management have changed radically in Lithuania. Live attenuated vaccines have been used for the control of PRRSV infection. Furthermore, genetics of the pigs bred on the farms have changed, as well as strict biosecurity measures have started to be applied. In order to explain how these changes had influenced the spread of PRRSV on pig farms in Lithuania and to assess the actual situation of PRRSV infection on the farms, it was necessary to determine the distribution of PRRSV

antibodies in different pig age groups, farms, and regions.

Wild boars (*Sus scrofa*) are considered to be a reservoir of the agents of many dangerous infectious diseases, which can infect domestic pigs, other animals, and even people (20, 21). Wild boars can also be a source of infectious diseases of pigs (1, 2, 15, 20). Available scientific publications provide not much information about persistence of PRRSV in wild boars. PRRSV antibodies in wild boars have been only discovered in a few countries and only in some individual cases (2, 17, 18, 22). At present, it is thought domestic pigs can infect wild boars with PRRSV rather than *vice versa*; however, an evidence is still lacking and opinions are often contradictory. The available information does not provide any conclusive evidence about wild boars being a reservoir of PRRSV (19, 20). It is likely that domestic pigs could have been infected by wild boars earlier before the strict biosecurity measures have been introduced to pig farms.

Therefore, the aim of the study was to determine serological prevalence and to provide more information on PRRSV infection in Lithuanian pig and wild boar populations over a period of the last five years.

Material and Methods

Collection of the samples. Blood samples were collected from 55 different pig farms in 30 regions of Lithuania in the period of 2009 to 2013. The blood was collected from clinically healthy sows and gilts ($n = 1683$), boars ($n = 1782$), piglets under three months of age ($n = 1549$), and fattening pigs ($n = 4842$), which have not been vaccinated against PRRSV. During the period of the study the samples were collected many times from individual farms. At one sampling per farm, 60-120 samples were collected. Detailed information relating to the age, sex, breed, region, and the farm of origin of the sampled pigs was obtained on the farms. The principle of simple random sampling was applied collecting blood samples of pigs from each group. Obtained serum samples were kept frozen at -18°C .

Random blood samples of hunted wild boars ($n = 1357$) were collected during the autumn hunting season between 2009 and 2013. Blood samples from juvenile wild boars under 12 months of age ($n = 452$), 12-24-month-old wild boars ($n = 406$), and 24-month-

old and older wild boars ($n = 499$) were collected. Serum samples were kept frozen until the investigations. The blood samples were collected in all ten districts of Lithuania, all 50 regions, and in more than 300 different hunting sites.

Serological examination. Commercial enzyme-linked immunosorbent assay kits (IDEXX PRRS HerdChek, IDEXX PRRS X3, INGEZIM PRRS Europa) were used for detection of PRRSV antibodies in serum samples. The tests were performed according to the manufacturer's instructions. A sample was considered to be positive for the antibodies if the sample-to-positive (S/P) ratio was ≥ 0.4 . All the schemes of the research methods of PRRSV antibodies used were accredited according to the requirements of ISO/IEC 17025:2005 standards (11).

Statistical analysis. The results were evaluated using the statistics software package GraphPrism 3.0TM and Microsoft Excel 2010. The 95% confidence interval (CI) for seroprevalence proportions was calculated, using apparent prevalence and sample size. The results were regarded to be statistically significant at $P < 0.05$.

Results

PRRSV antibodies in serum samples, which were collected between 2009 and 2013 on different pig farms in Lithuania, were determined in 4.32% of the pig population (95% CI 3.92-4.72). The largest significant ($P < 0.05$) amount of PRRSV-serologically positive samples was established in 2011 and accounted for 8.15% (95% CI 6.6-9.7) while the smallest 2.76% amount (95% CI 2.29-3.23) was determined in 2010 (Table 1).

Serological investigations of different groups of pigs with respect to PRRSV are presented in Table 2. PRRSV antibodies were found in 11.82% (95% CI 10.28-13.36) of all investigated blood serum samples of sows and gilts. As much as 8.2% of serologically positive samples (95% CI 6.83-9.57) were determined in the piglets under three months of age. The PRRSV prevalence in different groups of pigs had shown that PRRSV was circulating more actively in the groups of sows, gilts, and piglets ($P < 0.05$). The considerably smaller seroprevalence was detected in boars (0.62%) and in the fattening pigs (1.84%).

Table 1. PRRSV serological prevalence in Lithuanian pig farms between 2009 and 2013

Sampling year	Number of tested farms	Number of positive farms	Number of tested sera	Number of positive sera	Percentage of positive sera	95% confidence interval (%)
2009	11	3	1210	71	5.87*	4.55-7.19
2010	14	6	4743	131	2.76*	2.29-3.23
2011	11	4	1203	98	8.15*	6.6-9.7
2012	10	4	1548	73	4.72	3.66-5.78
2013	9	5	1152	53	4.6	3.39-5.81
Total	55	22	9856	426	4.32*	3.92-4.72

* $P < 0.05$

Table 2. PRRSV seroprevalence in different groups of pigs

Pig group	Number of tested sera	Number of positive sera	Percentage of positive sera	95% confidence interval (%)
Sows and gilts	1683	199	11.82*	10.28-13.36
Boars	1782	11	0.62*	0.26-0.98
Piglets under three months of age	1549	127	8.2*	6.83-9.57
Fattening pigs	4842	89	1.84*	1.46-2.22
Total	9856	426	4.32*	3.92-4.72

* $P < 0.05$ **Table 3.** PRRSV antibodies in wild boar sera

Sampling year	Number of tested sera	Number of positive sera	Percentage of positive sera	95% confidence interval (%)
2009	274	15	5.5*	2.8-8.2
2010	124	4	3.23	0.12-6.34
2011	363	22	6.06	3.61-8.51
2012	286	26	9.1*	5.77-12.43
2013	310	6	1.94*	0.4-3.48
Total	1357	73	5.38	4.18-6.58

* $P < 0.05$ **Table 4.** Prevalence of PRRSV antibodies in wild boar serum by age groups

Age group	Number of tested sera	Number of positive sera	Percentage of positive sera	95% confidence interval (%)
Juveniles up to 12 months	452	12	2.65	1.17-4.13
Subadults up to 24 months	406	11	2.71	1.13-4.29
Adults over 24 months	499	50	10.02	7.39-12.65
Total	1357	73	5.33	4.18-6.58

PRRSV positive samples were found in 12 out of 30 regions of the country. The investigations at 55 pig farms between 2009 and 2013 had shown that 40% of the farms had pigs with PRRSV antibodies.

The antibodies were detected in 5.38% (95% CI 4.18-6.58) out of 1357 serum samples of wild boars killed during the autumn-winter hunting seasons between 2009 and 2013. The largest number of PRRSV positive samples was determined in 2012 and accounted for 9.1 % (95% CI 5.77-12.43), and the smallest number 1.94% (95% CI 0.4-3.48) was established in 2013 (Table 3).

The results of PRRSV seroprevalence in different age groups of wild boars (from 12-month-old juveniles to 24 months and older) are presented in Table 4. Animals that had PRRSV antibodies were found in all age groups; however, the largest number, 10.02% (95% CI 7.39-12.65), was discovered in the adult wild boars. Results obtained were similar in the age group of juvenile wild boars under 12 months of age and 12-24 months old wild boars, 2.6% (95% CI 2.6-7.2) and 3.0% (95% CI 3.2-6.9) respectively. The results of examination of blood serum samples of 24-month-old and older wild boars were statistically significantly higher ($P < 0.05$) than those of juveniles and young wild boars (Table 4).

PRRSV positive samples in wild boars were found in 23 regions of all 10 districts of Lithuania.

Discussion

The study on pig farms in Lithuania conducted between 2009 and 2013 revealed that 4.32 % of the

animals tested were positive for PRRSV antibodies. A significant decrease in the number of PRRSV positive pigs was observed as compared to the results obtained between 2005 and 2007, when PRRSV antibodies were found in 13.7% of blood serum samples (24). Serological investigations conducted between 1998 and 2001, showed that 29.2%-40.7% of samples were PRRSV-positive (25), which points at this tendency even more clearly. Such a considerable decrease in the numbers of PRRSV-positive pigs can be explained by the fact that during recent years new owners have depopulated several large pig farms, and PRRSV-negative pig herds are bred there now. Modern pig breeding technologies have been implemented at Lithuanian pig farms paying a great attention to the biosecurity requirements, quarantining, and grouping, as well as laboratory investigations of pigs.

PRRSV epidemiological situation in the pig population in Lithuania could be evaluated positively taking into consideration data of other countries, which show that PRRSV antibodies were detected in 18%-82.5% of farms in the Netherlands (8). In UK, serologically PRRSV-positive serum samples were determined in 39.8% of unvaccinated pigs of different age (9). Serological investigations of recent years at pig farms in Thailand had shown that 87.5% of replacement gilts had PRRSV antibodies (26).

PRRSV prevalence in different groups of Lithuanian pigs had revealed that virus was more actively circulating among sows and gilts. The 11.82% of all sows and gilts had PRRSV antibodies, whereas in the groups of boars the number of serologically positive samples was considerably

smaller (0.62%). This indicates that in practice, PRRSV infection does not spread at Lithuanian pig farms through the semen or direct contact during mating.

Publications provide not much data about PRRSV prevalence in the populations of wild boars in other countries and available data is sometimes contradictory. PRRSV antibodies were detected in the population of wild boars only in 0.3%-3.6% of cases in the USA, France, and in Germany (2, 18, 22). In the USA, no positive samples were detected in the samples collected in 1976 and in 1993, and only two positive animals were found in 1994 by Lutz and Wurm (16). No PRRSV antibodies were found in wild boars in the neighbouring Poland and Russian Federation (10, 14). Blood serum investigations of boars were negative in Switzerland, Spain, Croatia and Slovenia (7, 21, 27, 28, 30). PRRSV was discovered in lung samples of boars in Germany by means of RT-PCR (18). This study proved for the first time that European (genotype 1) and American (genotype 2) strains of PRRSV could be directly determined by the RT-PCR in 15.9% of lung or tonsil samples. Another publication revealed the spread of genotype 2 PRRSV in the population of hybrid wild pigs (29).

The results of our study have demonstrated that comparatively large proportion of Lithuanian wild boars (5.38%) have PRRSV antibodies, which is even higher than the proportion of serologically positive domestic pigs (4.32%). These prevalence results also showed that in Lithuania there are more wild boars positive for PRRSV antibodies than in Spain (2.0%-3.0%) (4, 6) and Germany (3.82%), as it was demonstrated during recent PRRSV serological investigations in these countries (13). This high serological prevalence in the wild boars in Lithuania can be related to the changes in the wild boar population. Density of population of wild boars increased significantly in Lithuania between 2009 and 2013 from 1.84 to 2.66 boars per km². PRRSV prevalence in wild boars could have been determined by intense migration and additional feeding of the animals during winter months. An increase in the wild boar population in certain places in the forest could intensify the spread of PRRSV from one wild boar to another. The results of our investigations indicated that the prevalence of PRRSV among wild boars can be much higher than it has been thought until now.

The broad distribution of serologically positive PRRSV samples of wild boars emphasises the importance of this population in PRRSV epidemiology in Lithuania and possibly neighbouring countries. It is even more important that the PRRSV strains identified in Lithuanian wild boars are likely genetically and antigenically different from those in Lithuanian pigs (23). Data of the investigations conducted in other countries showed that either a relatively small number of wild boar population was

in contact with PRRSV, or they were serologically negative. This leads to the conclusion that PRRSV infection does not take place in these wild boar populations at all, or manifests itself quite sporadically (20).

High proportion of PRRSV positive wild boars in Lithuania established by our investigations, which was even higher than that among domestic pigs, provides the conclusion that wild boars in Lithuania can serve as a natural reservoir for PRRSV and pose a threat to domestic pigs.

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