

## SEROLOGICAL SURVEY ON PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IN LITHUANIAN PIGS AND WILD BOARS

Jūratė Buitkuvienė<sup>1,2</sup>, Jurgita Deltuvytienė<sup>1</sup>, Ramunė Čepulienė<sup>1,3</sup>, Virginija Žilionytė<sup>1</sup>, Jurgita Mozūraitytė<sup>2</sup>, Gediminas Pridotkas<sup>2</sup>, Arūnas Stankevičius<sup>1</sup>

<sup>1</sup>Department of Anatomy and Physiology, Veterinary Academy, Lithuanian University of Health Sciences  
Tilžės 18, LT-47181 Kaunas; e-mail: sarunas@lva.lt

<sup>2</sup>National Food and Veterinary Risk Assessment Institute  
J. Kairiūkščio 10, LT-08409 Vilnius; e-mail: jbuitkuviene@vet.lt, gpridotkas@vet.lt

<sup>3</sup>Department of Anatomy, Histology and Anthropology, Faculty of Medicine, Vilnius University  
M. K. Čiurlionio st. 21, LT-03101, Vilnius; email: ramune.cepulienė@mf.vu.lt

**Abstract.** The main purpose of the present study was to investigate the seroprevalence of porcine reproductive and respiratory syndrome virus (PRRSV) infection in Lithuanian pigs and wild boars. The porcine serum samples (n=8704), collected from 2008 to 2011, were tested with enzyme linked immunosorbent assay, using commercial IDEXX, INGENAZA tests. The presence of the antibodies against PRRSV was detected in 4.29 % (95% CI 3.48 – 5.1) of the samples. The seroprevalence in sows and gilts (16.98%) was significantly higher ( $P<0.05$ ) than in boars (0.48%), piglets (9.24%) and fattening pigs (1.79%). The detected seroprevalence differences in different study years were statistically insignificant ( $P>0.05$ ), however, the swine farms (n=55) with seropositive pigs were widely distributed in 12 out of 30 Lithuanian regions.

From 1022 examined wild boar sera, collected during the hunting seasons in 2008–2011, 6.36 % (95% CI 4.52 – 8.2) of samples were positive to PRRSV antibodies in 49 locations out of 61 investigated. The number of seropositive animals over 4 year period decreased from 9.1% in 2008 to 6.06 % in 2011, however estimated seroprevalence differences were statistically insignificant ( $P>0.05$ ). The analysis of seroprevalence in different age groups of wild boars showed that antibodies to PRRSV were present in all age groups, however it was significantly higher in adults than in juveniles, sub-adults and unknown age animal groups and reached 12.7% (95 % CI, 10.4 – 14.9). Our study showed that wild boars may play an important role in PRRSV transmission to the domestic pig population.

**Keywords:** PRRSV, seroprevalence, pigs, wild boars.

### Introduction

The porcine reproductive and respiratory syndrome virus (PRRSV) is widespread in pig populations of many countries and it is the cause of many reproductive problems in sows and diseases of the respiratory system in other pigs, the young ones in particular. Today, 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 the countries where pig-breeding is developed. It has been calculated that due to PRRSV infection losses per each adult pig can total from 3 to 10 English pounds per year (Lewis et al., 2007). In case of the acute outbreak of the disease, the productivity of a pig herd decreases by 5–20 per cent, as much as 75.6 per cent of the newborn young pigs die (part of them die when being weaned), others suffer from pathology of the respiratory system (Cho et al., 2006; Dee et al., 2000). Following 16 weeks of the acute course the disease usually becomes chronic (Pejsak et al., 1997). On the majority of pig farms chronic PRRSV infection turns into an endemic disease, which is also highly detrimental because diagnostics and treatment of the disease, adequate grouping of pigs and control of the breeding herd require additional funds and reproductive disorders develop in sows (Bierk et al., 2001; Corbellini et al., 2006).

Although the disease is highly detrimental and dangerous, PRRSV infection has not been investigated extensively in Lithuania thus far. Since 1997, when clinical symptoms characteristic of PRRSV were

observed and specific antibodies were determined for the first time (Janutėnaitė et al., 2000), further serological PRRSV prevalence has been studied several times episodically on different pig farms (Stankevičienė et al., 2002; Stankevičienė et al., 2008). Also, molecular diagnostics has been introduced and PRRSV strains have been characterised (Stadejek et al., 2002; Stankevičius et al., 2003; Stankevičius et al., 2008).

In the course of the last 10 years, the entire pig breeding technology and conditions have changed in essence in Lithuania, live attenuated vaccines have been used for the prophylactics of PRRSV infection, not only the breeds of the pigs kept on the farms have changed but also strict biological safety measures have been introduced. Seeking to elucidate how these changes influenced the spread of PRRSV on the pig farms in Lithuania and what the situation of PRRSV infection on the present-day Lithuanian pig farms is, it was necessary to carry out these investigations and to assess the distribution of PRRSV specific antibodies in different pig groups, farms and regions.

Wild boars are considered to be a reservoir of the agents of many dangerous infectious diseases, which can infect domestic pigs, animals of other kinds and also people (Ruiz-Fons et al., 2008). Wild boars can also be a source of infectious diseases of pigs (Laddomada, 2000; Al Dahouk et al., 2005). Interaction and exchange of infectious diseases between wild boars and domestic pigs have been studied most extensively and described in the

cases of classic swine fever and Aujeszki disease virus infection (Albina et al. 2000). Wild boars are considered to be the source of these viruses. Scientific publications provide very scanty information about PRRSV persistence in wild boars. Antibodies against PRRSV in wild boars have been discovered in several countries only and in individual cases only (Oslage et al., 1994; Saliki et al., 1998; Albina et al., 2000). Many other publications contained information about negative results of the investigations into PRRSV-specific antibodies (Vicente et al., 2002; Ruiz-Fons et al., 2006; Zupančič et al., 2002; Vengust et al., 2006 m.). At present, it is assumed that wild boars are infected with PRRSV by domestic pigs rather than the other way round. However, clear evidence is lacking and the available evidence is often contradictory. Currently available information does not provide any conclusive evidence about wild boars being a reservoir of PRRSV (Ruiz-Fons et al., 2007; Meng et al., 2009). It is likely that domestic pigs could have become infected by wild boars earlier before strict bio-safety requirements had been introduced to pig farms. These investigations were carried out seeking to assess the importance of PRRSV infection in the population of wild boars in Lithuania.

**The objective of the work** is to determine the prevalence of PRRSV infection in Lithuanian pig and wild boar populations by means of serological investigations.

#### Materials and methods

Blood serum samples collected from 55 different pig farms in 30 regions of Lithuania between 2008 and 2011 were used in carrying out investigations into PRRSV-specific antibodies. Blood serum was taken from clinically healthy pigs that had not been vaccinated against PRRSV. Prior to the beginning of the investigations all blood serum samples (n=8704) were kept frozen at  $-18^{\circ}\text{C}$ . To carry out the investigations blood serum samples were collected from sows and gilts (n=895), boars (n=1682), young pigs under 3 months of age (n=1385), and fattening pigs (n=4742). Precise and detailed information about these samples was obtained on the farms relating to the age, sex, breed, region and the farm of the pigs. When collecting blood serum samples of pigs from each group the principle of simple random sampling was adopted.

During the autumn hunting season in 2008–2011 random blood serum samples of hunted wild boars (n=1022) were collected to carry out investigations into PRRSV infection. Blood serum samples of juvenile wild boars under 12 months of age (n=341), 12 – 24-month old wild boars (n=266), adult 24-month-old and older wild boars (n=379) were collected and all the samples were kept frozen until the beginning of the investigations. Wild boar blood samples were collected in all ten counties of Lithuania and in its all 50 districts, in more than 300 different hunting sites.

To determine PRRSV specific antibodies commercial indirect immunofluorescence assay (IFA) kits IDEXX PRRS HerdChek, IDEXX PRRS X3, INGEZIM PRRS

Europa were used. The investigations were carried out according to the methodological recommendations of the manufacturers of IFA diagnostic kits. All the schemes of the research methods of PRRSV-specific antibodies employed in the investigations were either accredited or verified according to the requirements of LST EN ISO/IEC 17025:2005 standards.

The investigation results have been assessed with the help of statistical programme package GraphPrism 3.0<sup>TM</sup>. The 95% confidence interval has been calculated (CI). The student confidence level and the results obtained are considered to be statistically significant when  $p < 0.05$ .

The investigations were carried out following the Law on the Care, Keeping and Use of Animals of the Republic of Lithuania No. 8-500 of 6 November 1997 (*Valstybės žinios (Official Gazette)*, No. 108) and secondary legislation.

#### Investigation results

PRRSV-specific antibodies in blood serum samples, which were collected between 2008 and 2011 on different pig farms in Lithuania, were determined in 4.29 per cent of the pig population (95% CI 3.48–5.1). The largest amount of PRRSV-serologically positive samples was established in 2011 and accounted for 8.15 proc. (95% CI 6.99 – 9.31), and the smallest amount (2.76 per cent, 95% CI 1.92 – 3.6) was determined in 2010 (Table 1). The differences in the investigation results of PRRSV-specific antibodies between 2008 and 2011 were not statistically significant or when the data of different years were compared with the average ( $p > 0.05$ ).

Table 1. **PRRSV-specific antibody investigation results in pig blood serum samples collected between 2008 and 2011**

Year	Number of pigs studied	Found positive	%	95 PI, %
2008	1548	73	4.72	3.28–6.44
2009	1210	71	5.87	4.41–7.36
2010	4743	131	2.76	1.92–3.6
2011	1203	98	8.15	6.99–9.31
Total:	8704	373	4.29	3.48–5.1

Serological investigations into different groups of pigs with respect to PRRSV are presented in Table 2. PRRSV-specific antibodies were found in 16.98 per cent (95% CI 15.98 – 17.98) of all investigated blood serum samples (n=895) of sows and gilts. As much as 9.24 per cent of serologically positive samples (95% CI 8.14 – 10.34) were determined in the group of young pigs under three months of age. Our investigations into the prevalence of PRRSV carried out in different groups of pigs showed that PRRSV circulated more actively in the group of cows and young pigs Table 2 ( $p < 0.05$ ). During the investigations 16.98% of all the sows and young pigs under investigation (n=895) had PRRSV-specific antibodies, whereas the number of serologically positive samples in the group of boars and fattening pigs was considerably smaller (0.48 – 1.79%).

Table 2. PRRSV-specific antibody investigation results in different groups of pigs between 2008 and 2011

Group of pigs	Number of studied pigs	Found positive	%	95 CI, %
Sows and young pigs	895	152	16.98	15.98–17.98
Boars (of different age)	1682	8	0.48	0.03–0.93
Piglets under 3 months	1385	128	9.24	8.14–10.34
Fattening pigs	4742	85	1.79	0.95–2.74
Total:	8704	373	4.29	3.48–5.1

PRRSV positive samples were found in 12 districts of the country out of the 30 investigated ones. The largest number of PRRSV positive samples was discovered on the farms in Jonava (62.71 per cent), Vilkaviškis (60.17 per cent) and Radviliškis (43.24 per cent) districts. Investigation of 55 pig breeding farms between 2008 and 2011 showed that 40 per cent of the farms had PRRSV-specific antibodies.

The largest number of blood serums containing PRRSV antibodies was found in large pig complexes where the number of pigs kept totalled 15 000–30 000. As much as 71.13 per cent of the samples under investigation (n=194) had PRRSV-specific antibodies there (Table 3). On smaller pig breeding farms (up to 500 pigs) specific antibodies were determined in 5 per cent of the samples selected for investigations (n=80). The differences in the investigation results between large and small pig breeding farms were statistically significant ( $p<0.05$ ). On the medium-sized farms PRRSV-specific antibodies were determined in 38.81–58.6 per cent of the collected samples ( $p>0.05$ ).

Table 3. Influence of the size of a pig herd on PRRSV prevalence in the pig population of Lithuania

Size of a pig herds (number of pigs)	Number of pigs studied	Found positive	%
1–500	80	4	5.00
1 000–5 000	226	113	50.00
5 001–10 000	67	26	38.81
10 001–15 000	157	92	58.6
15 001–30 000	194	138	71.13

Table 4. PRRSV-specific antibodies investigation results in blood serum samples collected during the 2008–2011 hunting season

Year	Number of wild boars studied	Found positive	%	95 CI, %
2008	286	26	9.1	7.3–10.8
2009	274	15	5.5	3.14–7.86
2010	99	2	2.02	0.5–3.5
2011	363	22	6.06	3.58–8.62
Total:	1022	65	6.36	4.52–8.2

Out of 1022 blood serum samples of the wild boars killed during the autumn-winter hunting seasons between 2008 and 2011, PRRSV-specific antibodies were discovered in 6.36 per cent (95 % CI 4.52–8.2) of the

samples. The largest number of PRRSV positive samples was determined in 2008 and accounted for 9.1 per cent (95% CI 7.3 – 10.8), and the smallest number (2.02 per cent 95% CI 0.5–3.5) was established in 2010 (Table 4). The differences in the PRRSV-specific antibody investigation results in 2008–2011 were not statistically significant or when comparing the data of different years with the average ( $p>0.05$ ).

PRRSV antibody investigation results in different age groups of wild boars (from 12-month-old juveniles to 24 months and older) are presented in Table 5. Animals that had PRRSV antibodies were found in all age groups, however, the largest number, 12.7 per cent (95% CI 10.4–14.9), was discovered in the age group of adult, 24 months and older wild boars. The blood serum investigation results were similar in the age group of juvenile wild boars under 12 months of age and 12–24 months old wild boars, 2.6 per cent (95% CI 2.6–7.2) and 3.0 per cent (95% CI 3.2–6.9), respectively. No positively reacting ones were determined in the unknown age group of wild boars. The investigation results of blood serum samples of 24-month-old and older wild boars were statistically significantly higher than the data of serological investigations of the juveniles and young wild boars presented in Table 5 ( $p<0.05$ ).

Table 5. PRRSV-specific antibodies investigation results in different age groups of wild boars

Age group of wild boars	Number of wild boars studied	Found positive	%	95 CI, %
The young under 12 months of age	341	9	2.6	2.6–7.2
Young 12–24 month old wild boars	266	8	3.0	3.2–6.9
24-month old and older adult wild boars	379	48	12.7	10.4–14.9
Total:	1022	65	6.36	4.52–8.2

Serological investigations into wild boars to determine PRRSV were carried out in all 50 districts of Lithuania. PRRSV positive samples were found in 23 districts of the country.

PRRSV-positive blood serum samples were found in all districts of Lithuania without exception (Fig. 2) in which the number of positive samples ranged between 1.98 and 11.3 per cent.

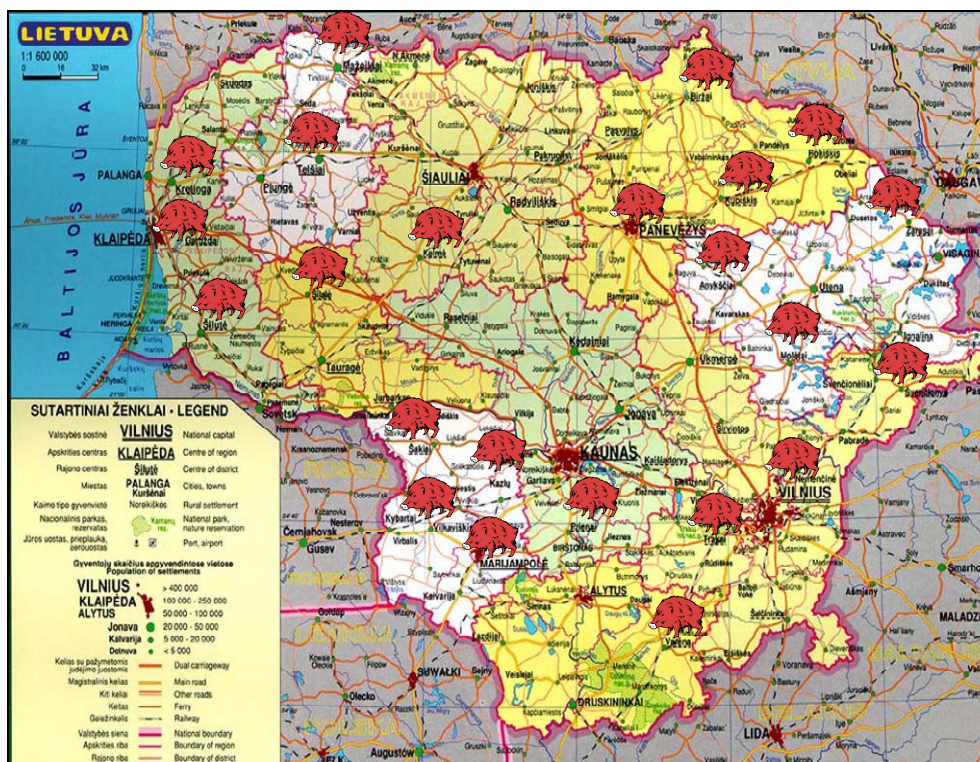


Fig.1. PRRSV prevalence in a wild boar population in Lithuania between 2008 and 2011 by districts. The sites where PRRSV-specific antibodies were discovered in blood serum samples are marked by wild boar silhouettes on the map.

The largest number of PRRSV-positive samples, as many as 16, were found among the samples collected from the wild boars hunted in the district of Utena ( $n=213$ ) and 12 PRSSV-positive samples were taken from the wild boars killed in the district of Vilnius ( $n=117$ ).

The largest percentage of PRRSV-positive wild-boars was determined in the districts of Marijampolė (11.3 per cent), Tauragė (10.46 per cent) and Vilnius (10.26 per cent), and the smallest one (1.98 per cent) – in the district of Šiauliai.

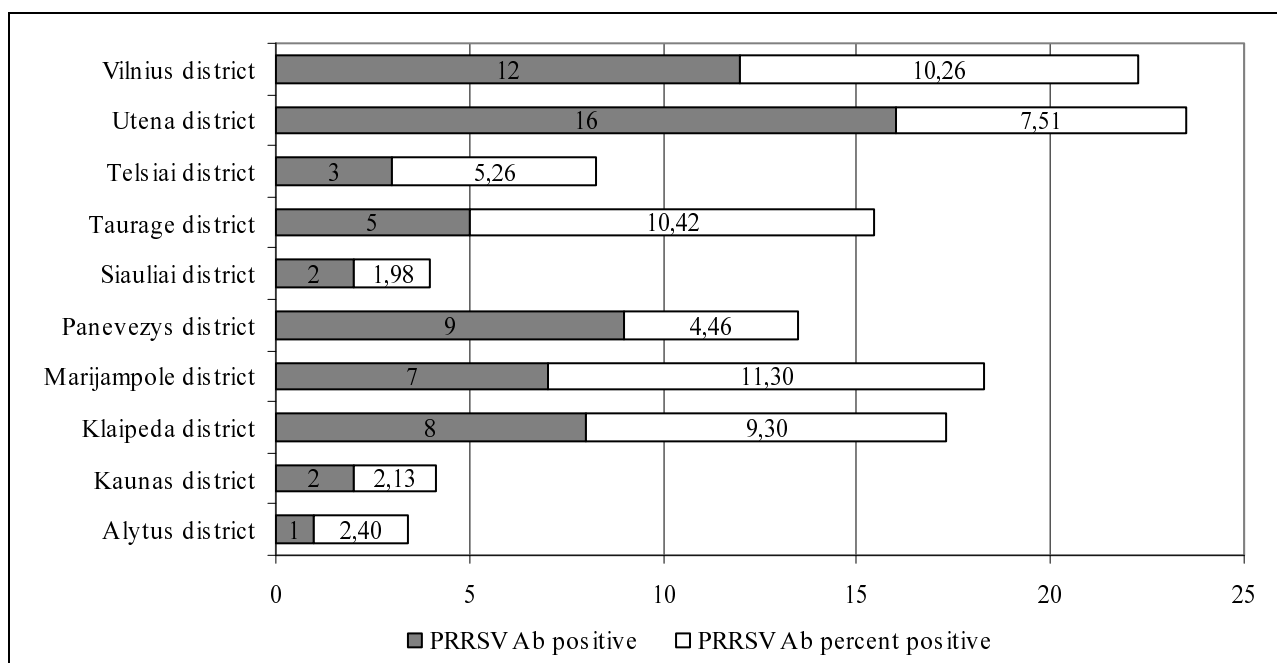


Fig. 2. PRRSV-specific antibody investigation results in blood serum samples of wild boars collected between 2008 and 2011 in 10 districts of Lithuania

**Discussion and conclusions.** The investigations into PRRSV prevalence on pig breeding farms in Lithuania carried out between 2008 and 2011 showed that 4.29 per cent of the animals under investigation had this virus-specific antibody. Attention should be drawn to the fact that unvaccinated young pigs and pigs, which displayed no symptoms characteristic of PRRSV infection, were investigated by means of the IFA method, and blood serum samples were collected at random for studying control of other important infectious diseases. During the investigation period, PRRSV-specific antibodies were detected in 2.76–8.15 per cent of blood serum samples of the pigs under investigation; however, no statistically significant changes were established during that period. Having compared the results obtained with the results of the investigations into the PRRSV infection carried out on breeding farms between 2005 and 2007 (Stankevičienė et al., 2008), where PRRSV antibodies were found only in 13.7 per cent of blood serum samples, a significant decrease in the number of PRRSV infected pigs can be observed. The serological investigations carried out between 1998 and 2001 during which 29.2–40.7 per cent of PRRSV-positive samples were detected (Stankevičienė et al., 2002) revealed this tendency even more clearly. Such a considerable decrease in the number of PRRSV-positive pigs can be accounted for by the fact that of recent years the new owners have depopulated several large pig breeding farms and at present PRRSV-negative pig herds are bred there. Modern pig breeding technologies have been started to be employed on pig breeding farms paying great attention to biological safety requirements, quarantining and grouping, as well as laboratory investigations of pigs. Of late, live attenuated vaccines have protected pigs against acute outbreaks of PRRSV infection, decreased efficiently the emission of the virus to the ambient environment and have increased immunological resistance of the herd to PRRSV infections.

The assessment of the PRRSV epidemiological situation in the pig population of Lithuania has yielded positive results. Yet the data from other countries, which show that PRRSV-specific antibodies in Holland were detected in 18 to 82 per cent of the farms (Duijnhof et al., 2011) should be taken into consideration. In England, 39.8 per cent of serologically PRRSV-positive blood serum samples of unvaccinated pigs of different age were determined (Evans et al., 2008). Serological investigations of recent years on pig farms in Thailand showed that 87.5 per cent of replacement gilts had PRRSV-specific antibodies (Tummaruk et al., 2012).

Our investigations into PRRSV prevalence in different groups of pigs showed that PRRSV circulated more actively among sows and gilts. During the investigations 16.98 per cent of all sows and gilts ( $n=895$ ) under investigation had PRRSV-specific antibodies, whereas in the groups of boars and fattening pigs the number of serologically positive samples was considerably smaller (0.48–1.79 per cent). Having analysed the data of serological investigations of different years it turned out that PRRSV infection in the group of boars was very

small not only in assessing the entire period under investigations on the whole but also the data of different years shows that only a small number (0.3–0.4 per cent) of pigs of this group had specific antibodies. This leads to the supposition that PRRSV infection practically does not spread on Lithuanian pig breeding farms through the semen or through direct contact during mating.

Investigations into PRRSV-specific antibodies on 55 pig breeding farms in 30 districts of Lithuania showed that serologically positive samples were found on pig breeding farms in 12 districts of the country. In our opinion these data also confirm a conditionally favourable epidemiological situation in different regions of Lithuania with respect to PRRSV infection because the largest number (60 per cent) of pig breeding farms in Lithuania had no PRRSV-specific antibodies, which means that PRRSV was not prevalent on these farms. The situation, which is improving with respect to PRRSV infection, can also be observed when comparing the data obtained with the results of the investigations carried out between 1997 and 2001 (Stankevičienė et al., 2002). More than ten years ago, pig farms in 23 regions had PRRSV-specific antibodies. Serological investigations in different districts of Lithuania also showed that in some districts of Lithuania a conditionally smaller number of PRRSV can be detected (from 60.17 to 43.24 per cent), however, to substantiate these data in more detail additional investigations are needed taking into consideration the size of a pig population in each region, the number of pig breeding farms and the number of pigs bred there.

The results of the investigations obtained also showed that the number of PRRSV positive blood serum samples was reliably smaller on small pig breeding farms (up to 500 pigs) than that on large or medium-sized farms. This allows concluding that the concentration of pigs in large pig complexes creates favourable conditions for PRRSV infection to spread. The results of our investigation also clearly showed that the number of PRRSV positive unvaccinated pigs can account for up to 71.13 per cent in pig complexes containing from 15 000 to 30 000 pigs. The earlier investigations revealed that 63.4–66.3 per cent of blood serum samples contained PRRSV-specific antibodies in large pig breeding complexes (Stankevičienė et al., 2002). The results of the investigations into PRRSV prevalence carried out by other authors are also similar. According to their data, the conditions for a spread of PRRSV on the farms of different size are better not only due to a large concentration of pigs but also due to a larger number of persistently infected pigs, to identify of which not only serological but also molecular research methods enabling the virus to be identified directly in the material under study, are needed (Evans et al., 2008; Stankevicius et al., 2008; Corbellini Luis et al., 2006).

Scientific publications provide very little data about PRRSV prevalence in the populations of wild boars (*Sus scrofa*) or the available data are rather contradictory. PRRSV antibodies were detected in the population of wild boars only in 0.3–3.6 per cent of cases in the USA, France and respectively in Germany (Saliki et al., 1998; Albina et al., 2000; Oslage et al., 1994). In the United

States of America, 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 (1996). No serologically positive PRRSV cases in 768 samples of wild boars chosen for investigations and collected in 1992–1993 and in 1995–1996 were detected. No PRRSV-specific antibodies were found in wild boars in neighbouring Poland and the Russian Federation (Fabisiak et al., 2013; Kukushkin et al., 2008). Blood serum investigations of boars were negative in Spain, Croatia and Slovenia (Vicente et al., 2002; D. Ruiz-Fons et al., 2006; Zupančič et al., 2002; Vengust et al., 2006 m.). By means of PCR (polymerase chain reaction) PRRSV was discovered in lung samples of boars in Italy and Germany (Bonilauri et al., 2006; Reiner et al., 2007). Investigations into the wild boars of Germany carried out by Reiner et al. (2007) by means of molecular methods proved for the first time that European (genotype 1) and American (genotype 2) strains of PRRSV could be directly determined by the RT-PCR (reverse transcription polymerase chain reaction) in 15.9 per cent of the lung or tonsils samples (Reiner et al., 2009). Also, one publication of recent years has been publicised about the spread of PRRSV of genotype 2 in the population of hybrid wild pigs (Wu et al., 2011). There is no much data about prevalence of PRRSV infection among wild boars available in literature, whereas the results of our investigations present new valuable epidemiological information about PRRSV infection in wild boars, which has been very little investigated thus far.

The results of our investigations conducted between 2008 and 2011 showed a comparatively large (6.36 per cent) number of wild boars that had PRRSV-specific antibodies, which was even larger than the number of serologically positive pigs (4.29 per cent) in the pig population. Also, the results of the investigations into the prevalence of PRRSV showed a larger number of positive wild boars than the results of recent PRRSV serological investigations into wild boars carried out in Spain (2.0–3.0 per cent; Bodella et al., 2011; Closa-Sebastia et al., 2011) and Germany (3.82 per cent; Kaden et al., 2009). This conditionally large number of serologically positive PRRSV animals can be accounted for by the conditions, which formed in the wild boar population in Lithuania. Between 2008 and 2011, the density of wild boars increased significantly in Lithuania – from 1.84 to 2.66 boars per km<sup>2</sup>. PRRSV prevalence in wild boars could have been determined by intense migration and additional feeding of the animals during winter months. This determines an increase in the wild boar population in certain places in the forest, which is conducive to a spread of PRRSV from one wild boar to another. The results of our investigations show that the spread of PRRSV among wild boars can be much more significant than it has been thought up till now; however, more detailed investigations into the wild boar populations in neighbouring Latvia, Belarus and Kaliningrad Region are necessary to prove that. The PRRSV serological investigations carried out in Lithuania are the first efficient and the only ones of this kind investigations conducted in Eastern Europe.

The investigation results in different age groups of wild boars conducted between 2008 and 2011 showed that PRRSV-specific antibodies in blood serum samples of adult 24-month old and older wild boars were detected more often than in a group of juveniles or 12–24 month old animals. In our opinion, this result shows that two-year old and older wild boars in Lithuania can be the main source of PRRSV infection in Lithuania, however, it is possible to verify this supposition conclusively only after studying clinical samples of wild boars by means of RT-PCR (reverse transcription polymerase chain reaction), which would allow cases of infection of this virus to be determined directly. As far as we know, the only investigations in Europe and the world, which directly proved PRRSV prevalence in the wild boar population, were carried out in Germany (Reiner et al., 2007).

PRRSV is widespread among wild boars in a large territory of Lithuania. This is also confirmed by detecting the presence of specific antibodies in the samples collected in 23 regions, which are attributed to 10 districts of Lithuania. This enables the supposition to be made that it was not by way of casual contact that PRRSV got into the wild boar population in some region or district of Lithuania. The broad distribution of serologically positive PRRSV samples of wild boars shows a regular circulation of this virus in the whole population of wild boars in Lithuania. The data of the investigations carried out by other researchers show that a relatively small number of wild boars that were in contact with PRRSV, or the detection of serologically negative animals on the whole, leads us to the conclusion that PRRSV infection does not practically manifest itself in the wild boar population at all or manifests itself sporadically (Ruiz-Fons et al., 2007).

The large number of PRRSV positive wild boars in Lithuania established by our investigations, which was even larger than that among domestic pigs, enables us to suppose that wild boars in Lithuania can serve as a natural reservoir for PRRSV. This changes the prevailing opinion that no sufficient evidence is available about the PRRSV endemic reservoir in the wild boar population (Meng et al., 2009). Also, it should be remembered that wild boars are universally considered to be an important source of classic swine fever and Aujeszky's disease virus infection, which can very easily infect pigs through direct contact (Albina et al., 2000).

## Conclusions

1. PRRSV serological investigations carried out on pig breeding farms in Lithuania between 2008 and 2011 showed that these virus-specific antibodies were present in 4.29 per cent (95% CI 3.48–5.1) of the pigs under investigation in 12 out of 30 Lithuanian regions. PRRSV-specific antibodies were detected in 16.98 per cent (95% CI 15.98–17.98) of blood serum samples of sows and gilts, and these results were statistically significantly larger than the data of serological investigations into boars (0.48 per cent 95% CI 0.03–0.93) and fattening pigs (1.79 per cent; 95% CI 0.95–2.74). A statistically significant difference established between the number of

PRRSV positive samples on large 15 000–30 000 pig breeding farms (71.13 per cent) and small (up to 500 pigs) farms (5 per cent) proves that the concentration of pigs in modern pig complexes in Lithuania creates favourable conditions for PRRSV infection to spread.

2. PRRSV infection investigations carried out between 2008 and 2011 showed, for the first time in Eastern Europe, a relatively large 6.36 per cent (95% CI 4.52–8.2) number of wild boars that had this virus-specific antibodies, which was larger than the number of serologically positive pigs determined in the pig population on Lithuanian pig breeding farms. The largest number of PRRSV-specific antibodies (12.7 per cent; 95% CI 10.4–14.9) was determined in the age group of 24-month old and older wild boars, which was significantly larger than the number of positive samples respectively determined in the age group of juveniles (2.6 per cent; 95% CI 2.6–7.2) or 12–24-month old pigs (3.0 per cent; 95% CI 3.2–6.9). Detection of PRRSV-specific antibodies in wild boar samples collected in 23 regions of Lithuania belonging to 10 districts presents the first preliminary evidence that wild boars can be a natural reservoir for PRRSV in Eastern Europe.

**Acknowledgments.** The investigation was funded by the Science Council of Lithuania (Contract No. MIP-065/2012).

## References

1. Al Dahouk S., Nockler K., Tomaso H., Splettstoesser W., Jungersen G., Riber U., Petry T., Homann D., Scholtz H., Hensel A., Neubauer H. Seroprevalence of brucellosis, tularemia, and yersiniosis in wild boars (*Sus scrofa*) from North-Eastern Germany. *Journal of Veterinary Medicine B*. 2005. 52. P. 444–455.
2. Albina E, Mesplede A, Chenut G, Le Potier MF, Bourbao G, Le Gal S, Leforban Y (2000) A serological survey on classical swine fever (CSF), Aujeszky's disease (AD) and porcine reproductive and respiratory syndrome (PRRS) virus infections in French wild boars from 1991 to 1998. *Veterinary Microbiology*. 77. P. 43–57.
3. Bierk M., Dee S. A., Rossow K. D., Collins J. E., Guedes M. I., Pijoan C., Molitor T. W. Diagnostic investigation of chronic porcine reproductive and respiratory syndrome virus in a breeding herd of pigs. *Veterinary Record*. 2001. 148. P. 687–690.
4. Boadella M., Ruiz-Fons F., Vicente J., Martin M., Segales J., Gortazar C. Seroprevalence evolution of selected pathogens in Iberian wild boar. *Transboundary emerging diseases*. 2011. 25. P. 136–142.
5. Cho J., Dee S. Porcine reproductive and respiratory syndrome virus. *Theriogenology*. 2006. 66. P. 655–662.
6. Closa-Sebastia F., Casas-Diaz E., Cuenca R., Lavin S., Mentaberre G., Marco I. Antibodies to selected pathogens in wild boar (*Sus scrofa*) from Catalonia (NE Spain). *European journal of wildlife research*. 2011. 57. P. 977–981.
7. Corbellini Luis G., Schwermer H., Presi P., Thur B., Stark Katharina D. C., Reist M. Analysis of national serological surveys for the documentation of freedom from porcine reproductive and respiratory syndrome in Switzerland. *Veterinary Microbiology*. 2006. 118. P. 267–273.
8. Dee S. A., Molitor T. W., Rossow K. D. Epidemiological and diagnostic observations following the elimination of porcine reproductive and respiratory syndrome virus from a breeding herd of pigs by the test and removal protocol. *Veterinary Record*. 2000. 146. P. 154–158.
9. Duinhof T., van Schaik G., van Esch E., Wellenberg G. Detection of PRRSV circulation in herds without clinical signs of PRRS: comparison of five age groups to assess the preferred age group and sample size. *Veterinary Microbiology*. 2011. 150. P. 180–184.
10. Evans C., Medley G., Green L. Porcine reproductive and respiratory syndrome virus (PRRSV) in GB pig herds: farm characteristics associated with heterogeneity in seroprevalence. *BMC veterinary research*. 2008. 4. P. 48–52.
11. Fabisiak M., Podgorska K., Skrypiec E., Szotka A., Stadejek T. Detection of porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) antibodies in meat juice samples from Polish wild boar (*Sus scrofa*). *Acta veterinaria Hungarica*. 2013. In press.
12. Janutėnaitė J., Ščerbavičius R., Blaževičius E. Current status of porcine reproductive and respiratory syndrome in Lithuania, sero-epidemiological study by ELISA. *Proceedings of the 5<sup>th</sup> International Congress of Veterinary Virology*. 2000. P. 244–245.
13. Kaden V., Lagen E., Hanel A., Hlinak A., Mewes L., Hergarten G., Irsch B., Deken J., Bruer. Retrospective serological survey on selected viral pathogens in wild boar populations in Germany. *European journal of wildlife research*. 2009. 55. P. 153–159.
14. Kukushkin S., Kanshina A., Timina A., Baybikov T., Mikhailishin V. Investigation of wild boar (*Sus scrofa*) for porcine reproductive and respiratory syndrome in some territories of Russia. *European journal of wildlife research*. 2008. 54. P. 515–518.
15. Laddomada A. Incidence and control of CSF in wild boar in Europe. *Veterinary Microbiology*. 2000. 73. P. 121–130.
16. Lewis C., Tahar A., Claperton M., Archibald A., Bishop S. Genetic perspectives on host responses to porcine reproductive and respiratory syndrome (PRRS). *Viral Immunology*. 2007. 20(3). P. 343–357.
17. Lutz W., Wurm R. Serological investigations to demonstrate the presence of antibodies to the viruses causing porcine reproductive and respiratory syndrome, Aujeszky's disease, hog cholera, and porcine parvovirus among wild boar (*Sus scrofa*) in North Rhine-Westphalia.

Zeitschrift fur Jagdwissenschaft. 1996. 42. P. 123–133.

18. Meng X. J., Lindsay D.S., Sriranganathan N. Wild boars as sources for infectious diseases in livestock and humans. *Philosophical transactions of the royal society B*. 2009. 364. P. 2697–2707.

19. Montagnaro S., Sasso S., De Martin L., Lango M., Iovane V., Ghiumino G., Pisanelli G., Nava D., Baldi L., Pagnini U. Prevalence of antibodies to selected viral and bacterial pathogens in wild boars (*Sus scrofa*) in campania Region, Italy. *Journal of wildlife diseases*. 2010. 46(1). P. 316–319.

20. Oslage U., Dahle J., Muller T., Kramer M., Beier D., Liess B. Prevalence of antibodies against the viruses of European swine fever, Aujeszky's disease and «porcine reproductive and respiratory syndrome» in wild boars in the federal states Sachsen-Anhalt and Brandenburg. *Deutsche Tierärztliche Wochenschau*. 1994. 101. P. 33–38.

21. Pejsak Z., Stadejek T., Markowsk-Daniel I. Clinical sings and economic losses by porcine reproductive and respiratory syndrome virus in large breeding farm. *Veterinary Microbiology*. 1997. 55. P. 317–322.

22. Reiner G., Fresen C., Bronnert S., Willems H. Porcine reproductive and respiratory syndrome virus (PRRSV) infection in wild boars. *Veterinary Microbiology*. 2009. 136. P. 250–258.

23. Ruiz-Fons F., Segales J., Gortazar C. A review of viral diseases of the European wild boar: Effects of population dynamics and reservoir role. *Veterinary Journal*. 2008. 176. P. 158–169.

24. Ruiz-Fons F., Vicente J., Vidal D., Holfe U., Villanua D., Gauss C., Segales J., Almeria S., Montoro V., Gortazar C. Seroprevalence of six reproductive pathogens in European wild boar (*Sus scrofa*) from Spain: The effect on wild boar female reproductive performance. *Theriogenology*. 2006. 65(4). P. 731–743.

25. Saliki J., Rodgers S., Eskew G. Serosurvey of selected viral and bacterial diseases in wild swine from Oklahoma. *Journal of Wildlife diseases*. 1998. 34. P. 834–838.

26. Stadejek T., Stankevicius A., Storgaard T., Oleksiewicz M., Belak S., Drew T., Pejsak Z. Identification of radically different variants of porcine reproductive and respiratory syndrome virus in Eastern Europe: towards a common ancestor for European and Amerocan viruses. *Journal of general Virology*. 2002. 83. P. 1861–1873.

27. Stankevičienė M., Stankevičius A., Stankevičius H., Liutkevičienė V., Černauskienė J., Šakalienė J., Petkevičius S. Kiaulių reprodukcinių ir kvėpavimo sindromo paplitimas, veislių jautrumas virusui. *Veterinarija ir zootechnika*. 2008. 42(64). P. 71–80.

28. Stankevičienė M., Stankevičius A., Kiaulių reprodukcinių ir kvėpavimo sindromo viruso (KRKSV)

seroepizootiniai tyrimai Lietuvos kiaulininkystės ūkiuose. *Veterinarija ir zootechnika*. ISSN 1392–2130. 20 (42). 2002. P. 51–58.

29. Stankevičius A., Čepulis R., Žilinskas H., Stadejek T., Pejsak Z. Persistence of European and American type PRRSV strains within Lithuanian pig herd. *Bulletin of veterinary Institute in Pulawy*. 2008. 52. P. 319–323.

30. Stankevičius A., Stankevičienė M., Pieškus J. Kiaulių reprodukcijos ir kvėpavimo sindromo viruso lietuviškų padermių genetiniai skirtumai ir filogenetinė analizė ORF5 ir ORF7 regionuose. *Veterinarija ir zootechnika*. 2003. 23:1–9.

31. Tummaruk P., Tantilertcharoen R. Seroprevalence of porcine reproductive and respiratory syndrome, Aujeszky's disease, and porcine parvovirus in replacement gilts in Thailand. *Tropical animal health and production*. 2012. 44. P. 983–989.

32. Vengust G., Valencak Z., Bidovec A. Serological survey of selected pathogens in wild boar in Slovenia. *Journal of Veterinary Medicine B*. 2006. 53. P. 24–27.

33. Vincente J., Leon-Vizcaino L., Gortazar C., Jose Cubero M., Gonzalez M., Martin-Atance P. Antibodies to selected viral and bacterial pathogens in European wild boars from southcentral Spain. *Journal of Wildlife Diseases*. 2002. 38. P. 649–652.

34. Wu J., Liu S., Zhou S., Wang Z., Li K., Zhang Y., Yu J., Cong X., Chi X., Xu S., Du Y., Ren S., Wang J. Porcine reproductive and respiratory syndrome in hybrid wild boars, China. *Emerging infectious diseases*. 2011. 17(6). P. 1071–1073.

35. Zupačič Z., Jukič B., Lojkič M., Čač Z., Jemeršič L., Starešina V. Prevalence of antibodies to classical swine fever, Aujeszký's disease, porcine reproductive and respiratory syndrome, bovine viral diarrhoea viruses in wild boars in Croatia. *Journal veterinary Medicine B*. 2002. 49. P. 253–256.

Received 4 April 2013

Accepted 10 September 2013