A SURVEY ON PRRS USING ELISA AND PCR METHODS ON LITHUANIAN SWINE FARMS IN 2001-2007

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ABSTRACT

The purpose of work was to perform a survey on porcine reproductive and respiratory syndrome (PRRS) using ELISA and PCR methods in Lithuanian swine farms in 2001-2007. Altogether 3766 swine serum samples were tested using ELISA in 2001-2007 and 1133 (30.1%) found to be positive. Respectively, 28.6%, (840/2933) – in 2001-2005, 34.3% (183/533) and 36.7% (110/300) positive samples were found in 2001-2005, 2006 and 2007.
KEY WORDS: PRRS, ELISA, PCR.

INTRODUCTION

PRRS is important swine disease throughout the world. The disease was first recognized in the United States in 1987 although its description had appeared in some countries years earlier, is now found in almost all swine producing countries. A number of PRRS outbreaks have occurred recently in South Africa, Russia, China and Viet Nam. The diseases, which represents and worldwide threat, is characterized by reproductive failure of sows and respiratory distress of piglets and fattening pigs which, combined with a potential for rapid spread , can cause significant production and economic losses. There are two antigenic types of the virus: the American and European. Some of the reports from Asia, where the PRRS virus has been isolated, describe a disease of swine with high mortality in different age group.

In Lithuania PRRS serology is carried out more than 10 years and PRRS virus is characterized using molecular biology methods [4, 5].

The prevalence of PRRSV antibodies positive animals was significantly higher (61.6%) in 1997 when PRRS disease was first recognized in Lithuania [3]. Later in 1998-2001, the number of positive swines was lower 38.7%, 40.7%, 36.1% and 29.2, respectively [3]. PRRS vaccination programs were introduced in some swine farms using the vaccines prepared with attenuated or inactivated PRRS virus strains to control reproductive performance. No change in epidemiological situation was observed because only sows were vaccinated.

No review about epidemiological situation for several year with respect to PRRS has been done. So the purpose of our work was to determine the changes of PRRS virus infection in Lithuania situation from 2001 to 2007.

MATERIAL AND METHODS

All samples were tested at Veterinary Institute of Lithuanian Veterinary Academy.

Serological methods.

ELISA. All serum samples were tested for PRRS using an ELISA (IDEXX) [1].

Molecular biology methods.

PCR – single tube nested RT-PCR was used [2, 4, 5].

Primers ORF5F (5' ATGAGATGTTCTCACAAATTGGGGCG 3') and
ORF5R (5' CTAGGCCTCCCATTGCTCAGCCGAAGT 3') were used for nested PCR [6] and primers ORF5B (5' CAATGAGGTGGGCIACAACC 3') and
ORF5C (5' TATGTIATGCTAAAGGCTAGCAC 3') were used for the first PCR [1].

Pathological material of stillbirths and 2-4 months aged piglets was tested.

RESULTS AND DISCUSSION

Altogether 3766 swine serum samples were tested by means of ELISA with respect to PRRSV antibodies in 2001-2007 and 1133 samples were found to be positive.

In Table 1 the data of serological investigation shows that, 2933 serum samples were tested and 840 (28.6%) ones were positive. Positive samples ranged from 7.4% iki 88.2% in different swine farms.

Table 1

Seroprevalence of PRRS in 2001-2005

No.	Farm	ELISA			PCR		
		Samples, n	Positive		Samples	Positive	
			n	%		n	%
1.	ABBb	120	34	28.3	1	0	0
2.	ABV	411	168	40.8	74	13	17.6
3.	UABAv	49	18	36.7	0	0	0
4.	UABBk	155	71	45.8	17	2	11.8
5.	UABB	296	22	7.4	4	0	0
6.	UABGb	34	5	14.7	8	0	0
7.	UABK	694	236	34.0	29	1	3.4
8.	UABL	643	73	11.3	0	0	0
9.	UABS	100	62	62.0	8	1	12.5
10.	UABSi	70	15	21.4	0	0	0
11.	UABZ	87	21	24.1	0	0	0
12.	UABBaltic	40	18	45.0	0	0	0
13.	UkVG	27	10	37.0	6	0	0
14.	UkSK	18	9	50.0	0	0	0
15.	ZUBB	10	1	10.0	0	0	0
16.	ZUBV	17	15	88.2	0	0	0
17.	ZUBD	162	62	38.2	81	1	1.2
Altogether		**2933**	840	28.6	228	18	7.9

Pathological material samples were tested using PCR method and 18 (7.9%) out of 228 were positive in 2001-2005. Positive samples ranged from 1.2% to 17.6% in different swine farms.

Comparison of the results of serological and molecular biology methods showed that in UABBk 45.8% (71/155) serum samples were positive and 11.8% (2/17) pathological material ones. In UABS 62.0% (62/100) serum samples were positive and 12.5% (1/8) pathological material ones. In ABV 40.8% (168/411) serum samples were positive and 17.6% (13/74) pathological material ones. UABK 40.7% (217/533) serum and 3.4% (1/29) pathologtical material samples were positive. ZUBD 38.2% (62/162) serum and 1.2% (1/81) pathological material samples were positive (Table 1).

In 2006 the investigation was done in 11 swine farms and 533 serum samples were tested by ELISA (Table 2). Also 32 pathological material samples were tested by PCR in 2006 (Table 2). It was found that 34.3% serum samples were positive by ELISA and no positive samples were found by PCR. Positive samples by ELISA ranged from 8.7% to 70.0% in different farms. The seropositive samples were found in 72.7% (8/11) swine farms.

Samples of 15 swine farms were tested in 2007 (Table 3), 300 serum samples of 12 swine farms were tested and 110 (36.7) ones were positive. No positive samples by PCR were

found. Seropositive samples ranged from 5.3% to 100.0% in different swine farms. Seropositive samples were found in 75.0% (9/12) swine farms.

Table 2

Seroprevalence of PRRS in 2006

No.	Farm	ELISA			PCR	
		Samples, n	Positive		Samples, n	
			n	%	Tested	Negative
1.	UABB	10	1	10.0	13	13
2.	UABGb	153	51	33.3	0	0
3.	UABINT	128	51	39.8	0	0
4.	UABKagr	16	3	18.8	4	4
5.	UABKa	26	16	61.5	5	5
6.	UABBaltic	20	14	70.0	0	0
7.	UABNa	12	0	0	7	7
8.	UABS”	70	36	51.4	0	0
9.	UABSi	15	0	0	0	0
10.	UABZ	30	9	30.0	3	3
11.	UABZk	30	0	0	0	0
		23	2	8.7	0	0
Altogether:		533	183	34.3	32	32

Table 3

Seroprevalence of PRRS in 2007

No.	**Farm**	**ELISA**			**PCR**	
		Tested samples, n	**Positive samples**			
			n	**%**	**Ištirta**	**Negative**
1.	UABB	17	1	5.9	6	6
2.	UABBb	10	5	50.0	0	0
3.	UABBr	0			2	2
4.	UABD	0			4	4
5.	UABJ	30	27	90.0	0	0
6.	UABK	99	10	10.1	17	17
7.	UABKa	12	0	0	0	0
8.	UABSi	10	1	10.0	0	0
9.	UABSa	19	1	5.3	0	0
10.	UABBaltic	40	40	100.0	0	0
11.	UABVet	13	0	0	0	0
12.	UABZk	10	0	0	0	0
13.	UABNa	0			5	5
14.	UkGCh	24	24	100.0	20	20
15.	UkICh	16	1	6.3	0	0
Altogether:		**300**	110	36.7	54	54

Detailed study in 2003 showed that number (61.6%) of seropositive samples was higher in 1997, when disease was first recognized. Later seropositive samples ranged - 38.7%, 40.7%,

36.1% and 29.2%, respectively, in 1998, 1999, 2000 and 2001. Our data shows, that no essential changes occurred since 1998. However, high prevalence of seropositive animals shows that a lot of swines had contact with virus and virus carriers are possible in swine farms [3].

Earlier it was estimated that the seroprevalance in endemic Lithuanian White swine breed was significant lower than in other herds in 1997-2001 [3]. However, in our opinion it is really difficult to confirm it because of the insufficient number of purebred Lithuanian White swine farms. It was found that in the large swine farms the number of positive animals was two times higher than in the small farms (63-66% versus 22.0-37%) [3]. However our data shows that it is difficult to confirm, because the immunological structure of farm can be different from time to time, from farm to farm.

REFERENCES

1. Oleksiewicz, M.B., Botner, A., Madsen, K.G., Storgard, T. Sensitive detection and typing of porcine reproductive and respiratory syndrome virus by RT-PCR amplification of whole viral genes. Vet Microbiol., 1998, 64(1), 7-22.
2. Stadejek, T., Stankevicius, A., Storgaard, T., Oleksiewicz, M.B., Belák, S., Drew, T. W., Pejsak, Z. Identification of radically different variants of porcine reproductive and respiratory syndrome virus in Eastern Europe: towards a common ancestor for European and American viruses Journal of General Virology, 2002, 83, 1861-1873.
3. Stankevičienė, M., Stankevičius, A. Kiaulių reprodukcinio ir kvėpavimo sindromo viruso (KRKSV) seroepizootiniai tyrimai Lietuvos kiaulininkystės ūkiuose. Veterinarija ir Zootechnika. 2002, 20 (42), 51-58.
4. Stankevičius, A., Stankevičienė, M., Pieškus, J. Kiaulių reprodukcinio ir kvėpavimo sindromo viruso lietuviškų padermių genetiniai skirtumai ir filogenetinė analizė ORF5 ir ORF7 regionuose. Veterinarija ir zootechnika. 2003, 23 (45), 36-46.
5. Stankevičius, A., Stankevičienė, M., Šalomskas, A., Pieškus, J. Europietiškojo ir amerikietiškojo genotipų kiaulių reprodukcinio ir kvėpavimo sindromo viruso (KRKSv) identifikavimas ir tipizavimas vieno mėgintuvėlio atvirkštinės transkripcijos lizdine polimerazine grandinine reakcija (AT-PGR). Veterinarija ir Zootechnika. 2003, 21(43), 29-36.
6. Suarez, P., Zardoya, R., Martin, M.J., Prieto, C., Dopazo, J., Solana, A., Castro, J.M. Phylogenetic relationships of European strains of porcine reproductive and respiratory syndrome virus (PRRSV) inferred from DNA sequences of putative ORF-5 and ORF-7 genes. Virus Research, 1996, 42, 159-165.