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SEROLOGICAL SURVEY ON SELECTED PATHOGENS ON 16 SMALL PIG FARMS IN SLOVENIA

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*SUMMARY: Blood samples were collected between 2012 and 2014 on 16 small one-site pig farms with 34 to 79 breeding animals. 1636 serum samples of breeding animals and 815 serum samples of fatteners were tested with IDEXX PRRS ELISA. 1054 samples (from 7 small farms) were screened with one step RT-PCR (Qiagen, Germany) using sequences based on the open reading frame 7 (ORF7), which detect Type 1 and Type 2 PRRSV strains respectively. 81 serum samples of breeding animals and 85 serum samples of fatteners were tested with Swine Salmonella Antibody Test Kit (IDEXX) and CHEKIT*APP-ApxIV (IDEXX). 80 serum samples of sows were assayed for leptospira antibody using a microscopic agglutination test (MAT). Antibodies against porcine reproductive and respiratory virus (PRRSV) were detected in 67.5% of breeding animals and in 41.1% fatteners. By RT-PCR, PRRSV was detected in 7.9% of serum samples. The seroprevalence to salmonella in breeding animals was 21% and in fatteners 5.8%. The prevalence against Actinobacillus pleuropneumoniae (APP) antibodies was 87.6% in breeding animals and 49.4% in fatteners. Three farms were positive to leptospirosis (serovar hardjo, serovar grippotyphosa). We suggested farms free of PRRS to continue with biosecurity practices and 11 farms with PRRS we had suggested biosecurity measures and herd closure. Almost all breeding animals had antibodies against APP, though clinical signs were not present. Seroprevalence of salmonella in Slovenia is low. 3 pig farms had leptospirosis. All tree farms treated infected pigs.*

Key words: *pig, health status, small pig farms, control measures.*

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INTRODUCTION

After 2004 a significant decrease in pig meat production in Slovenia was noticed; also the number of animals and number of large farms has been decreasing for several years (Statistical office of the Republic of Slovenia, data from 1.1.2010). Most pig farms in Slovenia are small-sized and one site production farms. We have 3909 farms with 1 to 20 breeding pigs, 206 farms with 21 to 50 breeding pigs, 31 farms with 51 to 100 breeding pigs, 12 farms with 101 to 200 breeding pigs, 2 farms with 501 to 1000 breeding pigs and only 2 farms with more than 1000 breeding pigs (base VOLOS, Ministry for a agriculture, forestry and food, UVHVVR, data from 1.2.2013).

Before Slovenia joined EU in 2004 pig production in our country was for years under systematic monitoring and control for many important diseases. Representative numbers of pig sera were tested each year for the following disease: Aujeszky's disease, transmissible gastroenteritis, porcine reproductive and respiratory syndrome (PRRS) and pig brucellosis. All testings were obligatory and paid by government. In period 1995 to 2003 seroprevalence of PRRS was stable and low (3%), Slovenia was free of PRRS (Valenčak, 2004). Epidemiological situation in our country is different now. Majority of large farms became seropositive to PRRS what is not surprising due to fact that new breeding animals and fatteners were introduced to the farms without quarantine or serological testing.

Now in Slovenia systematic monitoring is implemented only for classical swine fever and Aujeszky's disease. The health status for other diseases on small pig farms is mostly unknown.

The objective of this study was serological survey on PRRS, salmonella, *Actinobacillus pleuropneumoniae* (APP) and leptospirosis at selected small farrow-to-finish pig farms in Slovenia. After completed serological testings we had proposed farmers appropriate control measures.

MATERIAL AND METHODS

Blood samples were collected between 2012 and 2014 on 16 small one-site pig farms with 34 to 79 breeding animals, free of classical swine fever and Aujeszky's disease. At the beginning of the research pigs from 14 small farms were vaccinated against *Mycoplasma hyopneumoniae*, pigs at 4 farms were vaccinated against porcine circovirus diseases and porcine parvovirosis, pigs from 3 farms were vaccinated against atrophic rhinitis, pigs from 2 farms were vaccinated against PRRS and pigs from 1 farm were vaccinated against *Erysipelothrix rhusiopathiae*.

Blood samples were drawn from the anterior *vena cava* by venipuncture. Serum was harvested by centrifugation for 10 min at 3000 rpm and stored at – 20 °C until testing for the presence of antibodies.

PRRS: 1636 serum samples of breeding animals and 815 serum samples of fatteners were tested with IDEXX PRRS ELISA (HerdChek X3, IDEXX Laboratories Westbrook, Maine, USA). The presence or absence of antibody to PRRS virus (PRRSV) is determined by calculating the S/P ratio for each sample. If the S/P ratio was less than 0.40, the sample was classified as negative for PRRS virus antibodies. 1054 samples (from 7 small farms) were screened with one step RT-PCR (Qiagen, Germany) using sequences based on the open reading frame 7 (ORF7), which detect Type 1 and Type 2

PRRSV strains respectively (Donadeu et al., 1999; Torremorell et al., 2002). The PRRS strain VR-2332 (Type 2) and the Lelystad viruses (Type 1) were used as positive controls. Reaction mixtures without RNA served as negative controls. PRRSV positive samples were directly sequenced in both directions using the Macrogen sequencing service (Macrogen, South Korea) and the RT-PCR amplification primers. For each sample, 258 nucleotide long sequences were aligned with the published data using BLAST (available at http://www.ncbi.nlm.nih.gov/) at the National Centre for Biotechnology Information (NCBI), and PRRSV sequences obtained were compared using the sequence analysis software Lasergene® (DNASTAR Inc., Madison, WI, USA).

APP: 81 serum samples of breeding animals and 85 serum samples of fatteners were tested with ELISA CHEKIT*APP-ApxIV (IDEXX). The diagnostic relevance of the result was obtained by comparing the OD of the samples, with OD of the positive control. Samples were considered positive when the value (%) was equal or higher than 40%. Samples were considered negative when the value (%) was lower than 30%. If the samples were suspect (≥30% to <40%) they were tested in a second run.

SALMONELLA: 81 serum samples of breeding animals and 85 serum samples of fatteners were tested with ELISA Swine Salmonella Antibody Test Kit (IDEXX). The results were calculated in OD% referring to a set of standard sera, defined according to the Danish Mix-ELISA system. Samples with "OD%" equal or greater than 20% (S/P = 0.5) were classified as positive (more stringent screening).

LEPTOSPIROSIS: 80 serum samples of sows were assayed for leptospirosis antibody using a microscopic agglutination test (MAT). As a positive result the titer ≥ 1:100 was estimated.

RESULTS

Table 1. Presence of antibodies against PRRS on small farms.

Farm	Tested		Positive No. / %	
	Breeding animals	Fatteners	Breeding animals	Fatteners
1	10	19	0 / 0	0 / 0
2	310	100	187 / 60.3	49 / 49
3	337	162	305 / 90.5	85 / 52.4
4	44	41	41 / 93.2	20 / 48.8
5	20	20	0 / 0	0 / 0
6	17	13	0 / 0	0 / 0
7	127	75	29 / 22.8	7 / 9.3
8	114	39	64 / 56.1	31 / 79.4
9	64	5	64 / 100	5 / 100
10	68	21	68 / 100	21 / 100
11	44	31	35 / 79.5	21 / 67.7
12	15	15	0 / 0	0 / 0
13	211	137	179 / 84.8	87 / 63.5
14	30	50	0 / 0	0 / 0
15	157	75	67 / 42.6	0 / 0
16	68	12	66 / 97	9 / 75
Total	1636	815	1105 / 67.5	335 / 41.1

Antibodies against PRRS virus were detected in 67.5% of serum samples of breeding animals and in fatteners in 41.1% (Table 1).

Table 2. Results of PRRSV detection by RT-PCR on 7 small farms.

Farm	Tested	Positive No./%
2	141	20 / 14.1
3	436	56 / 12.8
7	72	0 / 0
8	52	0 / 0
11	20	3 / 15
13	247	5 / 2
15	86	0 / 0
Total	1054	84 / 7.9

By RT-PCR, PRRSV was detected in 7.9% of serum samples (Table 2). The nucleotide identity (ORF7) between reference strain Lelystad and PRRSV strain detected from 4 farms was only 89.5% - 93.4%.

Table 3. Presence of antibodies against salmonella and APP on small farms.

Farm	Tested		Salmonella Positive No.		APP Positive No.	
	Breeding animals	Fatteners	Breeding animals	Fatteners	Breeding animals	Fatteners
1	5	5	0	0	3	0
2	5	5	3	0	5	2
3	5	5	2	0	5	1
4	5	5	0	0	0	2
5	5	5	0	0	5	4
6	5	5	0	0	5	4
7	5	5	2	0	5	2
8	5	5	0	0	5	1
9	5	5	1	0	5	4
10	6	4	1	0	3	4
11	5	5	0	0	5	1
12	5	5	0	0	5	0
13	5	11	3	1	5	8
14	5	5	0	0	5	4
15	5	5	2	1	5	0
16	5	5	3	3	5	5
Total	81	85	17 / 21%	5 / 5.8%	71 / 87.6%	42 / 49.4%

The prevalence of serum samples with salmonella antibodies in breeding animals was 21% and in fatteners 5.8%. The prevalence against APP was 87.6% in breeding animals and 49.4% in fatteners (Table 3).

Table 4. Results of leptospirosis detection.

Farm	Tested	Positive No.
1	5	0
2	5	0
3	5	2
4	5	0
5	5	1
6	5	0
7	5	0
8	5	0
9	5	0
10	5	0
11	5	0
12	5	0
13	5	0
14	5	1
15	5	0
16	5	0
Total	80	4

Three farms were serologically positive to leptospirosis (serovar hardjo, serovar grippotyphosa).

DISCUSSION

Slovenia was free of PRRS before joining EU in 2004 (Valenčak, 2004). Only few years later the detected seroprevalence was 44.8%, as indicated by the data of a study on antibody prevalence in 194 herds in 2010 (Toplak et al., 2010). PRRS is endemic in most swine-producing countries and leads to major economic losses (Stadejak et al. 2003). Besides that, increased prevalence of endemic diseases on the farm after introduction of the PRRS to the farm is important also from welfare perspective. Therefore the elimination of the disease is the most justified decision. Elimination of a disease is disappearance of all clinical cases of a specific disease (Toma et al., 1991) which is the consequence of desistance of virus replication and circulation in the population of pigs. No single strategy for elimination will work on infected farms; therefore, the program must be individually designed based on the unit's pig flow and facility design as well as serological results (Gillespie et al., 1999). In our study 11 small pig farms had antibodies against PRRSV and PRRSV was detected in breeding animals in 67.5% of serum samples and in fatteners in 41.1%. We suggested to PRRS free farms to continue with biosecurity practices and to 11 farms with PRRS we suggested biosecurity measures and herd closure or serum inoculation. Herd closure is required to achieve herd stability. In the period of herd closure new pigs cannot be introduced to the farm. This applies also to internal replacements of gilts to the breeding herd. PRRSV elimination through herd closure is based on the fact that naturally developed immunity eliminates virus infection from the farm (Torremorell et al., 2002). Serum inoculation is intramuscular injection of virus derived from serum of viremic pigs, which contains a farm-specific strain of PRRSV. Procedures that expose pigs to the homologous herd strain have repre-

sented successful approach being implemented in many countries (Batista et al., 2002). The success of PRRS elimination depends on the biosecurity practices and the cooperative work (Toma et al., 1991). On five small farms we successfully eliminated PRRS. On one farm we eliminated PRRS by serum inoculation, herd closure (a period of year and 7 months) and biosecurity measures, on other farms only with herd closure (immunization with natural exposure) and strict biosecurity protocols. Biosecurity measures included: entering the farm after changing clothes; having personnel aid in the changing of coveralls and boots; the washing of hands; using footbaths; maintaining individual responsibility for each pig category; use of the all in/all out system; one age category of pigs in one room; one way pig flow; the cleaning and disinfection of pens, pig equipment kept on the farm; deratization and disinsection (Pitkin et al., 2011). One farm which eliminated PRRS with herd closure and biosecurity measures quit vaccination against PRRS, the nucleotide identity (ORF7) between reference strain Lelystad and PRRSV strain detected on 3 farms was only 89.5% to 93.4%. The complete protection in PRRS is only against the same or homologous type of virus (Batista et al., 2002). The detected high heterogeneity between field and vaccine strain in Slovenian pig population is likely to be the main obstacle for the effective elimination of PRRS virus by vaccination, hence the detected PRRSV in Slovenia shared an 88.0% to 93.4% nucleotide identity with the Lelystad virus (Štukelj, 2013).

If those five farms will continue with biosecurity measures the PRRS eradication will be achieved in one year. Eradication of a disease is disappearance of the clinical case of a disease and of the pathogen, as well as antibodies and virus (Toma et al., 1991).

Pleuropneumonia is one of the important bacterial diseases of the respiratory tract of the pig and occurs in most pig-keeping countries (Gottschalk et al., 2003). Its importance derives from the fact that can cause pneumonia that results in death, clinical disease that may become chronic, or subclinical disease in successive batches of pigs and causes losses from death, reduced production, and increases costs of medication or vaccination. Early identification of subclinical infected herds is important for the control of the disease because carrier animals are the main source of transmission between herds (Gottschalk and Taylor, 2006). In our research almost all breeding animals had antibodies against APP, though clinical signs were not present. The ApxIV ELISA does not differentiate serotypes of APP. The previous survey made on large farms proved that in Slovenia pathogen serotypes are present (Golinar, 2002). Vaccination against APP in Slovenia is not used.

Salmonella infections of swine are concerned for two major reasons. The first is the clinical disease (salmonellosis) in swine that may result, and second is that swine can be infected with broad range of salmonella serotypes that can be a source of infection of pork products (Griffith et al., 2006). Although salmonella contamination of poultry and beef products exceeds that of pork, salmonella control programmes in swine will continue to be a primary focus of food safety initiatives. Seroprevalence of salmonella in Slovenia is low. From 2006 to 2008 we had started the salmonella control of fatteners at one large farm. In 2008 the level of samples with optical density (OD) % equal or greater than 20% was 24.8% (Štukelj et al., 2009). In present study at small farms OD 20% in fatteners was only 5.8%. Comparison of the seroprevalence between large and small farms shows that the number of positive fatteners is higher at large farms.

Leptospirosis is a cause of reproductive loss in breeding herds and is an occupational zoonosis of those who work with pigs. Endemic infection in a herd of swine may

produce little evidence of clinical disease, but when it is first introduced into susceptible breeding herd, or during periods of waning herd immunity, it can cause very appreciable losses through abortion, the full-term birth of dead pigs or weak pigs or reduced viability, or infertility (Ellis, 2006). In our survey 3 small pig farms had antibodies against leptospira. All tree farms had started with treatment prescribed by their veterinarians and they are now free of leptospirosis. We had additionally proposed strict biosecurity and rodent control programs instigated in and around the farm complex.

CONCLUSION

By monitoring and enforcing measures against various diseases as well as following strict biosecurity protocol we could improve the wellbeing of these animals, decrease the necessity of medications and other pharmaceutical additives which would result in a more economically sound husbandry and consequently safer food for consumers.

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SEROLOŠKE PRETRAGE ODABRANIH UZROČNIKA NA 16 MANJIH UZGOJA SVINJA U SLOVENIJI

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Izvod

Uzorci krvi uzimani su u godinama 2012 do 2014 na 16 manjih uzgoja svinja koje drže 34 do 79 priplodne životinje. 1636 uzoraka seruma priplodnih životinja i 815 uzoraka tovljenika je bilo testirano IDEXX PRRS ELISA testom. 1054 uzoraka (sa 7 uzgoja) bilo je skenirano sa RT-PCR (Qiagen, Nemačka) upotrebom sekvencija koje temelje na "open reading frame" 7 (ORF7) koji utvrđuje tip 1 odnosno tip 2 PRRS virusni antigen. 81 uzoraka seruma priplodnih životinja i 85 uzoraka tovljenika testirali smo sa Swine Salmonella Antibody Test Kit (IDEXX) i CHEKIT*APP-ApxIV (IDEXX). 80 seruma krmača testirali smo na prisustvo protivtela leptospire upotrebom testa mikroskopske aglutinacije (MAT). Protivtela protiv virusa reproduktivnog i respiratornog sindroma svinja (PRRS) utvrdili smo kod 67,5% priplodnih životinja i 41,1% tovljenika. Upotrebom RT-PCR, PRRS virus je bio dokazan kod 7,9% uzoraka seruma. Seroprevalencija na salmonelu kod priplodnih životinja bila je 21% a kod tovljenika 5,8%. Prevalencija na *Actinobacillus pleuropneumoniae* (APP) protivtela je bila 87,6% kod priplodnih i 49,4% kod tovnih životinja. Tri uzgoja bila su pozitivna na leptospiru (serovar hardjo, serovar

grippotyphosa). Na farmama slobodnih od PRRS savetovali smo održavanje postojećih mera biosigurnosti a na zaraženim farmama preporučili smo biosigurnosne mere i zatvaranje stada. Gotovo sve priplodne životinje su imale protivtela protiv APP ali bez kliničkih znakova. Serprevalencija salmoneloze je u Sloveniji niska. 3 uzgoja imala su leptospiru. Na svima se zaražene svinje leče.

Ključne reči: svinje, zdravstveni status, manji uzgoji, mere nadzora.

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