

PREVALENCE OF ANTIBODIES AGAINST SELECTED PATHOGENS IN WILD BOARS (*SUS SCROFA*) IN SLOVENIA

Marina Štukelj¹, Ivan Toplak², Gorazd Vengušt^{3*}

¹Institute for the Health Care of Pigs, ²National Veterinary Institute, Institute for Microbiology and Parasitology, ³National Veterinary Institute, Institute for Breeding and Health Care of Wild Animals, Fish and Bees, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia

*Corresponding author, E-mail: gorazd.vengust@vf.uni-lj.si

Summary: One hundred eighty-four blood samples were collected from wild boars (*Sus scrofa*) shot in Slovenia during the hunting season 2010/2011. Samples were tested by enzyme immunoassays for antibodies against four viral and two bacterial diseases. 83 samples (45.1%) had antibodies against Aujeszky's disease virus (ADV), 165 (89.7%) against porcine circovirus type 2 (PCV2), 29 (15.8%) against *Mycoplasma hyopneumoniae* (Mhyo), 52 (28.3%) against *Actinobacillus pleuropneumoniae* (APP). Antibodies against classical swine fever virus (CSFV) and against porcine reproductive and respiratory syndrome virus were not detected (PRRSV).

Key words: bacterial infection; serologic survey; Slovenia; viral infections; wild boar

Introduction

In Slovenia wild boars are present all over the country; however, the highest density can be found in the southwest part of the country (1). The wild boar is one of the most important big game species in Slovenia with a hunting bag of around 8.000 pigs per year. A drastic increase in the population density of wild boars in Slovenia occurred during the last decade despite the hunting-related reduction of their population.

Since 2005 the hunting bag of wild boar has increased from 6.892 to 8.742 in 2010 (2).

The highest density of the domestic pig population is located in the eastern part of the country (Fig. 1). Moreover, the majority of the Slovenian pig industry involved traditional small pig farms (17494) with less than 20 pigs; 761 farms with 21 to 50 pigs; 330 farms with 51 to 100 pigs; 246 farms with 101 to 200 pigs; 151 farms with 201 to 500 pigs; 40 farms with 501 to 1000 pigs; and only 13 farms with more than 1001 pigs. From a sum total of 312.373 pigs in Slovenia, 31.309 are breeding sows; 996 are boars; and 280.068 are primarily fatteners (data from National animal

registry; owner Ministry of Agriculture and the Environment). Domestic pigs live mostly without any close contact with the wild boar population. Nevertheless, these “backyard” pig operations might be potential points for the introduction and spread of diseases from wild boars to domestic pigs.

In recent years there has been a growing interest in the role of wild boar populations within the epidemiology of important infectious diseases of swine. This interest occurs due to an increase in the wild boar population density worldwide, leading to a higher probability of disease transmission (3). Wildlife can act as a reservoir for pathogens shared with their related domestic

species, being able to transmit and maintain them even without the presence of the domestic reservoir (4). Many pathogens are shared by wild boars and domestic swine such as classical swine fever (CSF) (5), Aujeszky's disease (AD) (6), porcine circovirus type 2 (PCV2) (3), porcine reproductive and respiratory syndrome (PRRS) (7), *Mycoplasma hyopneumoniae* (Mhyo) (8) and *Actinobacillus pleuropneumoniae* (APP) (9).

CSF is caused by an infection with CSF (hog cholera) virus (CSFV). CSF is a disease listed by The World Organisation for Animal Health (OIE) (10). Under natural conditions, the infection occurs in domestic pigs and wild boar causing

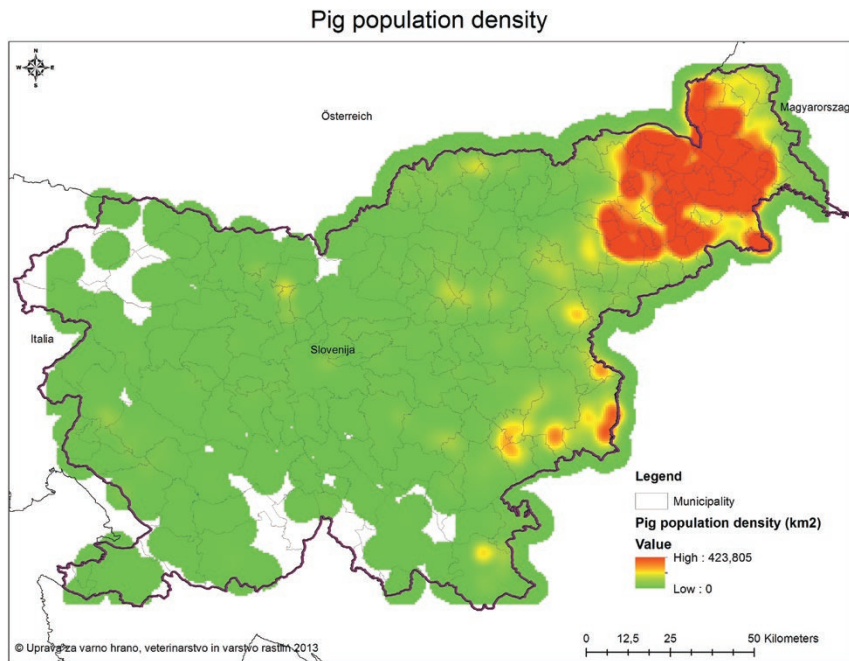


Figure 1: Domestic pig density in Slovenia per km² (National animal registry; owner Ministry of Agriculture and the Environment)

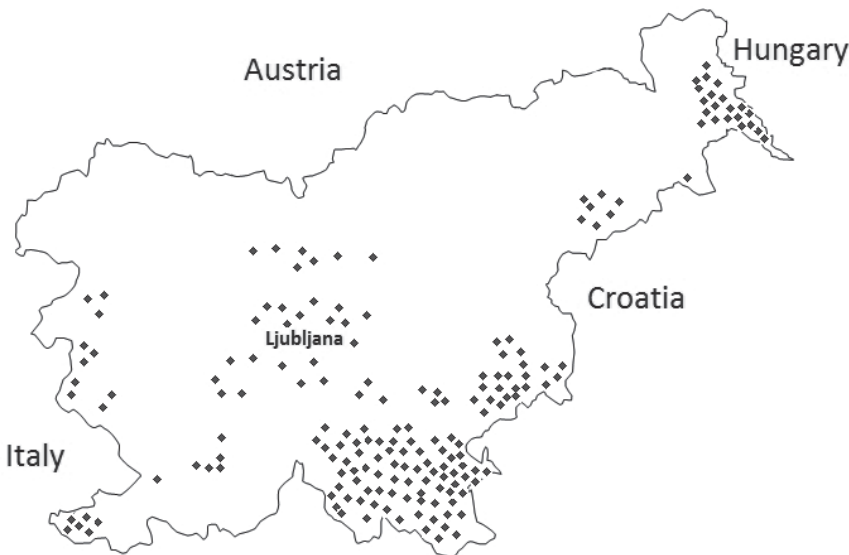


Figure 2: Sampling locations (squares) of wild boar (*Sus scrofa*) in Slovenia. Blood samples were collected from 184 shot wild boars throughout the country during the hunting season 2010/2011

major economic losses especially in countries with an industrialized pig production (5).

AD or pseudorabies virus is worldwide distributed swine alphaherpes virus that infects wild and domestic swine as natural host (14). AD virus (ADV) also infects a wide range of other hosts except humans and primates. Mammals other than swine are considered dead-end hosts because infection is fatal before virus excretion. ADV has the ability of establishing a lifelong latent infection in neuronal and non-neuronal cells in swine (6). This particularity of herpes viruses can lead to virus persistence at herd level due to the reactivation of latent infections and consequent virus excretion. This feature remains one of the most important issues regarding ADV epidemiology in the domestic pig and wild boars (15).

PCV2 is a member of the *Circoviridae* family. The virus is ubiquitous in domestic swine population with antibody prevalence reaching almost 100% (3). PCV2 causes postweaning multisystemic wasting syndrome (PMWS) in domestic pig and other diseases and conditions referred to as porcine circovirus diseases (16). Due to relatively unspecific clinical signs, the establishment of a final diagnosis of PMWS is based on three different criteria: clinical signs, the presence of very specific lesions in lymphoid tissues and the presence of PCV2 in these tissues (17, 18). PRRS is one of the economically most important diseases in domestic swine (19). Porcine reproductive and respiratory syndrome virus (PRRSV) is an *Arterivirus* of swine, spread quickly and became enzootic in the pig population in most countries all over the world (20). Rates of spread and infection are advanced in areas with high herd and population densities (21). Aerosol and insects are able to bridge up to 3km (22). Mhyo is the principal etiological agent responsible for enzootic pneumonia in pigs. The

clinical outcome of Mhyo infection depends on environment and management conditions and the production system in operation (23). APP is an important pathogen of the porcine respiratory tract and is considered an obligate parasite of the respiratory tract (24). There are no other natural hosts. Two biotypes and several serovars exist. All serovars are capable of causing disease (9). Despite the enormous role of APP distribution in domestic swine production, a very few published data about prevalence and distribution of APP infections in wild boars are available.

The aim of the study was to estimate the prevalence of selected pathogens in wild boar population in Slovenia and estimating the risk of infection for domestic swine.

Material and methods

Blood samples were collected from 184 shot wild boars throughout the country (Fig. 2) during the hunting season 2010/2011.

Immediately after shoot blood was collected from the animal into sterile serum separation tubes (Vacuette; Greiner Bio-one, Kremsmunster, Austria) and sent to the laboratory. Serum was obtained by centrifugation (at 1300 x g at 4°C for 10 minutes) and frozen at -20°C until analysed.

For all serology the Enzyme-Linked ImmunoSorbent Assay (ELISA) from different manufacturers were used (Table 1). Tests were carried out in accordance with the manufacturer's manual. The results were expressed as positive or negative based on producer's recommended cut off value.

For statistical purposes, the data were divided into age groups (young (≤ 1 yr) and old (≥ 1 yr)). Statistical analysis for potential age effects on antibody prevalence was performed by χ^2 .

Table 1: Employed ELISAs for selected pathogens

Pathogen	ELISA	Sensitivity	Specificity
CSFV	CHEKIT-CSF-SERO, Idexx Laboratories, former Dr. Bommeli	93-98%	99%
ADV	Pseudorabies/Aujeszky disease virus gB PRV-gB-Ab (Svanova)	99.6%	99.3%
PCV2	Porcine Circo Virus type 2 antibody test kit (BioChek)	85%	95,6%
PRRS	HerdChek PRRS X3 antibody test kit (IDEXX)	98.9%	99.9%
Mhyo	Mycoplasma hyopneumoniae antibody test kit (BioChek)	85%	99%
APP	APP-ApxIV antibody test kit (IDEXX)	95%	99%

Table 2: Prevalence of antibodies against selected pathogens in wild boars (*Sus scrofa*) in Slovenia (n=184)

Pathogen	No. of positive	P (%)	95% CI
CSFV	0	0	0-2
ADV	83	45.1	37.7-52.6
PCV2	165	89.7	84.2-93.6
PRRSV	0	0	0-2
Mhyo	29	15.8	10.8-21.9
APP	52	28.3	21.8-35.4

P: prevalence; CI: confidence interval

Results

In total of 184 sera examined, in 83 (45.1%) sera we detected antibodies against ADV, in 165 (89.7%) sera antibodies against PCV2, in 29 (15.8%) sera antibodies against Mhyo and in 52 (28.3%) sera antibodies against APP. Antibodies against CSFV and PRRSV were not detected. The results are summarised in table 2. Statistically significant differences were noted regarding seroprevalence to ADV across age ($\chi^2 = 12.67$, $df = 1$, $P < 0.001$), with more positive adults (85%) than juveniles (40%). We did not find statistically significant age-related differences for PCV2, Mhyo and APP.

Discussion

CSFV has no known zoonotic potential; however, its presence in the region has serious economic implications for domestic pig farming and hunting tourism. It is a serious and contagious viral disease affecting pigs and wild boars that can be directly or indirectly transmitted from infected wild boar to domestic pigs. In this study no antibodies to CSF were detected in tested wild boar. The last outbreak of CSF in wild boar were reported in 1965 (12) and domestic swine in 1996 (11), respectively. Reports on CSF outbreaks in domestic pig in years 2006-2008 (25) and wild boar population in years 2009-2010 in Croatia (26) remind us about constant risk for introduction of CSF in Slovenia from Balkan area therefore, constant monitoring is an important part of control of CSF in wild boar (13) to remain free of CSF.

In many parts of the world, efforts are being carried out to control ADV in domestic pigs. In Europe, most countries have implemented strict national eradication programs based on initial

large scale vaccination of pigs with attenuated glycoprotein E (gE)-deleted vaccines. In countries that have reached the AD-free status, vaccination against ADV is forbidden (27). Slovenia is country officially free of AD from year 2010 in domestic pigs (28). The prevalence of antibodies against ADV found in the present study was 45.1%, which is higher than seroprevalence (31%) previously reported by Vengust et al. (29) in Slovenia. The increase of the population density of the wild boar in recent years could be the reason for higher prevalence; however, more studies are needed to determine the main cause. Lower seroprevalence was also reported from Germany (40%) (30), Italy (30.7%) (31, 32), Poland (11%) (33), France (3.5%) (34), Croatia (38.5%) (35) and Switzerland (2.8%) (36), whereas higher seroprevalence was established in Spain (49%) (27). Our data indicate that the risk of infection increases with age, and this is consistent with results from other studies (37, 38). Sample sizes per individual site were small, especially in east region (Goriško). The limited sample size means that results, particularly regarding areas, need to be taken with caution. However, wild boar AD antibodies seroprevalence in this study and study conducted in 2006 in the same region, indicate that AD remains endemic at low prevalences in the east Slovenia wild boar populations. Domestic pigs in Slovenia are free of AD. There is an evidence that ADV infection in domestic pigs and wild swine represents epidemiologically distinct infection cycles (32, 39) and experimental studies suggest that there is no possibility that infected wild swine can shed virus in sufficient amounts to trigger infection (40, 41). Taken together, these findings indicate that the virus isolated from wild boar is highly adapted to the population. Furthermore, the molecular

biological characterization of the wild boar ADV clearly supports the hypothesis that the infectious cycle within the wild boar population in eastern Germany is independent of that in domestic pigs (40). We can speculate that this observation can be the reason why our domestic pigs remain free of ADV.

It is known from serological surveys that PCV2 is ubiquitous with serological prevalence close to 100% in finishing pigs worldwide (17, 42). According to the Golinar-Oven (43) all tested domestic pigs in Slovenia were also positive. Thus, considering the ubiquitous distribution of PCV2 among domestic pig populations, and the known risk factors for PMWS in domestic pigs such as poor hygiene and crowding (18), we expected that the seroprevalence to PCV2 will be much lower in wild boar populations. In contrast the prevalence of antibodies against PCV2 in the present study was 89.7% higher than that reported by Ruiz-Fons et al. (44) (between 20 and 48%) in the wild boar population of Europe. Roic et al. (37) reported very low seroprevalence (15.1%) in Croatia. In Slovenia the prevalence is slightly lower in wild boars than in domestic pigs; but the fact that factors such as living conditions, age of infection, extent of PCV2 shedding, early weaning, and vaccinations (45), are the factors that may enhance the spread of the virus in commercial swine, may not be applicable to wild boar populations and may not be the key issues of transmission of the virus. Also the transmission between domestic pigs and wild boars is quite difficult while the high density of domestic pigs takes place in the eastern part of the country and the majority of wild boars live in the southwestern part of Slovenia. On the other hand, Toplak et al. (46) reported that the high diversity of strains of PCV2 detected in wild boars could be evidence for the persistence of PCV2 infection for a longer period and for the introduction of the virus from different sources. Furthermore, the population density, the infection pressure and the specificity of the biotope where the wild boar families live could play an important role in the effective transmission of virus between the boar and domestic pigs in the area. The results of sequencing analysis in Slovenia and Serbia, showing identical or very similar sequences of PCV2 strains in wild boar and domestic pigs, confirm this last possibility (47).

In the present study there was no detection of antibodies against PRRSV in wild boar population

in Slovenia which concur with the previous report on wild boar by Vengust et al. (29). Contrary antibodies against PRRSV have been found in Germany (0.4%) (48), France (1.3%) (36), Croatia (6.3%) (37) Italy (37.7%) (33) and the United States of America (USA) 1.7% (49). The detected prevalence of antibodies in 2010 against PRRSV in Slovenia from farms with more than 30 breeding sows was 48% (50). The sequencing results of 258 nucleotides in ORF7 from 30 representative herds with PRRSV-positive samples revealed the circulation of six genetically different strains of PRRSV, all belonging to the EU subtype 1 (51). The negative result of PRRSV observed in this study was in agreement with previous publications and suggests that the wild boars may be a low-risk reservoir for the transmission of the virus to domestic pigs (6, 45).

Prevalence of Mhyo obtained in this study (15.8%) was lower to that obtained previously in Slovenia (21%) (29) and lower to that observed in France (58%) (52) and the USA (32%) (53). However, the lack of consistent gross lesions compatible with enzootic pneumonia (EP) in studied animals shows that the effect of Mhyo probably remains subclinical in this species (8). Examination of the 36 sera from domestic swine in Slovenia has revealed antibodies against Mhyo in 83.3% (43). Based on the relatively high prevalence of Mhyo in conventional pig farms (43), the fact that the domestic pig could be the real reservoir for the wild boar should not be ruled out. The wild boar may only represent a potential Mhyo threat for Mhyo free farms (8).

Prevalence of APP seropositive pigs obtained in this study (28.3%) was lower to that obtained previously in Slovenia (52%) (29). A similar prevalence was reported by Reiner et al. (9) in Germany where more than one-third of the tested animals were infected. APP is a highly contagious and economically significant respiratory disease in domestic swine with significant negative impact on the pig production because of increased medication and decreased weight gain. Serological evidence for APP infection has recently been reported in domestic swine in Slovenia, with a seroprevalence rate of 100% (43). APP is the airborne disease and it seems that there could be some correlation between APP in wild boars and domestic pigs; however, this still need to be evaluated.

Detection of antibodies against ADV, PCV2, Mhyo and APP in the wild boar of the present study

supports the hypothesis that these animals may be reservoirs of swine diseases for domestic swine. It is very difficult to conclude if there is any association between the infectious agent that appear in both domestic and wild boar populations. In the case of ADV we assume that transmission between populations does not occur although the positive wild boars origin from all parts of Slovenia. PRRS is widely disseminated in domestic pig population and is not present in wild boar population. These data suggest that there is no transmission of diseases from wild boars to the domestic swine or vice versa in pig production system used in Slovenia. With outdoor raised population of domestic pigs the possibility of disease transmission from wild boars to domestic pigs will increase. Permanent control of wild and domestic swine populations may be important measure on national level for minimizing the spread and transmission of diseases among these two populations.

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POJAVNOST PROTITELES PROTI NEKATERIH POVZROČITELJEM BOLEZNI PRI DIVJIH PRAŠIČIH V SLOVENIJI

M. Štukelj, I. Toplak, G. Vengušt

Povzetek: Odvzetih je bilo 184 krvnih vzorcev divjih prašičev (*Sus scrofa*), odstreljenih v lovski sezoni 2010/2011 v Sloveniji. Vzorce smo testirali s testom ELISA za dokaz protiteles proti štirim virusnim in dvema bakterijskima boleznima prašičev. Pri 83 vzorcih (45.1 %) smo potrdili protitelesa proti bolezni Aujeszkega, pri 165 (89.7 %) vzorcih smo potrdili protitelesa proti prašičjemu cirkovirusu tipa 2, pri 29 (15.8 %) vzorcih protitelesa proti *Mycoplasma hyopneumoniae* in pri 52 (28.3 %) vzorcih protitelesa proti *Actinobacillus pleuropneumoniae*. Protiteles proti klasični prašičji kugi in protiteles proti prašičjem reprodukcijskem in respiratornem sindromu pri testiranih divjih prašičih nismo potrdili.

Ključne besede: bakterijske infekcije; serologija; Slovenija; virusne infekcije; divji prašiči