

## A Serological Survey of Selected Pathogens in Wild Boar in Slovenia

G. VENGUST<sup>1,3</sup>, Z. VALENCAK<sup>2</sup> and A. BIDOVEC<sup>1</sup>

Addresses of authors: Institutes for <sup>1</sup>Breeding and Health Care of Wild Animals, Fishes and Bees and <sup>2</sup>Health Care of Pigs, Veterinary Faculty, University of Ljubljana, Gerbiceva 60, 1000 Ljubljana, Slovenia; <sup>3</sup>Corresponding author: Tel.: +386 1 4779196; fax: +386 1 2832243; E-mail: gorazd.vengust@vf.uni-lj.si

Received for publication July 26, 2005

### Summary

Serum samples collected from 178 shot wild boars (*Sus scrofa*) were tested for the presence of antibodies against classical swine fever virus, Aujeszky's disease virus (ADV), porcine reproductive and respiratory syndrome virus, porcine respiratory coronavirus (PRCV), transmissible gastroenteritis virus, swine influenza virus, porcine parvovirus (PPV), swine vesicular disease virus, *Actinobacillus pleuropneumoniae* (APP), *Mycoplasma hyopneumoniae*, *Salmonella* spp., *Brucella* spp. and *Haemophilus parasuis* (HPS) throughout Slovenia during the hunting season 2003/2004. The number of samples corresponds to 3% of the total hunting bag. By enzyme-linked immunosorbent assay (ELISA) antibodies against ADV were detected in 55 sera (31%), against PRCV in five sera (3%), PPV in 87 sera (49%), APP in 93 sera (52%), *M. hyopneumoniae* in 38 sera (21%), *Salmonella* spp. in 85 sera (47%) and HPS in 33 sera (18%).

### Introduction

Geographically, Slovenia is a Central-European country situated between Italy, Austria, Hungary and Croatia. In its entirety, different species of wildlife can be found and biodiversity in this area is very high (Mršič, 1997). The wild boar (*Sus scrofa*) is one of the most important big-game species in Slovenia with a hunting bag of around 6000 pigs per year. The population density of wild boars in Slovenia has increased drastically during the last decade despite hunting. Wild boars are present all over the country; however, the highest densities are situated in the southwest part of the country. As in Slovenia, wild boar population has increased both in number and density across Europe (Artois et al., 2002). In the last decade, evidences accumulated show that in some situations wild boars can act as a reservoir for infectious diseases of domestic pigs (Elbers et al., 2000; Laddomada, 2000; Al Dahouk, et al., 2005) and movement of these animals can potentially result in dissemination of these diseases. Currently there is no recorded data regarding prevalence and distribution of the most important infectious agents among wild boars in Slovenia. Therefore, our objective was to determine the seroprevalence against selected infectious pathogens in wild boars from Slovenia.

Data regarding exposure to different infectious agents and movement of wild boar populations may play an important role in minimizing the exposure of domestic pig to these infectious diseases and may also help to perform studies of risk assessment of infectious diseases.

### Materials and Methods

Blood samples were collected from 178 shot wild boars throughout the country during the hunting season 2003/2004. Hunters had previously been trained to collect the blood. Immediately after a shoot, blood was collected from the animal into sterile tubes and sent to the laboratory. Serum was obtained by centrifugation and frozen at –20°C until examination. Only sera with limited haemolysis (178 samples out of 208) and absence of protein denaturation were selected for analysis.

The following tests were carried out in accordance with the manufacturer's instructions.

#### Serological investigations against viral diseases

*Enzyme immunoassay for detection of antibodies against CSFV*  
Chekit-CSF-sero (Bommeli Diagnostics, Bonn, Switzerland), an enzyme immunoassay designed to detect antibodies against the glycoprotein E2 of the classical swine fever virus (CSFV) in serum of pigs, was used.

*Enzyme immunoassay for the detection of antibodies against glycoprotein gII of ADV*

Svanovir, Pseudorabies-gII-Ab EIA Test Kits, (Svanova Biotech, Uppsala, Sweden), was used. Antibodies against glycoprotein gII of Aujeszky's disease virus (ADV) may be expected in the serum from infected or vaccinated pigs but absent in sera of non-infected and non-vaccinated pigs.

*Enzyme immunoassay for the detection of antibodies against PRRSV*

HerdChek PRRSV Antibody Test Kit (IDEXX, Westbrook, ME, USA), an enzyme immunoassay for the detection of antibody against porcine reproductive and respiratory syndrome virus (PRRSV) in swine serum using PRRS and normal host cell antigens, was used.

*Enzyme immunoassay for the detection of antibodies against TGEV and PRCV*

Ingezim Corona Differential (Ingenasa, Madrid, Spain), blocking immunoenzymatic assay for the specific detection and differentiation of transmissible gastroenteritis virus (TGEV)

and porcine respiratory coronavirus (PRCV) in pig serum, was used. Kit applies a recombinant antigen and a couple of specific monoclonal antibodies, one to recognise epitopes to TGEV and PRCV and the other to recognise a specific epitope from TGEV.

*Enzyme immunoassay for the detection of antibodies against SIV*  
HerdChek SIV Antibody Test Kit – H1N1 (IDEXX), an enzyme immunoassay designed to detect the presence of antibody to swine influenza virus (SIV) subtype H1N1 in swine serum, was used. To a limited extent the SI enzyme-linked immunosorbent assay (ELISA) may detect antibody against other swine influenza subtypes.

*Enzyme immunoassay for the detection of PPV*

Svanovir, PPV-Ab ELISA kits (Svanova Biotech), which detect porcine parvovirus (PPV)-specific antibodies in serum, were used. The kit procedure was based on a competitive linked immunosorbent assay.

*Enzyme immunoassay for the detection of antibodies against SVDV*

Ceditest SVDV kit (Cedi – Diagnostics B.V., Lelystad, The Netherlands) for detection of antibodies to swine vesicular disease virus (SVDV) was used. Major components of the test are monoclonal antibody and inactivated SVDV antigen.

#### Serological investigations against bacterial diseases

*Enzyme immunoassay for the detection of antibodies against APP*

Chekit APP – ApxIV enzyme immunoassay kit (Bomelli Diagnostics), a rapid, sensitive and specific method for detecting antibodies against *Actinobacillus pleuropneumoniae* (APP), the causative pathogen for swine pleuropneumonia, in serum and plasma of swine independent of the APP serotype, was used. Microtitre plates are pre-coated with recombinant bacterial Apx IV antigen.

*Enzyme immunoassay for the detection of antibodies against Mycoplasma hyopneumoniae*

HerdChek *Mycoplasma hyopneumoniae* Antibody Test Kit (IDEXX), an enzyme immunoassay for the detection of antibodies against *M. hyopneumoniae* in swine serum and plasma, was used.

*Enzyme immunoassay for the detection of antibody to Salmonella spp.*

HerdChek Swine Salmonella Antibody Test Kits (IDEXX), which allows rapid screening for the presence of antibodies against a broad range of Salmonella serogroups, were used according to the test protocol.

*Rose bengal agglutination test (RBT) for detection of antibodies to Brucella spp.*

*Brucella abortus/melitensis/suis* rose bengal test (RBT) antigen (OIE Brucellosis Reference Centre, VLA, Weybridge, UK)

and serum sample were placed on a plastic plate and mixed. The mixture was agitated for 4 min at room temperature on an agitator and then read for agglutination. Any visible reaction was considered positive.

*Enzyme immunoassay for the detection of antibodies against HPS*

Biovet HPS Antibody Test Kit (ELISA) HPS (Biovet, Saint-Hyacinthe, Canada) an immunoenzymatic assay for the detection of antibodies against *Haemophilus parasuis* in porcine serum, was used. The porcine serum samples and the controls were diluted and incubated in wells coated with HPS antigen and in wells coated with a cell lysate that serve as negative control.

#### Results

Examination of the 178 sera from wild boars has revealed antibodies against ADV in 55 sera (31%), PRCV in five sera (3%), PPV in 87 sera (49%), APP in 93 sera (52%), *M. hyopneumoniae* in 38 sera (21%), *Salmonella* spp. in 85 sera (47%) and HPS in 33 sera (18%). There was no indication of antibodies against CSFV, PRRSV, TGEV, SIV, SVDV and *Brucella* spp. within this wild boar population.

#### Discussion

Sera were collected during the hunting season 2003/2004, which is the only way to obtain a large sample size of wild boar sera. However, such sampling has some disadvantages because of haemolysis and dilution of the samples as described by Müller et al. (1998) and Van Der Leek et al. (1993). Wild boar samples were distributed throughout the region of Slovenia and corresponded to 3% of the shot boars.

Aujeszky's disease is an economically important disease of pigs, for which several European countries (Elbers et al., 2000; Müller et al., 2003, 2005; Martini et al., 2003) and the USA (Hahn et al., 1997; Corn et al., 2004) have implemented national program for elimination of the disease. The prevalence of antibodies against ADV found in the present study (31%) is higher than that reported from Eastern (8.9%) and Western (9.9%) Germany (Müller et al., 1998; Lutz et al., 2003), France (5.5%) (Albina et al., 2000), Italy (30%) (Capua et al., 1997) and lower than that reported from south-central part of Spain (56%) (Gortazar et al., 2002), Croatia (54%) (Župančić et al., 2002), Tunisia (54%) (Jridi et al., 1996) and Corsica with prevalence up to 61% (Albina et al., 2000). Prevalence of ADV antibodies in wild boars from the southern part of the USA was found to be 29% (Nettles and Erickson, 1984), 35% (Van Der Leek et al., 1993), 38% (Corn et al., 2004) and 61% (Gresham et al., 2002). Hahn et al. (1997) estimated that the potential source for reinfection in the USA is the large population of wild boars where prevalence of ADV is variable but can be exceeded by up to 60% as was estimated later in South Carolina (Gresham et al., 2002). The relatively high prevalence of antibodies against ADV in wild boars in Slovenia is surprising because our domestic pig population is free of AD. In the last two decades, there was only one outbreak of AD in 1996 in a very limited area where the disease was eradicated by slaughtering of all seropositive pigs (Valenčak, 2002).

Porcine parvovirus is known to be involved in early foetal death, stillbirths and weak births and it is common in domestic swine population in Slovenia (Šabec and Valenčak, 2000). Antibodies were present in 87 (49%) sera. Seroprevalence was lower than that reported in Germany (77%) (Lutz and Wurm, 1996) and Italy (99%) (Mignone et al., 1995) and higher than that reported in Spain (10%) (Vicente et al., 2002) and USA where in different studies Saliki et al. (1998) and New et al. (1994) reported 17% and 14% positive samples respectively.

No information exists on prevalence of APP and PRCV in wild boars so far. APP is the etiological agent of porcine pleuropneumonia, 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 (Vigre et al., 2003), spread worldwide. Our results indicated that 93 sera (52%) had antibodies against APP. As *A. pleuropneumoniae* is either airborne or transmitted directly to the animal by a carrier animal (Nicolet, 1994) the number of positive cases is expected.

Porcine respiratory coronavirus is a significant pathogen in swine respiratory disease and it may play a substantial role in swine pneumonia in synergism with mycoplasma, bacteria, or viruses (Paul et al., 1994). The virus is probably a deletion mutant of TGEV (Rasschaert et al., 1990). PRCV does not appear to be an important primary pathogen but it has considerably complicated the diagnosis of TGE, particularly by serological means. In our study antibodies to PRCV were detected in five wild boar sera (3%). On the contrary, research in domestic pig in Slovenia showed very high seroprevalence (65%) (Valenčak, 2002).

*Mycoplasma hyopneumoniae* is the most economically significant porcine bacterial respiratory pathogen. It is a frequently isolated pathogen in combination with PRRSV (Thacker et al., 1999). In our case, we have detected antibodies against *M. hyopneumoniae* in 38 sera (21%); however there were no antibodies against PRRSV. A similar study was also conducted in Spain but no antibodies were detected against *M. hyopneumoniae* and PRRSV (Vicente et al., 2002). In domestic pigs in Slovenia overall seroprevalence exceeded 70%, in some regions seroprevalence can reach up to 100% (Valenčak, 2002).

In our study, antibodies to *Salmonella* spp. were detected in 85 wild boar sera (47%). Seroprevalence was higher than that reported by Vicente et al. (2002) in Spain. They detected antibodies against *Salmonella* serotype B in 4% and *Salmonella* serotype C in 3% of the cases. Disease control among wild boar population in Texas (USA) using faecal specimens revealed no positive cases (Corn et al., 1986). *S. choleraesuis* was revealed by bacteriological examination in juvenile wild boars in Germany causing haematogenous osteomyelitis (Müller et al., 2004). *S. choleraesuis* was also the cause of disease and death of farmed wild boars in Spain (Perez et al., 1999). In Japan, *Salmonella* spp. was isolated from two wild boars meat samples (Kanai et al., 1997). *Salmonella* spp. is presented in domestic swine as well as in wild boar population (Perez et al., 1999; Vicente et al., 2002) and could, as a zoonotic agent, play an important role in transmission to humans via infected carcasses and pork product. Seroprevalence of *Salmonella* spp. in domestic pigs in Slovenia is low. Established seroprevalence in fatteners ranged between 6 and 25% (Štukelj and Valenčak, 2004).

Little information is available on *H. parasuis* in wild boars. *H. parasuis* is one of the major causes of nursery mortality in domestic swine herds worldwide. In our study antibodies to *H. parasuis* were detected in 33 wild boar sera (18%). A similar study was also performed in Spain but without positive cases (Vicente et al., 2002).

There was no evidence for exposure to CSF, PRRS, TGE, SIV, SVD and *Brucella* spp. within this wild boar population in Slovenia. As these diseases appear in neighbouring countries, the spread of pathogens because of migration of wild boars across the borders is possible. If the above is taken into consideration, regular monitoring of wild boar diseases becomes essential.

From the results of 178 examined wild boar blood samples from all over Slovenia it can be anticipated that there are pathogens in European countries that are not present in Slovenian domestic pigs or wild boars (CSFV, PRRSV, TGEV, SVDV, *Brucella* spp.). ADV is present in wild boars but not in domestic pigs. There are agents which are present in domestic pigs but not in wild boars (PRCV, SIV) and, finally, we have found agents that are present in both domestic and wild populations but seroprevalence in each population is different (PPV, APP, *M. hyopneumoniae*, *Salmonella* spp., HPS).

At present, it is very difficult to conclude if there is any association between the infectious agent that appears in both domestic and wild populations. However, we assume that in the case of ADV, transmission between populations does not occur although pig farms are in regions where positive wild boars live. Nevertheless, we agree with Albina et al. (2000) that it is difficult to assess if wild boars are a potential source of infection for domestic pigs.

## Acknowledgements

The authors gratefully acknowledge hunters. Without their cooperation these studies would not have been possible. The research was funded with a research grant from the Veterinary Administration of the Republic of Slovenia and the Ministry of Education and Sport.

## References

- Al Dahouk, S., K. Nöckler, H. Tomaso, W. D. Spletstoesser, G. Jungersen, U. Riber, T. Petry, D. Homann, H. C. Scholz, A. Hensel, and H. Neubauer, 2005: Seroprevalence of brucellosis, tularemia, and yersiniosis in wild boars (*Sus scrofa*) from North-Eastern Germany. *J. Vet. Med. B* **52**, 444–455.
- Albina, E., A. Mesplede, G. Chenut, M. F. Le Potier, G. Bourbao, S. Le Gal, and Y. A. Leforban, 2000: 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. *Vet. Microbiol.* **77**, 43–57.
- Artois, M., K. R. Depner, V. Guberti, J. Hars, S. Rossi, and D. Rutili, 2002: Classical swine fever (hog cholera) in wild boar in Europe. *Rev. Sci. Tech. Off. Int. Epizoot.* **21**, 287–303.
- Capua, I., C. Casaccia, G. Calzetta, and V. Caporale, 1997: Characterisation of Aujeszky's disease viruses isolated from domestic animals and from a wild boar (*Sus scrofa*) in Italy between 1972 and 1995. *Vet. Microbiol.* **57**, 143–149.
- Corn, J. L., P. K. Swiderek, B. O. Blackburn, G. A. Erickson, A. B. Thiermann, and V. F. Nettles, 1986: Survey of selected diseases in wild swine in Texas. *J. Am. Vet. Med. Assoc.* **189**, 1029–1032.

- Corn, J. L., D. E. Stallknecht, N. M. Mechlin, M. P. Luttrell, and J. R. Fischer, 2004: Persistence of pseudorabies virus in feral swine populations. *J. Wildlife Dis.* **40**, 307–310.
- Elbers, A. R. W., L. J. M. Dekkers, and J. W. B. Van der Giessen, 2000: Sera-surveillance of wild boar in the Netherlands, 1996–1999. *Rev. Sci. Tech. Off. Int. Epizoot.* **19**, 848–854.
- Gortazar, C., J. Vicente, Y. Fierro, L. Leon, M. J. Cubero, and M. Gonzalez, 2002: Natural Aujeszky's disease in a Spanish wild boar population. *Ann. N. Y. Acad. Sci.* **969**, 210–212.
- Gresham, C. S., C. A. Gresham, M. J. Duffy, C. T. Faulkner, and S. Patton, 2002: Increased prevalence of *Brucella suis* and pseudorabies virus antibodies in adults of an isolated feral swine population in coastal South Carolina. *J. Wildl. Dis.* **38**, 653–656.
- Hahn, E. C., G. R. Page, P. S. Hahn, K. D. Gillis, C. Romero, J. A. Anelli, and E. P. J. Gibbs, 1997: Mechanisms of transmission of Aujeszky's disease virus originating from feral swine in the USA. *Vet. Microbiol.* **55**, 123–130.
- M. Jridi, H. Bouzghaia, and B. Toma, 1996: Maladie d'Aujeszky chez le sanglier en Tunisie. *Epidemiol. Sante Animal* **30**, 99–105.
- Kanai, Y., H. Hayashidani, K. Kaneko, M. Ogawa, T. Takahashi, and M. Nakamura, 1997: Occurrence of zoonotic bacteria in retail game meat in Japan with special reference to Erysipelothrix. *J. Food Protect.* **60**, 328–331.
- Laddomada, A., 2000: Incidence and control of CSF in wild boar in Europe. *Vet. Microbiol.* **73**, 121–130.
- Lutz, W., and R. Wurm, 1996: 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*, L., 1758) in Northrhine-Westfalia. *Z. Jagdwiss.* **42**, 123–133.
- Lutz, W., D. Junghans, D. Schmitz, and T. Müller, 2003: A long-term survey of pseudorabies virus infections in European wild boar of western Germany. *Z. Jagdwiss.* **49**, 130–140.
- Martini, M., M. Drigo, M. Dalla Pozza, G. Ferrari, F. Martinello, and B. Sona, 2003: A study of the progress of the Aujeszky's disease control programme in Italy using survival analysis. *J. Vet. Med. B* **50**, 191–195.
- Mignone, W., C. Ercolini, M. Perruchon, and M. Poggi, 1995: Stato sanitario die cinghiali selvatici (*Sus scrofa*) in Liguria: indagini sierologiche. *BIPAS* **12**, 69–71.
- Mršič, N., 1997: Species richness in Slovenia and in other countries of the world. In: Mršič, N. (ed.), *Biotic Diversity in Slovenia: Slovenia – the 'hot spot' of Europe*, pp. 111–113. Ministry of the Environment and Spatial Planning, Ljubljana.
- Müller, T., J. Teuffert, K. Ziedler, C. Possardt, M. Kramer, C. Staubach, and F. J. Conraths, 1998: Pseudorabies in the European wild boar from Eastern Germany. *J. Wildl. Dis.* **34**, 251–258.
- Müller, T., H. J. Batza, H. Schluter, F. J. Conraths, and T. C. Mettenleiter, 2003: Eradication of Aujeszky's disease in Germany. *J. Vet. Med. B* **50**, 207–213.
- Müller, M., A. Weber, R. Tucher, and L. Naumann, 2004: Case report: *Salmonella choleraesuis* as a cause of haematogenous osteomyelitis in a wild boar (*Sus scrofa*). *Tierärztl. Umschau.* **59**, 700–702.
- Müller, T., Teuffert, J., Staubach, C., Selhorst, T., and K. R. Depner, 2005: Long-term studies on maternal immunity for Aujeszky's disease and classical swine fever in wild boar piglets. *J. Vet. Med. B* **52**, 432–436.
- Nettles, V. F., and G. A. Erickson, 1984: Pseudorabies in wild swine. In: *Proceedings of the 88th Annual Meeting of the United States Animal Health Association*, pp. 505–506.
- New, J. C., K. Delozier, C. E. Barton, P. J. Morris, and L. N. D. Potgieter, 1994: A serologic survey of selected viral and bacterial diseases of European wild hogs, Great-smoky mountains national-park, USA. *J. Wildl. Dis.* **30**, 103–106.
- Nicolet, J., 1994: *Actinobacillus pleuropneumoniae*. In: Straw, B. E. (ed.), *Diseases of Swine*, 8th edn, pp. 401–407. Iowa State University Press, Ames.
- Paul, P. S., P. G. Halbur, and E. M. Vaughn, 1994: Significance of porcine respiratory coronavirus infection. *Comp. Cont. Educ. Pract.* **16**, 1223–1234.
- Perez, J., R. Astorga, L. Carrasco, A. Mendez, A. Perea, and M. A. Sierra, 1999: Outbreak of salmonellosis in farmed European wild boars (*Sus scrofa ferus*). *Vet. Rec.* **145**, 464–465.
- Rasschaert, D., M. Duarte, and H. Laude, 1990: Porcine respiratory coronavirus differs from transmissible gastroenteritis virus by a few genomic deletions. *J. Gen. Virol.* **71**, 2599–2607.
- Šabec, D., and Z. Valenčak, 2000: Okužba s prašičjim parvovirusom (Porcine parvovirus infection). In: Šabec, D., and Z. Valenčak (eds), *Bolezni prašičev: repetitorij*, pp. 109–110. Veterinary faculty, Ljubljana.
- Saliki, J. T., S. J. Rodgers, and G. Eskew, 1998: Serosurvey of selected viral and bacterial diseases in wild swine from Oklahoma. *J. Wildl. Dis.* **34**, 834–838.
- Štukelj, M., and Z. Valenčak, 2004: Control of salmonellosis in Slovenia – preliminary results. In: Blaha, T., and C. Pahlitzsch (eds), *Proceedings of the 18th International Pig Veterinary Society Congress, June 27–July 1, 2004 Hamburg, Germany*, p. 688. International Pig Veterinary Society, Osnabrück.
- Thacker, E. L., P. G. Halbur, R. F. Ross, R. Thanawongnuwech, and B. J. Thacker, 1999: *Mycoplasma hyopneumoniae* potentiation of porcine reproductive and respiratory syndrome virus-induced pneumonia. *J. Clin. Microbiol.* **37**, 620–627.
- Valenčak, Z., 2002: The pig health control in Slovenia. In: Morrison, R., and J. Zimmerman (eds), *The 17th International Pig Veterinary Society Congress, June 2–5, Ames, Iowa USA. Proceedings, International Pig Veterinary Society*, Ames, IA, **2**, 301.
- Van Der Leek, M. L., H. N. Becker, E. C. Pirtle, P. Humphrey, C. L. Adams, B. P. All, G. A. Erickson, R. C. Belden, W. B. Frankenberg, and E. P. J. Gibbs, 1993: Prevalence of pseudorabies (Aujeszky's-disease) virus-antibodies in feral swine in Florida. *J. Wildl. Dis.* **29**, 403–409.
- Vicente, J., L. Leon-Vizcaino, C. Gortazar, M. J. Cubero, M. Gonzalez, and P. Martin-Atance, 2002: Antibodies to selected viral and bacterial pathogens in European wild boars from southcentral Spain. *J. Wildl. Dis.* **38**, 649–652.
- Vigre, H., A. K. Ersboll, and V. Sorensen, 2003: Decay of acquired colostral antibodies to *Actinobacillus pleuropneumoniae* in pigs. *J. Vet. Med. B* **50**, 430–435.
- Župančič, Ž., B. Jukic, M. Lojkic, Z. Cac, L. Jemersic, and V. Staresina, 2002: Prevalence of antibodies to classical swine fever, Aujeszky's disease, porcine reproductive and respiratory syndrome, and bovine viral diarrhoea viruses in wild boars in Croatia. *J. Vet. Med. B* **49**, 253–256.