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Authors: Sedlak, Kamil, Bartova, Eva, and Machova, Jirina

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Antibodies to Selected Viral Disease Agents in Wild Boars from the Czech Republic

Kamil Sedlak,[1] Eva Bartova,[2,3] and Jirina Machova[1] [1]Department of Virology and Serology, State Veterinary Institute Prague, Sidlistni 136/24, 165 03 Prague 6, Czech Republic; [2]Department of Biology and Wildlife Diseases, University of Veterinary and Pharmaceutical Sciences, Palackeho 1/3, 612 42 Brno, Czech Republic; [3]Corresponding author (email: bartovae@vfu.cz)

ABSTRACT: Blood samples were collected from wild boar (*Sus scrofa*) shot during the hunting season from 1999 to 2005 in the Czech Republic. Sera were tested by enzyme-linked immunosorbent assay for the presence of antibodies against classical swine fever virus (CSFV), swine vesicular disease virus (SVDV), Aujeszky's disease virus (ADV), and bovine viral diarrhea virus (BVDV). Indirect fluorescence antibody test was used for detection of antibodies against porcine circovirus type 2 (PCV-2) and transmissible gastroenteritis virus (TGEV). Antibodies against ADV, BVDV, PCV-2, and TGEV were detected in 30% (101 of 338), 1% (2 of 352), 43% (57 of 134), and 1% (1 of 134) of wild boars, respectively. Sera of 6,471 and 362 tested wild boars were negative for the presence of antibodies against CSFV and SVDV, respectively. This is the first survey of TGEV antibodies in wild boars and the first serologic survey of viral diseases in wild boars in the Czech Republic. Wild boars in the Czech Republic may act as a potential reservoir of ADV and thus have a role in the epidemiology of this disease.

Key words: Bovine viral diarrhea, classical swine fever, postweaning, pseudorabies, *Sus scrofa*, swine vesicular disease, transmissible gastroenteritis, wasting syndrome, wild boar.

There are expanding populations of wild boars (*Sus scrofa*) in almost all European countries (Saezroyuela and Telleria, 1986; Laddomada, 2000), including the Czech Republic. In 1995, hunters shot 35,312 wild boars in the Czech Republic; in 2000, 67,858 wild boars were harvested; and in 2005, this number increased to 100,030. Based on this indication of population increase, there is a potential risk of wild boars spreading and acting as reservoirs for several viral diseases. The objective of our study was to conduct a serologic survey of the wild boar populations focusing on six selected viral diseases that are epidemiologically and economically important to the health of both wild boar and domestic swine populations.

Between 1999 and 2005, serum samples were collected from hunted wild boars in 13 districts in Bohemia, the Czech Republic. All sera were collected under the national Classical Swine Fever Virus Surveillance Program and, therefore, no age or sex data were available for these animals. Serologic assays were completed at the State Veterinary Institute, Prague.

Antibodies against classical swine fever virus (CSFV), swine vesicular disease virus (SVDV), Aujeszky's disease virus (ADV, *Suid herpesvirus-1*), and bovine viral diarrhea virus (BVDV) were detected by Ceditest CSFV enzyme-linked immunosorbent assay (ELISA) (Cedi Diagnostics B.V., Lelystad, Netherland), Ceditest SVDV ELISA (Cedi Diagnostics B.V.), AD Ab ELISA (Test-Line, Brno, the Czech Republic), and Ceditest BVDV ELISA (Cedi Diagnostics B.V.), respectively. Antibodies against porcine circovirus type 2 (PCV-2) and transmissible gastroenteritis virus (TGEV) were detected by an indirect fluorescence antibody test (IFAT) using glass slides coated with swine testicle cells (PT-1) infected with the respective viruses (VMRD, Pullman, Washington, USA) and conjugate anti-swine FITC (Sigma Aldrich). Sera were diluted in a twofold series starting at 1:40 as the basic dilution; positive and negative control sera were used. Titers $\geq$40 were considered positive, and final titers were defined as the highest dilution producing distinct fluorescence in foci of infected cells.

The results of serologic examination of wild boars are summarized in Table 1. For PCV-2, the range of antibody titers is also

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available (Table 2). One of 134 examined wild boars had antibodies against TGE, with a titer of 80.

Classical swine fever is a highly contagious disease caused by CSFV in the genus *Pestivirus* of the family *Flaviviridae*. Pigs and wild boars are natural reservoirs of CSFV. Classical swine fever is a disease with high economic importance, and in the Czech Republic, CSFV was detected in October 1990; it had not been detected for 15 yr prior to this. During 1990–1999, CSFV was annually detected in the population of wild boars in the Czech Republic. As many as six CSFV outbreaks were detected in domestic pigs during the years 1994–1997 (Holejsovsky, 2003). A previous serologic and virologic survey of hunted wild boar in the Czech Republic from 1994–2002 reported 3.2% antibody prevalence and 1.7% prevalence of infection; differences in prevalence were observed in individual districts (Holejsovsky, 2003). Our results confirm that this virus does not circulate in the wild boar populations in the middle and western part of the Czech Republic. The possibility that CSFV can persist in wild boar populations has been shown in France with 0.7% antibody prevalence (Albina et al., 2000), in Croatia with 38.6% antibody prevalence (Zupancic et al., 2002), and also in the Netherlands, Germany, and Italy (cited in Laddomada, 2000).

Swine vesicular disease is a highly contagious disease of pigs caused by SVDV in the genus *Enterovirus* of the family *Picornaviridae*. The current strains of SVDV circulating in the population of pigs in Europe can be divided into two groups: Cluster A includes Italian strains that cause only asymptomatic infection (Brocchi, et al., 1997); cluster B includes more pathogenic strains causing outbreaks in the Netherlands (1992), Spain (1993), and more recently in Portugal (2007). In the Czech Republic, the virus has not yet been detected in domestic pigs. Samples tested for antibodies to SVDV in our study were restricted to 1999 and 2001, and sample size was limited by serum quality; we used only serum samples without hemolysis and contamination. However, none of 362 serum samples tested seropositive, and from these results, it does not appear that SVDV is present in the wild boar populations.

Aujeszky's disease virus belongs to the genus *Varicellovirus* in the family *Herpesviridae*. This virus primarily causes neurologic disorders, but is also associated with reproductive and respiratory disorders in several mammal species, excepting humans. The primary ADV reservoirs are domestic pigs and the wild boar. Antibody prevalence rates for ADV in populations of wild boars have been reported for Germany (0.6–9.4%; Dahle et al., 1993; Muller et al., 1998; Lutz et al., 2003), Spain (3.6%–44%; Vicente et al., 2002,

TABLE 1. Antibodies to selected viral disease agents in wild boars (*Sus scrofa*) from the Czech Republic.

	Virus[a] (Number seropositive/number tested)					
Year	CSFV	SVDV	ADV	BVDV	PCV-2	TGEV
1999	0/741	0/264				
2000	0/669					
2001	0/661	0/98				
2002	0/1000					
2003	0/969					
2004	0/1154		41/128	1/150		0/4
2005	0/1277		60/210	1/202	57/134	1/130
Total	0/6471	0/362	101/338	2/352	57/134	1/134

[a] CSFV = classical swine fever virus; SVDV = swine vesicular disease virus; ADV = Aujeszky's disease virus; BVDV = bovine viral diarrhea virus; PCV-2 = porcine circovirus type 2; TGEV = transmissible gastroenteritis virus.

TABLE 2. Range of antibody titers against porcine circovirus type 2 in wild boars (*Sus scrofa*) from the Czech Republic.

Antibody titer	40	80	160	320	640	1,280	2,560	5,120	10,240
Number of positive animals	10	5	7	7	11	8	7	1	1

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2005; Ruiz-Fons et al., 2007), Croatia (54.5%; Zupancic et al., 2002), Italy (0–51%; Giovannini et al., 1988; Lari et al., 2006), Slovenia (26%; Vengust et al., 2005), and France (3.5%; Dahle et al., 1993). In the Czech Republic, the national pseudorabies eradication program was completed in 1988, at which time domestic pigs were considered to be free of ADV. However, wild boars may represent a potential reservoir for ADV, and contact between wild boars and domestic pigs occasionally occurs. In March 2004, there was a case of infection in domestic pigs after contact with wild boar (Sedlak and Machova, 2005). This transmission event initiated the present serologic surveillance; sera from that time (March 2004 to December 2005) and sera from January and February 2004 were examined for antibodies against ADV. Of 338 samples tested, 101 tested positive in ELISA, and 88 of these ELISA-positive serum samples tested positive in the virus neutralizing test with titers of 4 to 32; the results of 13 sera could not be interpreted.

Bovine viral diarrhea is a contagious disease caused by BVDV the genus *Pestivirus* of the family *Flaviviridae*. In the Czech Republic, BVD is prevalent in cattle. From our results, it is evident that wild boars in the Czech Republic are rarely exposed to this infection. Cross-reaction with CSFV can be excluded because all sera were negative for this virus. However, it is possible that antibodies relate to exposure to a related pestivirus other than BVDV (e.g., border disease virus). In Germany and Croatia, the reported prevalence of BVDV antibodies in wild boar is 0.8% and 4.5%, respectively (Dahle et al., 1993; Zupancic et al., 2002).

Postweaning multisystemic wasting syndrome in domestic pigs is caused by PCV-2. Recently, Schulze et al. (2004) and Vicente et al. (2004) reported a case of clinical disease in wild boars in Germany and Spain, respectively. Antibodies to PCV-2 were not detected in wild boars sampled in Croatia (Zupancic et al., 2002). The positive results observed in this study are consistent with reports of seropositve wild boars sampled in France (3.6%; Albina et al., 2000) and Spain (47.9%; Vicente et al., 2004).

Transmissible gastroenteritis is an acute, highly contagious disease of pigs caused by TGEV in the genus *Coronavirus* of the family *Coronaviridae*. From our results, it is evident that infection caused by this virus is not widespread in wild boars from the Czech Republic. Data from other countries are not available; to our knowledge, this is the first serologic survey of TGEV in wild boars.

In conclusion, our data confirm that wild boar populations in the Czech Republic are free of CSFV and SVDV infections. However, this situation can rapidly change with regard to changing ecology (especially the movement of wild boars and their increasing population density). The present serologic survey suggests a widespread exposure of wild boars to ADV and PCV-2. Aujeszky's disease in wild boars presents a high risk of transmission to domestic pigs as well as to domestic and wild carnivores. Serologic examination of wild boars enables easy and economically accessible monitoring of important infections. Nevertheless, surveys to detect virus, especially one causing endemic infections, are highly recommended.

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