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Authors: Roić, Besi, Čajavec, Stanislav, Tončić, Josip, Madić, Josip, Lipej, Zoran, et al.

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Prevalence of Antibodies to Porcine Parvovirus in Wild Boars (*Sus scrofa*) in Croatia

Besi Roić,[1,5] Stanislav Čajavec,[2] Josip Tončić,[3] Josip Madić,[4] Zoran Lipej,[1] Lorena Jemeršić,[1] Mirko Lojkić,[1] Željko Mihaljević,[1] Željko Čač,[1] and Branko Šoštarić[1] [1]Croatian Veterinary Institute, Savska cesta 143., 10000 Zagreb, Croatia; [2]Veterina Ltd, Svetonedeljska 2, Kalinovica, 10436 Rakov Potok, Croatia; [3]Institute of Forestry, Cvjetno naselje 41, 10450 Jastrebarsko, Croatia; [4]Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Croatia; [5]Corresponding author (email: besiroic@hotmail.com)

ABSTRACT: Serologic evidence of exposure to porcine parvovirus (PPV) in the wild boar (*Sus scrofa*) in Croatia was investigated. Serum samples from 219 wild boars captured during 2003 from 12 different locations in the Republic of Croatia were tested by using a commercial enzyme-linked immunoassay (ELISA) and a hemagglutination inhibition (HI) test. Antibodies to PPV were detected in 91 (41.6%) of tested samples and positive results were detected in wild boar from all sample locations. Adults had a significantly higher prevalence (70%) than juveniles (31%; $P<0.01$). Our results indicate that wild boar populations throughout the Republic of Croatia are exposed to PPV.

Key words: Croatia, porcine parvovirus, serology, *Sus scrofa,* wild boar.

The wild boar (*Sus scrofa*) is the most abundant wild ungulate species in Europe (Artois et al., 2002), and because of their high density in some parts of Europe, these populations may play an important role in the maintenance and transmission of diseases affecting domestic swine. However, information on the prevalence and distribution of these potentially important infectious disease agents among wild boar populations is currently limited (Vicente et al., 2002).

Porcine parvovirus (PPV) infections in swine can result in fetal mummification and abortion, usually in the absence of outward maternal clinical signs (Mengeling, 1999). Infections with PPV represent one of the major causes of reproductive failure in domestic pigs in Croatia, and from 1985 through 1995, increasing economic losses in large production swine farms were detected (Roić et al., 1996). During this period, systematic monitoring and vaccination programs were implemented (Markuš-Cizelj et al., 1996). Serologic testing during 1985 indicated that antibodies to PPV, as determined by the hemagglutination inhibition (HI) test, were present in 38% of domestic swine; in 1995 the seroprevalence was less than 1%. From 1995 to the present, approximately 30% of the breeding domestic herds have been vaccinated, and during the past few years several outbreaks of PPV in domestic herds have been detected. Since wild boars can share habitat with domestic swine, contact and subsequent PPV transmission between these populations may occur. The objective of this study was to determine the distribution and prevalence of PPV antibodies in Croatian wild boar populations.

Between July and December 2003, serum samples from 219 wild boars were collected from 12 hunting areas (Fig. 1). These areas are located in the continental part of Croatia (between 45°12′ to 45°55′N and 15°8′ to 19°27′E), representing an area of 917 km^2. The wild boar population in this area is estimated at 4,200 animals. Estimates are derived from direct counts as part of Directive 10/94 and 5/95 ordered by the Ministry of the Agriculture and Forestry of the Republic of Croatia. Samples were stratified according to age groups and gender. Age was determined based on tooth eruption and wear pattern (Boitani and Mattei, 1992). Individuals <12 months old were classified as juveniles, those from 13 to 24 months of age were considered subadults, and those >24 months old were classified as adults. All animals were captured in cage traps and anesthetized with xylazine hydrochloride (7.5 mg/kg, Rompun® 2%, Bayer AG, Leverkusen, Germany) and ketamine hydrochloride (5 mg/kg, Narketan 10®, Chassot

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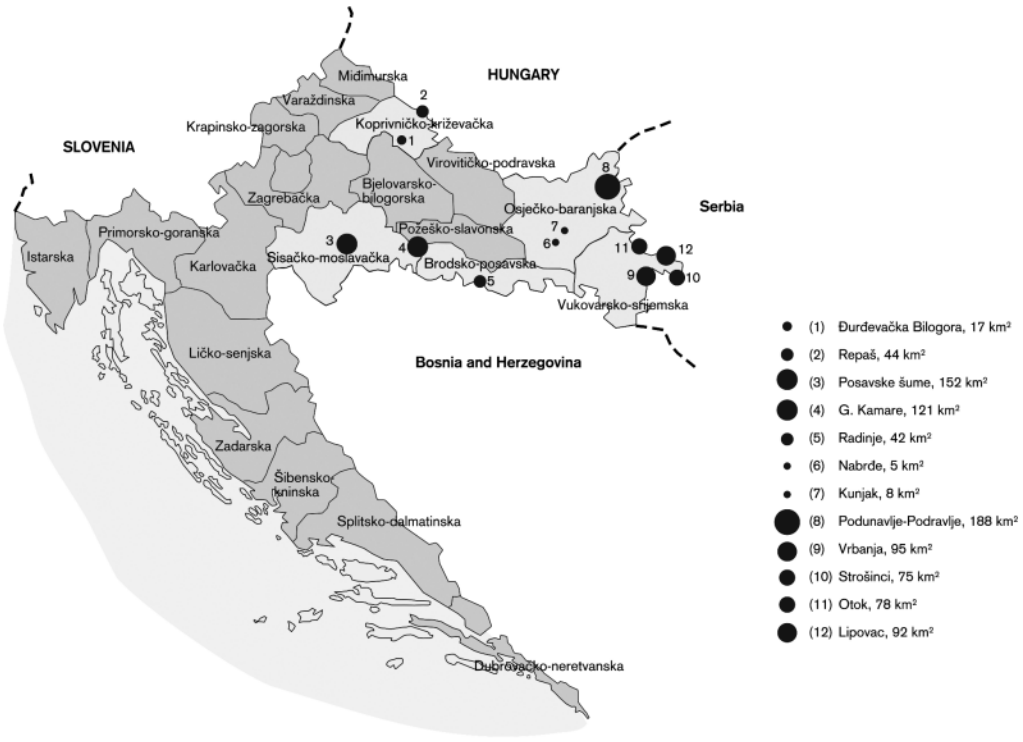


FIGURE 1. Hunting areas in the Republic of Croatia from which wild boar sera were collected.

AG, Belp Bern, Switzerland) or with tiletamine-zolazepam (9 mg/kg, Zoletil®, Virbac, France) intramuscularly (IM). Blood samples were taken from each animal by vena cava cranialis puncture during chemical immobilization. Samples were immediately centrifuged in the field, and were stored at −20 C until they were delivered to the Virological laboratory of the Croatian Veterinary Institute in Zagreb.

The detection of PPV antibodies in wild boars sera was done by using a commercially available blocking ELISA kit (INGEZIM PPV COMPAC, Ingenasa, Hnos. Garcia Noblejas, 41, Madrid, Spain) according to the manufacturer's instructions. Results were interpreted based on the manufacturer's recommendations; sera were considered negative if the blocking percentage was less than 20%, positive when the value was greater than 30%, and ambiguous when the value was between 20% and 30%. Optical density was determined on a Behring ELISA Processor II spectrophotometer (Behring AG, Marburg, Germany) at 405 nm. To confirm the ELISA results and to determine antibody titers, a HI test also was used (Joo et al., 1976). Multivariate logistic regression models were used to identify risk factors associated with PPV-seropositive results. Each model was applied at the individual level using the epidemiologic data (age, sex, and locations) as independent variables and the serologic status as a dependent variable. For assessing statistical significance of confounding and interaction, we used likelihood ratio test (LRT). All statistical analysis was performed using STATA 6.0 (StataCorp. 2003. Stata Statistical Software: release 6, College Station, Texas, USA).

Antibodies to PPV were detected in all age groups and in both sexes and from all of sample areas (Table 1). Of 219 sera examined, 91 (41.6%) were positive to PPV by blocking ELISA. Positive results were observed in 39 of 126 (31.0%) of juveniles,

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TABLE 1. Serologic results of 219 wild boar sera from Croatia tested by blocking ELISA and HI test by age class, sex, and location.

Location	Number positive[a]/number sampled			Total tested (M/F)[b]	Total positive (%)
	Juveniles	Subadults	Adults		
Podunavlje	2/33			33 (21/12)	2/33 (6)
Repaš	2/9	1/4	6/6	19 (11/8)	9/19 (47)
D. Bilogora	22/30	2/3	3/3	36 (16/20)	27/36 (75)
G. Kamare	2/11			11 (7/4)	2/11 (18)
Radinje	11/35			35 (17/18)	11/35 (31)
Nabrde	0/4	0/3	4/4	11 (6/5)	4/11 (36)
Kujnjak	0/2	1/4	4/5	11 (5/6)	5/11 (45)
P. šume	0/1	1/7	5/9	17 (9/8)	6/17 (35)
Strošinci		1/1	5/9	10 (3/7)	6/10 (60)
Vrbanja			5/6	6 (4/2)	5/6 (83)
Otok	0/1	2/6	2/5	12 (7/5)	4/12 (33)
Lipovac		4/8	6/10	18 (10/8)	10/18 (56)
Total positive	39/126 (31.0%)	12/36 (33.3%)	40/57 (70%)	219 (116/103)	91/219 (41.6)

[a] ELISA positive value and HI-test positive (≥1:320)
[b] M=males, F=females.

12 of 36 (33%) subadults, and 40 of 57 (70%) adults. All ELISA-positive samples tested positive on HI with titers ranging from 320 to 40,960; most were between 1,280 and 2,560. A significantly higher seroprevalence was detected in older animals as compared with juveniles; odds ratio (OR)=1.2 in subadults and 5.24 in adults, $P<0.01$). No significant difference in prevalence between males and females was detected ($P>0.05$). Significant differences were detected between hunting areas ($P<0.01$) and the highest ORs were observed for Vrbanja (77.5), Đurđevačka Bilogora (46.5), Strošinci (23.3), and Lipovac (19.4). Further analysis indicated a significant ($P<0.05$) interaction between the hunting areas and age ($P<0.05$). Adjusting for this interaction, the highest ORs relating to location were recorded for Đurđevačka Bilogora (38.3) and Radinje (7.1); seroprevalence was still dependent on age with an OR of 3.67 for subadults and 20.3 for adults.

Serologic evidence of PPV infection has been previously described in the European wild boar populations by several authors. In Germany, Liebermann et al. (1986) found that 65.5% of sampled boar had an antibody titer of ≥20. Lutz and Wurm (1996) reported a PPV antibody prevalence of 77% from the wild boar population in Germany. A prevalence of 41.3% is reported from the Republic of Serbia (Gagrčin et al., 1990). In Italy, 56.7% of the tested sera reacted positively against PPV (Cordioli et al., 1993) as compared with a prevalence of 10% in wild boar from Spain (Vicente et al., 2002). Antibodies to PPV have also been reported in wild swine populations in the USA: a seroprevalence rate of 14% was reported for swine sampled in The Great Smoky Mountains National Park (New et al., 1994), and 17% of tested animals were seropositive in Oklahoma (Saliki et al., 1998).

In the present study, 100% of the 91 ELISA-positive samples also tested positive by the HI test, which validates the specificity of this ELISA as applied to serologic testing of wild boar populations. Our results showed a significantly higher seroprevalence in the adult wild boars (70.2%) than in juveniles. A similar relationship was observed in Serbia with 65% of the animals >1 yr old testing positive (Gagrčin et al., 1990). This age relationship may be explained by repeated exposure to the virus, increasing the probability

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of infection with age. This study demonstrated a significant difference in the seroprevalence between the hunting areas ($P<0.01$) with the highest prevalence of seropositive animals (ranging from 33% to 83%) associated with the hunting areas Strošinci, Vrbanja, Otok, and Lipovac. These are located within the region of Spačva, which is bordered by the Republic of Serbia. We believe that this is associated with the tradition of feeding domestic pigs in open grounds within in this area, thus increasing possible contact and transmission of diseases between wild boars and domestic swine. Seropositive boar were detected in areas with more developed domestic pig production. Transmission of PPV between wild boar and domestic swine managed under such conditions may be facilitated by involvement of domestic swine herds that are under less developed management or, as suggested by Gipson et al. (1999), through wild boar hunters who have contact with domestic swine farms. Our data indicate that wild boar populations in Croatia are commonly exposed to PPV and could represent a potential threat to domestic swine, especially gilts.

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