

## A serological survey of classical swine fever virus in wild boar (*Sus scrofa*) from Croatia

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### ABSTRACT

During 2003, serum samples from 259 captured wild boars were collected. This field study was performed in the continental part of the Republic of Croatia in an area of 920 km<sup>2</sup> with approximately 4200 animals. In order to investigate the CSF prevalence in wild boars in this area the sera were tested for the presence of antibodies to classical swine fever virus (CSFV). An enzyme linked immunoassay (ELISA) was used. Out of 259 sera examined 121 (46.71%) were found positive for the presence of antibodies to classical swine fever (CSF) virus. A significant difference in CSF seropositivity was recorded between age groups and yearlings had the highest odds rate - OR (15.37).

**Key words:** wild boar (*Sus scrofa*), classical swine fever, ELISA

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### Introduction

Classical swine fever (CSF) is an economically important infectious disease of pigs caused by an enveloped RNA virus. CSF virus (CSFV) belongs to the family *Flaviviridae*, genus *Pestivirus* (HEINZ et al., 2000). It is known that domestic pigs (*Sus scrofa domestica*) and wild boar (*Sus scrofa*) are equally susceptible to infection with the classical swine fever virus and it has been shown that the virus can be transmitted

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from wild boar to domestic pigs (SCHNYDER et al., 2002). In countries with intensive pig production and a high wild boar density, CSF can have a serious impact on agriculture and forestry (KADEN and LANGE, 2004). Even though vaccination against CSF is banned in most European countries (especially in the European Union), it is still used as one of the measures for CSF control (VAN OIRSCHOT, 2003). In Croatia, a new strategy based on non-vaccination policy for CSF control was implemented at the beginning of 2005 (ANONYMOUS, 2004).

To date, the classical swine fever virus (CSFV) detection and seroprevalence in the wild boars in the central part of Croatia (in the region of Moslavačka gora), has been reported by JEMERŠIĆ et al. (2000) and ŽUPANČIĆ et al. (2002). They found that out of 44 sera tested 17 (38.63%) were positive for CSF antibodies. Based on these initial results we planned to determine the serological prevalence of CSFV in the wild boar population from a large area of Croatia.

## Materials and methods

*Serum samples.* During 2003, between July and December, serum samples from 259 wild boars were collected at 10 different localities in the Republic of Croatia. The hunting areas are located in the continental part of Croatia (Fig. 1).

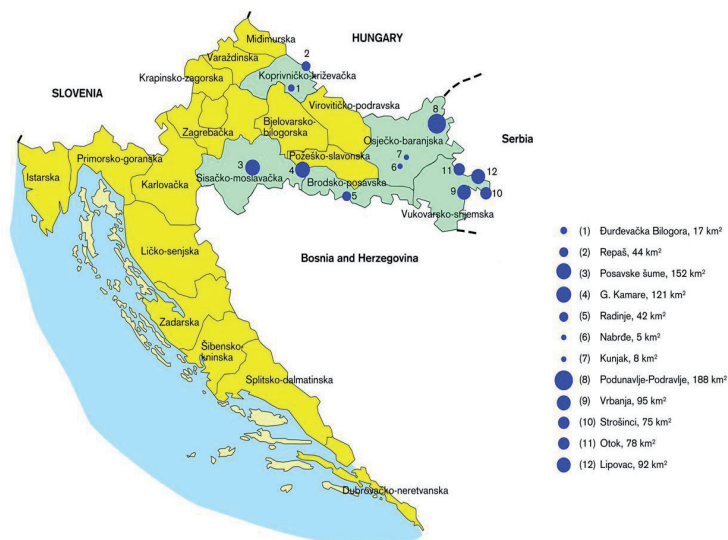


Fig. 1. Hunting areas in Croatia included in this study

The investigated localities have a territorial area of c. 920 km<sup>2</sup> (general map coordinate 45°12' to 45°55' N and 15°58' to 19°27' E) with a density of the wild boar population of approximately 4200 animals. The number of animals is estimated by counting during the feeding time (3 times per day) over the entire year according to the Directive 10/94 and 5/95 ordered by the Ministry of the Agriculture, Forestry and Water Management of the Republic of Croatia. These hunting areas are the property of Croatian Forests, which is the largest owner of hunting areas in the country.

Blood samples were taken from each wild boar by *vena cava cranialis* puncture during chemical immobilization. All the 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 AG, Belp Bern, Switzerland) or with a tiletamine-zolazepam (9 mg/kg, Zoletil®, Virbac, France) i/m. For each of the combinations, the respective doses of anesthetics expressed in mg/kg of body mass depended on the age and weight of each animal. Samples were immediately centrifuged in the field. After centrifugation serum samples were stored at – 20°C until they were delivered to the Virology laboratory of the Croatian Veterinary Institute in Zagreb. Serum samples were obtained from young piglets 3-12 months of age, subadults 2 years of age, boars 4-5 years of age and sows 3-4 years of age. Age was determined based on the tooth eruption and wear pattern (BOITANI and MATTEI, 1992).

*CSF serology.* All sera were tested by a commercially available competitive ELISA kit CHEKIT CSF-SERO (dr. Bommeli AG, Switzerland) to evaluate the antibody prevalence. The test based upon the detection of antibodies against the glycoprotein E2 (gp55) of the classical swine fever virus (CSFV) in serum and plasma of pigs. Due to the high specificity of CHEKIT-CSF-SERO, the cross reactivity with other pestiviruses such as Bovine Viral Diarrhoea virus (BVDV) or Border Disease virus (BDV) is insignificant. The procedure was carried out according to the manufacturer's recommendation. The reaction was read on a Behring ELISA Processor II spectrophotometer (Behring AG, Marburg, Germany), at a wavelength of 405/492 nm. Sera with an optical density (OD)-values  $\leq 40\%$  and  $\geq 50\%$  of the OD<sub>positive control</sub> were considered negative and positive, respectively. Intermediate OD-values were considered doubtful and classified as negative in the data analysis.

*Statistical analysis.* Each model was applied at the individual level using the epidemiological data (age and locations) as independent variables and the serological 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, TX, USA).

## Results

A total of 259 sera from captured wild boars, distributed in ten different hunting areas, were tested serologically. The age of wild boars was also included in the analysis. As presented in Table 2, of the 259 sera tested 121 (46.71%) were positive by the CSF ELISA. Table 1 summarizes the results for the CSF antibody prevalence according to the age groups of wild boars.

Table 1. Prevalence (%) of CSF antibody in wild boar sera according to age class

Animals	Number of sera examined	Positive sera	
		Number	%
Yearlings	147	94	63.94
Subadults	29	3	10.34
Sows	47	16	34.04
Boars	36	8	22.22
Total	259	121	46.71

Table 2. Results of CSF antibody detection in sera of wild boar and the localities from which wild boar sera were collected

Localities	Number of sera examined	Positive sera	
		Number	%
Podunavlje-Podravlje	48	35	72.91
Repaš	26	8	30.76
Đurđevačka Bilogora	35	23	65.71
Grede Kamare	11	5	45.45
Radinje	40	27	67.50
Posavske šume	53	12	22.60
Strošinci	10	4	40.00
Vrbanja	6	1	16.66
Otok	12	2	16.66
Lipovac	18	4	22.22
Total	259	121	46.71

A significant difference in CSF seropositivity was recorded between age groups when all the recorded animals are considered. The category that includes animals less than one year of age, which was most frequently represented, had the highest odds rate (OR) 15.37 for being seropositive. Significant difference between the hunting areas ( $P < 0.01$ ) were also observed and the highest OR for being CSF sera positive in descending order was found in Podunavlje-Podravlje (6.05), Radinje (4.67), Đurđevačka Bilogora (4.31), Grede Kamare (1.87) and Strošinci (1.5). Further analysis indicated a significant ( $P < 0.05$ ) interaction between the hunting areas and age. Taking into account the interaction observed the highest OR was recorded in Repaš (2.97). Also, appreciating the spatial pattern of CSF, younger animals had a higher OR being seropositive (OR was 14.1 in yearlings).

## Discussion

In the past two decades, CSF in wild boar has emerged as a serious problem in Europe (ARTOIS et al., 2002). During this period CSF was diagnosed in wild boars in some European countries such as France (ALBINA et al., 2000), Germany (OSLAGE et al., 1994, HERGARTEN et al., 2001), Italy (LADDOMADA, 2000) and Switzerland (SCHNYDER et al., 2002).

This serological analysis was performed to determine the CSF situation in wild boars from different parts of Croatia and present the first large-scale study. The serological diagnosis of CSF is important for surveys and the detection of hidden clusters of CSF, especially in wild boar (MOENNING, 2000). The number of collected samples from wild boars in this study corresponded to approximately 6% of the total number of wild boars in those areas. Our results demonstrate that 46.71% of wild boar sera were positive for CSF antibodies in comparison to 38.63% detected in previous investigations by JEMERŠIĆ et al. (2000) and ŽUPANČIĆ et al. (2002). The age distribution of wild boars tested for CSF antibodies was also determined. The category less than one year (3-12 month) was most frequently represented and 36.29% were found positive whereas 9.26% of the adults were positive. The results of the serological study show that the seroprevalence higher than 50% occurred in three hunting areas: Podunavlje-Podravlje (72.91%), Radinje (67.50%) and Đurđevačka Bilogora (65.71%). In the region of Spačva (hunting areas: Strošinci, Vrbanja, Otok and Lipovac), which is located on the border with the Republic of Serbia, the concentration of seropositive animals was up to 22.22%. In Serbia the disease has been persistent since 1996 (Handistatus II, Multiannual animal disease status, [oie.int/hs2/report.asp](http://oie.int/hs2/report.asp)). Most of the investigated hunting areas are from open hunting grounds and that fact indicates that there is a possibility of contact between wild boars and the domestic pig population as well as spread of the infection between them. Studies carried out in Germany have shown that 46% of the primary outbreaks of CSF occurring in

domestic pigs were caused by direct or indirect contact with infected wild boar or swill feeding (FRITZEMEIER et al., 1997; FRITZEMEIER et al., 2000). One of the unresolved issues is also how to prevent the disease from being reintroduced by wild boars from neighboring countries when there are no physical barriers to animal movements.

The animals in the observed localities were irregularly vaccinated against CSF and most of them were not vaccinated at all. The study does not include animals less than three months of age because of the possibility of detecting maternal antibodies due to vaccination of female wild boars (KADEN and LANGE, 2004). The results of our CSFV antibody detection support the hypothesis that the prevalence of infection is much higher in piglets of less than one year of age than in older animals, according to some previous results (LADDOMADA, 2000).

Our results obtained for CSFV serology in the present study indicate that CSFV persists in the wild boar population in Croatia. The data suggested that young wild boars might play a major role in the epidemiology of CSF in domestic pigs as well. Due to the importance of the wild boar in the epidemiology of CSF, specific control measures are recommended that include monitoring program for virus and/or specific antibody detection that should be applied in the future as the first step for the eradication of CSF in Croatia.

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**SAŽETAK**

Tijekom 2003. godine prikupljeni su uzorci krvi 259 uhvaćenih divljih svinja. Istraživanje je provedeno u kontinentalnom dijelu Republike Hrvatske na površini od oko 920 km<sup>2</sup> s ukupnom populacijom od približno 4200 divljih svinja. Cilj istraživanja bio je utvrditi prisutnost protutijela na virus klasične svinjske kuge u divljih svinja. Prikupljeni uzorci pretraženi su metodom imunoenzimnog testa (ELISA). Od ukupno 259 pregledanih uzoraka, 121 (46,71%) uzorak bio je pozitivan na virus svinjske kuge. Značajna razlika zabilježena je među pojedinim dobnim skupinama, s najvišim omjerom vjerojatnosti (OR) od 15,37 u nazimadi.

**Cljučne riječi:** divlja svinja, *Sus scrofa*, klasična svinjska kuga, ELISA

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