

## Serosurveillance for selected infectious disease agents in wild boars (*Sus scrofa*) and outdoor pigs in Switzerland

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**Abstract** The large abundance of free-ranging wild boars (*Sus scrofa*) and a trend towards animal friendly outdoor management of domestic pigs lead to an increasing probability of disease transmission between those animal populations. In 2001, an active monitoring was started for classical swine fever (CSF), Aujeszky's disease (AD) and porcine brucellosis (PB) in wild boars in Switzerland. The objective of this programme was to document the serological status of wild boars regarding the selected pathogens. To continue this serosurveillance, 1,060 wild boar samples were collected during two regular hunting seasons in 2004–2005. Furthermore, in a pilot study, 61 outdoor pigs from 14 farms located in areas with high wild boar densities were sampled in 2004 and serologically tested for AD and PB. All wild boar samples were negative for CSF. Seroprevalence for AD was 2.83% (95% CI 1.91–4.02%). Seroprevalence for PB was 13.5% (95% CI 10.7–16.7%) for the Rose Bengal test and 11.05% (95% CI 8.82–13.61%) for the indirect ELISA. There was no serological evidence for

AD in domestic pigs. All tested animals from 13 piggeries were seronegative for PB, but three pigs from the same farm showed doubtful results. Further investigations on the farm did not indicate the presence of PB in the herd. These findings urge the need for better diagnostic tools to obtain reliable results concerning PB prevalence. Since contact and following transmission of infectious agents between infected wild boars and outdoor pigs might occur in the future, it is advisable to include outdoor pigs in areas at risk in routine surveillance programmes.

**Keywords** Aujeszky's disease · Classical swine fever · Outdoor reared pigs · Porcine brucellosis · Serosurveillance · Wild boar (*Sus scrofa*)

### Introduction

In the past few years, Switzerland has witnessed a considerable increase in its wild boar (*Sus scrofa*) population. Despite of intensification of hunting, damage to farmland, crops and forests have caused substantial costs. The hunting bag increased by 250% over the last decade (<http://www.wild.unizh.ch/jagdzt/>) and in 2004 the annual costs of damage compensation reached 1.0 million Euro (<http://www.umwelt-schweiz.ch/>). At the same time, intensification of pig farming (outdoor rearing on pasture) became more and more popular because of extra compensation for organically produced meat or compliance with additional requirements on animal welfare that are promoted as incentives for farmers. Wild boars are known to be susceptible to diseases of domestic pigs and intra-specific interactions between free-ranging wildlife and domestic pigs can occur. High wild boar densities and increasing popularity of outdoor pigs in Switzerland also intensify the

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risk of contacts between wild boars and domestic pigs and, therefore, of transmission of disease agents. In Switzerland, domestic pigs are free of most notifiable infectious diseases such as classical swine fever (CSF), Aujeszky's disease (AD) and porcine brucellosis (PB), but these diseases were reported in wild boars.

Classical swine fever is one of the most important contagious diseases either of domestic pigs or of wild boar (Albina et al. 2000). It occurs worldwide and causes enormous economic losses (Nielen et al. 1999). In 1998, an outbreak of CSF occurred in the wild boar population in the south of Switzerland, but thanks to hunting management strategies, the epidemic could be controlled and the disease was eradicated (Schnyder et al. 2002). Serosurveillance for this disease in Switzerland between 2001 and 2003 revealed a mean seroprevalence of 0.20% (CI 0.06–0.55%) in wild boars from this region (Leuenberger 2004) indicating successful disease eradication.

Aujeszky's disease (Pseudorabies) is caused by an alpha-herpes virus and causes stillbirth and high mortality in piglets. Regulations of the Terrestrial Animal Health Code (Anonymous 2005b) imply that countries officially free from AD should either prove the disease absence in their wild boar population or take measures to prevent the transmission of virus between wild boars and domestic pigs. In Germany, seroprevalence in wild boar reaches 25% and higher in areas where the disease is endemic (Muller et al. 1998; Lutz and Junghans 2003). In Italy, Capua et al. (1997) confirmed the occurrence of AD in wild boars. A recent study performed in Switzerland by Leuenberger (2004) did not identify any confirmed seropositive wild boars. However, laboratory results were unsatisfying due to methodological problems and refinements of diagnostic tests were needed to assess a more realistic seroprevalence of AD in Swiss wild boars.

Porcine brucellosis (caused by *Brucella suis* biovars 1, 2 or 3) is a bacterial infectious disease causing abortion and stillborn or weak piglets. Italy reported a seroprevalence of up to 10% in wild boars (Gennero et al. 2005). In Spain and France, the disease appears to be even more prevalent with a seroprevalence of 17% and >50%, retrospectively (Rossi 2005; Ruiz-Fons et al. 2005). In Germany, Al Dahouk et al. (2005) reported a seroprevalence of 22%. A previous study carried out in different Swiss regions revealed a mean seroprevalence of 6% in 2002 and 14% in 2003 in hunted wild boars and the presence of *B. suis* biovar 2 in the reproductive organs (uterus, accessory glands) of 8% of the animals submitted to a bacteriological examination (Leuenberger et al. 2006).

Transmission of PB, AD and/or CSF to domestic pigs would compromise the freedom-from-infection status in Switzerland. Monitoring of the disease situation in wild boars and knowledge on the possible sanitary consequences

of intra-specific contacts are essential for the development of management and long-term surveillance strategies (Leuenberger 2004; Anonymous 2005a).

The aim of this study was to continue the serosurveillance of CSF, AD and PB in wild boars to (1) confirm CSF eradication, (2) re-investigate the seroprevalence of AD with refined testing methods and (3) monitor the suspected upward trend for PB. Furthermore, outdoor pigs from areas with strong wild boar presence were tested serologically for AD and PB. The goal of this pilot study was to detect sanitary consequences of possible interactions between domestic pigs and wild boars.

## Materials and methods

### Wild boars

The study area for wild boar sampling was comparable to the one chosen by Leuenberger (2004) including the same ten political regions (cantons). The study sites were characterised by high wild boar density and localised at the Swiss border to Germany (north), France (west), and Italy (south). The study sites harboured the majority of the Swiss wild boar population as assessed from population size estimates based on the national hunting statistic of 2002 and knowledge of local experts.

An approximate target sample size was calculated using the national hunting statistic (Federal Office for the Environment (FOEN), <http://www.umwelt-schweiz.ch/>). Data of the past 10 years showed figures between 2,200 and 6,300 wild boars shot per year in Switzerland. Assuming a population increment of 100–200% and considering that the hunting bag represents 50–100% of the population increment (<http://www.wildschwein-sangler.ch/>), the population size in the selected study areas was estimated to be about 6,700–13,400 in the northern part and 1,200–2,400 in the southern part. The median of the extreme values of each population estimate (10,000 and 1,800, respectively) was used for sample size calculation. A sample size of 300 for the northern and 250 for the southern Swiss wild boar population was obtained, considering a minimal expected prevalence of 1% and test characteristics of 98% sensitivity and 100% specificity, as indicated for domestic pigs (FreeCalc Version2 (c) Angus Cameron 1999).

Blood samples were collected by hunters and game-keepers during the regular hunting season from August to the end of February. Kits containing a numbered data sheet, blood tubes and stamped envelopes addressed directly to the laboratories involved in the diagnostic analysis were distributed to the hunting authorities. Hunters were advised to collect blood of shot animals either directly from the

## Statistical analysis

Data management and descriptive statistics were conducted in MS Access and MS Excel (Office 2000 Professional, Microsoft). For CSF and AD, the prevalence of infection was estimated using confirmed positive samples of the combined testing procedure (ELISAs and VNTs). For brucellosis in wild boar, the prevalence of infection was estimated based on the RBT and the ELISA results. RBT prevalence was used for comparison with studies in other countries and to statistically analyse associations with other variables because most other authors rely on RBT prevalence. ELISA prevalence was used for comparison with the results for brucellosis serology of the previous study (Leuenberger 2004) where samples were analysed by ELISA only.

Samples showing non-interpretable results in the RBT, doubtful results in the brucellosis ELISA and doubtful VNTs were excluded from the statistical analysis. Differences in the apparent prevalence (AP) between regions and between sampling rounds were assessed using the  $\chi^2$  test or when there were less than six observations per category, by the Fisher's exact test. Because the test characteristics for wild boar sera were hardly determinable and most authors used the AP estimate, the AP was used for further analysis and for comparison with other studies. The 95% confidence intervals of AP were calculated as exact binomial confidence intervals. The AP was analysed across age groups, between regions and between years using the  $\chi^2$  test or when there were less than six observations per category, by the Fisher's exact test. All statistical calculations were performed with NCSS (Hintze J., 2004, NCSS and PASS (Number Cruncher Statistical Systems. Kaysville, UT, USA, <http://www.NCCS.com>). Geographical data management was done in the ArcMap 8.2 (ESRI, USA).

## Results

### Wild boars

A total of 1,060 sera were obtained from ten Swiss cantons (Fig. 1a and b) during sampling rounds 1 and 2. Due to misdirection of some samples from the field, the total number of wild boar sera tested in the Institute of Veterinary Bacteriology (810 sera) was different from the number of sera tested at the Institute of Virology (1,060 sera). Most samples were collected between October and December with a peak in December. The average number of samples per canton was 58 (range 10–219). Sex distribution was balanced and there were 10.6% juveniles, 43.0% sub-adults and 46.4% adults. Data on sex and age were not provided for 186 and 53 animals, respectively. Sampled wild boars weighed on average 41.6 kg (range: 5–110 kg,  $n=790$ ).

## Serology

Results on all investigated agents per sampling round are summarized in Table 1.

No antibodies against CSF were detected in any of the 1,060 tested wild boars, neither north nor south of the Alps.

Out of the 1,060 samples analysed, 30 (2.83%) were seropositive for AD and 18 revealed doubtful results. Positive blood samples originated from seven different cantons situated north and south of the Alps. No significant difference in the seroprevalence between the populations north and south the Alps was observed. Most positive wild boars were sub-adult and adult animals. However, the difference in seroprevalence between juveniles and older animals was not significant ( $p>0.05$ , Fisher's exact test). There was no significant difference ( $p>0.05$ ) between prevalence in males (1.65%) and females (1.99%).

A total of 810 blood samples were tested for antibodies against *B. suis*. Geographical distribution of seropositive samples is shown in Fig. 1. Overall seroprevalence for PB was 13.5% (95% CI 10.7–16.7%) for the Rose Bengal test and 11.05% (95% CI 8.82–13.61%) for the indirect ELISA. Although differences in prevalence were obvious between the north and south with the ELISA results, they were almost absent when considering the RBT results. Seroprevalence (RBT results) in the different cantons ranged from 2.4% to 32.0%. The highest prevalences (>25%) were observed in the north-west and south-west of the country. Overall and separately for both sampling rounds, there was no significant difference in the prevalence between the wild boar populations located north and south from the Alps ( $p>0.05$ , Fisher's exact test). Differences in prevalence between age classes varied from one sampling round to the other: in sampling round 1, seroprevalence in juvenile animals was only slightly lower than seroprevalence in the sub-adult and adult age-classes, respectively ( $p=0.056$ , Fisher's exact test). In contrast, difference in seroprevalence between the adult and the sub-adult age-classes was statistically significant ( $p=0.006$ ). In sampling round 2, there was no significant difference in seropositivity between age-classes. No significant difference in prevalence was observed between the two sampling rounds, neither overall, in the north nor in the south (all  $p>0.05$ ). Prevalences (ELISA results) observed in the present study were similar to those reported earlier by Leuenberger (2004) in both compartments ( $p>0.05$ ).

## Bacteriology

No bacteriological isolation of both *B. suis* and *Y. enterocolitica* was achieved in any of the ten genital organs submitted to culture.

**Table 1** Seroprevalence and 95% confidence interval (CI) for classical swine fever (CSF), Aujeszky's disease (AD) and porcine brucellosis (PB) in wild boar in Switzerland as assessed during two sampling rounds in 2004 and 2005

| Sampling round              | 2004–2005  |             | 2005       |            |
|-----------------------------|------------|-------------|------------|------------|
| Number of samples collected |            |             |            |            |
| North of the Alps           | 624        |             | 309        |            |
| South of the Alps           | 84         |             | 43         |            |
|                             | Prevalence | 95% CI      | Prevalence | 95% CI     |
| CSF                         |            |             |            |            |
| North                       | 0.00       | 0.00–0.60   | –          | –          |
| South                       | 0.00       | 0.00–4.45   | –          | –          |
| AD                          |            |             |            |            |
| North                       | 3.73       | 2.38–5.45   | 1.99       | 0.74–4.32  |
| South                       | 0.00       | 0.11–5.45   | 2.33       | 0.06–12.3  |
| PB iELISA                   |            |             |            |            |
| North                       | 12.53      | 9.45–15.81  | 9.38       | 5.69–13.5  |
| South                       | 2.63       | 0.32–9.18   | 0.00       | 0.00–8.2   |
| PB RBT                      |            |             |            |            |
| North                       | 14.05      | 10.90–17.71 | 13.54      | 9.37–18.66 |
| South                       | 17.81      | 9.84–28.53  | 11.91      | 3.98–25.63 |

iELISA: indirect ELISA, RBT: Rose Bengal test.

## Outdoor pigs

### Questionnaire

Of the 25 farms fitting the selection criteria, 14 were willing to participate in the study. Six farms were classified as breeding farms, eight as finishing farms and one as a mixed breeding–finishing farm. Surface of the premises ranged from 1–45 ha (mean: 22.4 ha). Out of the 14 farms, 10 were located near wooded areas. In these farms, observations of wild boars were common and damage on farmland was reported to be considerable in 75% of the farms. The average number of pigs was 64 animals (range: 25–120) in finishing farms and 5 sows in breeding farms (range: 1–12). All farmers were conscious about the permanent presence of wild boars in their proximity and all of them protected the pig enclosures with single electric fences, wire netting or palings.

### Serology

A total of 61 animals from the 14 farms were serologically examined for antibodies against AD and PB. Considering disease clustering at herd level, this sample size allowed to demonstrate that herd-prevalence of brucellosis in the selected farms was <5% at a 95% confidence level (Cameron 1999). All samples were negative for AD. For brucellosis, 51 serum samples from 13 farms turned out negative in the RBT and indirect ELISA. In one farm, two out of ten serum samples showed a positive result in the indirect ELISA, but both were negative in the RBT. One of

these serum samples gave positive results in the CFT and the SAT, the second one was positive in the SAT only. A third serum sample showed a doubtful result in the indirect ELISA and a negative result in the RBT. This serum sample gave a positive result in the SAT, but was negative in the CFT. All other seven samples from this farm were negative in the indirect ELISA. Additional epidemiological investigations and clinical inspections on this farm provided no evidence of a *Brucella* infection. Attempts to isolate both *B. suis* and *Y. enterocolitica* through bacterial culture from the genital organs of five subsequently sampled pigs from this same farm were unsuccessful.

## Discussion

Samples from free-ranging wild boars were collected from both Swiss populations and analysed by means of serological tests to detect antibodies against CSF, AD and PB. Whenever possible, testing methods were improved compared to a former study performed in Switzerland (Leuenberger et al. 2006). Furthermore, outdoor pigs from farms in zones with remarkable presence of wild boars were sampled and serologically tested for AD and PB.

Number, geographical distribution and sex and age ratios of sampled wild boars from the two sampling rounds reflected the regular hunting bag. The expected sample size was reached in the population in the north but not in the south, although much effort was made to motivate the hunters to participate in the study.

The number of sampled pigs per farm was very low. Rearing pigs outdoor in Switzerland has regained popularity in recent years, but there is only a small number of professional breeding or fattening farms with large numbers of animals. Most of the farmers own small herds (see Results section) and adopted this system in addition to their main focus in farming. Participation in the study was voluntary, which had also a direct influence on the feasible sample size. Furthermore, criteria to select eligible outdoor piggeries were restrictive, reducing the number of farms to be considered for the study. However, in this way, pigs from selected farms corresponded to the population at highest risk.

### Classical swine fever

No positive samples were detected in any of the sampling rounds. Positive wild boars were identified for the last time in Switzerland in 2002 (Leuenberger 2004). They were two adults from southern Switzerland, which most probably acquired their antibodies during the outbreak of CSF in 1998. Thus, the repeated surveys confirmed the current absence of CSF in wild boars in Switzerland. However,

interactions are likely to become a serious threat for the maintenance of the freedom-from-infection status of domestic pigs for the three infectious diseases considered in this study. Indeed, interviews of pig farmers revealed that piggeries at highest risk are not protected by double-fencing systems, as recommended by several agricultural organisations and scientific experts (Anonymous 2004b).

Better diagnostic tools have to be developed to obtain more accurate results concerning PB prevalence and studies are needed to identify risk factors for interactions between wild boars and domestic pigs. However, some measures can already be recommended to reliably protect outdoor pigs from transmission of the three infectious agents (especially ADV and *B. suis*): (1) double-fencing the pastures with electric wires, (2) penning-in sows during heat and (3) a thorough clarification of any abortion causes in pigs.

Targeted serosurveillance of free-ranging wild boars allowed to document their serological status and to identify high risk areas for domestic pig populations regarding selected infectious agents. Evidence of pathogens considered as a threat for domestic species point out the importance of health monitoring in wildlife. Regarding PB, further investigations in wild boars should be carried out as soon as reliable diagnostic tools are available. In addition, surveillance of outdoor pigs in areas at risk should be included in routine surveillance programmes.

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