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## Prevalence of and risk factors associated with viral and bacterial pathogens in farmed European wild boar

Outi Hälli<sup>a,\*</sup>, Eve Ala-Kurikka<sup>a</sup>, Tiina Nokireki<sup>b</sup>, Teresa Skrzypczak<sup>b</sup>, Mirja Raunio-Saarnisto<sup>b</sup>, Olli A.T. Peltoniemi<sup>a</sup>, Mari Heinonen<sup>a</sup>

<sup>a</sup>University of Helsinki, Faculty of Veterinary Medicine, Department of Production Animal Medicine, Paroninkuja 20, FIN-04920 Saarentaus, Finland

<sup>b</sup>Finnish Food Safety Authority Evira, Research Department, Mustialankatu 3, FIN-00790 Helsinki, Finland

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### ABSTRACT

The aim of this study was to estimate in farmed European wild boars the prevalence of and risk factors associated with a range of common porcine viral and bacterial infections, namely, porcine parvovirus (PPV), porcine circovirus type 2 (PCV2), swine influenza virus (SIV), Aujeszky's disease virus (ADV), classical swine fever virus (CSFV), swine vesicular disease virus (SVDV), coronavirus causing transmissible gastroenteritis (TGEV), porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae*, *Lawsonia intracellularis*, *Brucella* spp., and *Leptospira* spp. A sampling frame was compiled based on a national record of wild boar farmers, and 32 farms were surveyed.

Serological screening was carried out on 303 samples from animals slaughtered between 2005 and 2008, and random-effect logistic regression models were developed for pathogens with a 'non-zero' prevalence. The apparent animal prevalence for PPV, PCV2, and *L. intracellularis* was 46.5% (95% confidence interval [CI] 41–52%), 51.1% (95% CI 45–57%) and 59.2% (95% CI 54–65%), respectively. Apparent farm seroprevalence rates for PPV, PCV2 and *Lawsonia intracellularis* were 56.3% (95% CI, 39–73%), 21.9% (95% CI, 8–36%) and 78.1% (95% CI, 64–92%), respectively. No antibodies were detected against SIV, ADV, CSFV, SVDV, TGEV, PRRSV, *Leptospira* spp., *Brucella* spp., or *M. hyopneumoniae*. Increasing herd size, proximity to dense populations of domestic swine and later sampling times within the survey period were found to be risk factors. Overall, the seroprevalence of these pathogens in farmed wild boar was similar to that in the farmed domestic pig population in Finland. However, it is possible that the rearing of wild boars in fenced estates may predispose them to particular infections, as reflected in higher antibody titres.

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### Introduction

The farming of European wild boar (*Sus scrofa scrofa*) is a growing industry in Finland and the economics of boar meat production has resulted in the development of intensive farming techniques. Such a development may pose an increased risk of disease transmission between boars (Ruiz-Fons et al., 2006, 2008), as well as between boars and contiguous domestic pigs (*Sus scrofa domestica*), and possibly wildlife. Wild boar farms could become potential reservoirs of infectious disease for domestic pigs since these species share many pathogens (Ruiz-Fons et al., 2008; Phillips et al., 2009; Sibila et al., 2009).

The aim of our study was to estimate the seroprevalence of commonly occurring porcine viral and bacterial pathogens in farmed wild boar populations in Finland, and to assess what role (if any) such populations might play as disease reservoirs. The

pathogens surveyed were porcine parvovirus (PPV), circovirus type 2 (PCV2), swine influenza virus (SIV), Aujeszky's disease virus (ADV), classical swine fever virus (CSFV), swine vesicular disease virus (SVDV), the coronavirus causing transmissible gastroenteritis (TGEV), porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae*, *Brucella* spp., *Lawsonia intracellularis*, and *Leptospira* spp. A supplementary objective was to evaluate the risk of certain management practices on disease prevalence on these farms.

### Materials and methods

#### Farm selection

A nationwide serological survey was carried out between 2005 and 2008. Based on an official national record of wild boar farmers, a sampling frame was compiled ( $n = 117$ ). Every farm was contacted by mail and non-respondents ( $n = 25$ ) received a phone call from a member of the research group. Ultimately, a response was obtained from 104 farmers: 33 indicated that they no longer farmed wild boar, 39 did not wish to participate, and 32 volunteered to take part.

\* Corresponding author. Tel.: +358 400 803312.

E-mail address: [outi.halli@helsinki.fi](mailto:outi.halli@helsinki.fi) (O. Hälli).

All farms used a similar production system. The boars were kept in fenced enclosures and were fed regularly at specific feeding sites. Outside breeding and farrowing periods, the females and young were kept in one or sometimes two separate areas. Adult boars were most commonly held in a separate, smaller enclosure. During the breeding season in late autumn/early winter, selected boars were housed with the adult sows. There was some variation in sow management during the farrowing season. The majority of farms divided late-gestation sows into smaller groups of animals familiar to each other. However, this approach was not practiced particularly in Southern Finland where animal numbers were larger. Piglets/young animals were generally housed initially with their dam, and then with other adult sows and their offspring until time of slaughter. Two farms in Eastern Finland specialised in growing/finishing animals (slaughter type farm). Vaccination was not practiced on any of the farms.

#### Sampling procedure

Farmers collected blood samples at 'on-farm' slaughter and provided details of animal identification number, age, and gender as well as basic information about their enterprise. Samples were mailed to the laboratory, where serum was extracted by centrifugation and stored at  $-18^{\circ}\text{C}$  until analysed. A sample size of 300 was required to find at least one seropositive animal at a 95% confidence level assuming a disease prevalence of 1%. In analysing risk factors, a sample size of 230 was required to find a 0.15 difference in proportions with a power of 0.9 and a 95% confidence level.

A total of 303 samples were collected from 32 farms with an average of 10 samples/farm (range 1–63). Of these 303 samples, 294, 272, 289, 280 and 274 were of sufficient quality to assess serologically for PCV2, SIV, *Leptospira* spp., *Brucella* spp. and *M. hyopneumoniae*, respectively. For PPV, ADV, CSFV, SVDV, TGEV, PRRSV, and *L. intracellularis*, 301 samples were suitable for analysis.

#### Serological examination

Most of the serological assessment was carried out at The Finnish Food Safety Authority Evira laboratories. Serology for *L. intracellularis* was performed at the Danish Institute for Food and Veterinary Research in Copenhagen. The Rose-Bengal agglutination test was used to detect antibodies to *Brucella* spp. A *Brucella abortus/melitensis/suis* Rose-Bengal test antigen (OIE Brucellosis Reference Centre, Institut Porquier), and the serum samples under test were placed on a plastic plate and mixed. The mixture was agitated for 4 min at room temperature and samples with any visible agglutination were considered positive. To detect antibodies to *L. interrogans* serovars Pomona, Tarrasovi, and Bratislava, a microscopic agglutination test (MAT) was used as detailed in the OIE manual (Anonymous, 2008). Samples with titres  $\geq 100$  were considered positive.

Serological screening for *L. intracellularis* was carried out using an ELISA as described by Boesen et al. (2005), and for *M. hyopneumoniae*, PPV, SIV, CSFV, PRRSV, ADV, TGEV and SVDV using commercially available ELISAs (*M. hyopneumoniae* ELISA, Oxoid; SVANOVIR PPV-Ab ELISA, Svanova Biotech AB; ID Screen Influenza A Antibody Competition, ID VET; PrioCHECK CSFV Ab, Prionics; HerdCheck PRRS Virus Antibody Test Kit, IDEXX Laboratories; PRV-gB-Ab, SVANOVIR; TGEV/PRCV-Ab, SVANOVIR, Svanova Biotech; and PrioCHECK SVDV Ab, Prionics).

Antibodies against PPV were also detected using a haemagglutination inhibition test (Joo et al., 1976) and against PRV and TGE/PRCV using serum neutralisation tests (Anonymous, 2008). Antibodies against PCV2 were detected with an immunoperoxidase monolayer assay. Sera were serially diluted and applied to fixed, PCV2-infected PK15 cell cultures and incubated for 15 min at  $37^{\circ}\text{C}$ . The cells were then washed and a peroxidase-conjugated anti-pig immunoglobulin (Ig) G added prior to a further 15 min incubation at  $37^{\circ}\text{C}$ . After washing, a peroxidase enzyme substrate was applied at room temperature for 15 min. The cells were then washed and viewed under white light.

#### Statistical analysis

Apparent animal and farm prevalences were calculated. The 95% confidence intervals (CI) for seroprevalence proportions were calculated, using apparent prevalence and sample size. Statistical modelling was carried out for pathogens with calculated prevalences  $>0$ . The outcome variables (seroprevalences) were coded as dichotomous variables (i.e. yes/no). The unit of interest was one wild boar. The herd identification number was used as a group-level variable (random effect). The explanatory variables used in the analysis were: herd type (slaughter or combined), herd size; surface area of enclosure and animal density in a herd; age ( $\leq 12$  months, 12–24 months, and  $\geq 24$  months) and gender of animal.

For each farm, the Finnish National Centres for Economic Development, Transport, and the Environment, were used as location codes (Southern, Central and Western, Eastern and Northern centres), which facilitated the identification of the number of domestic pig farms in the area. The year of sampling was included in the models as a categorical variable and the data were uploaded into STATA 9.2 (StataCorp) software.

During initial data handling, correlations and unconditional associations between variables were calculated. The initial model contained variables that fulfilled the inclusion criteria of a  $P$  value of 0.3 in unconditional association analysis. For the

PPV model, these variables were 'herd type', 'animal density', 'animal age' and 'gender', and 'year of sampling'. For the PCV2 model, these variables were 'herd type' and 'herd size', 'animal age' and 'year of sampling'. For the *L. intracellularis* model, these variables were 'surface area of enclosure', 'animal density', 'herd size', 'animal age' and 'gender' and 'year of sampling'. After fitting the initial model, a backward stepwise elimination model building technique was used. The significance of each variable was evaluated against the exclusion criteria (likelihood ratio test,  $P < 0.2$ ). The terms of interaction between the significant variables were entered into the model. None of these was found to be significant and were thus excluded from the final model. The possibility of confounding variables was considered, but was judged to be non-existent among the known variables.

The final model included the variables: 'herd size' and 'year of sampling' for PPV; 'age of animal', 'location', and 'year of sampling' for PCV2; and 'animal gender', 'herd size', 'location', and 'year of sampling' for the *L. intracellularis*, respectively. The reliability of the final model estimation procedure (maximum likelihood estimation) was evaluated using different numbers of quadrature points. The estimates changed  $<0.01\%$  by different variants of the numerical integration procedure. When the magnitude of the effect of exceptional observations and data points was scrutinised, no reason for concern was identified.

## Results

### Descriptive data

On average, boar farms had  $49.8 \pm 28.7$  animals (range 5–100) with an average area of  $9.2 \pm 10.2$  ha (range, 1–65 ha). Average animal density was  $6.2 \pm 5.8$  animals/ha (range, 1.5–25/ha). The distribution of the explanatory categorical variables is detailed in Table 1. Half of the samples represented approximately 65% of annually slaughtered animals. The samples originated from 32 farms (27% of farms within the sampling frame: 31% of 'responders' and 45% of 'active' farmers). Farms that did not participate in the study were smaller in size compared to those of responders (8.7 vs. 49.8 boars/farm). The Northern part of Finland was slightly over-represented in our sample population, although geographical location did not significantly influence farm participation ( $\chi^2 = 6.04$ ,  $P = 0.1$ , d.f. = 3). Most wild boar farming occurs in Eastern Finland, while smaller numbers of boars are held elsewhere on 'mixed type' farms as tourist attractions.

In Finland, official farm registration is obligatory, and all types were included in our sampling frame. We concluded that farms unwilling to participate in our survey were likely to be small, mixed enterprises, and that our participating farms were representative of those engaged in wild boar production.

### Prevalence rates and outcomes from statistical modelling

All samples were serologically negative for SIV, ADV, CSFV, SVDV, TGEV, PRRSV, *Leptospira* spp., *Brucella* spp., and *M. hyopneumoniae*. The apparent animal prevalences for PPV, PCV2 and *L.*

**Table 1**

Distribution of categorical variables relating to the 303 European wild boars surveyed.

Variable	Level	n	%
Herd type	Slaughter	115	38.0
	Combined	188	62.1
Centre <sup>a</sup>	Southern, Central and Western	47	15.5
	Eastern	201	66.3
	Northern	55	18.2
Age, months	$\leq 12$	29	9.6
	12–24	47	15.5
	$\geq 24$	108	35.6
Gender	Male	184	67.9
	Female	87	32.1
Sampling year	2005	65	22.0
	2006	12	4.1
	2007	57	19.3
	2008	162	54.7

<sup>a</sup> Centres for Economic Development, Transport, and the Environment.

**Table 2**

Effects of different factors on the seroprevalence of porcine parvovirus in 266 European wild boars according to the random-effect logistic regression model.

Variable	Odds ratio	Standard error	P	95% Confidence interval
Herd size	1.04	0.02	0.01	1.02–1.08
Sampling year 2006	17.5	16.0	0.00	3.0–104.1
Sampling year 2007	19.8	13.8	0.00	5.1–77.7
Sampling year 2008 (sampling year 2005)	117.1	81.3	0.00	30.1–456.5

**Table 3**

Effects of different factors on the seroprevalence of porcine circovirus type 2 in 291 European wild boars according to the random-effect logistic regression model.

Variable	Odds ratio	Standard error	P	95% Confidence interval
Eastern centre <sup>a</sup>	0.2	0.13	0.02	0.03–0.75
Northern centre <sup>a</sup> (Southern, Central, and Western centres <sup>a</sup> )	1.8	1.4	0.4	0.4–8.2
Age (12–24 months)	2.8	2.0	0.1	0.7–11.1
Age ( $\geq$ 24 months)	0.8	0.5	0.7	0.2–3.0
Age ( $\leq$ 12 months)				
Sampling year 2006	1.7	1.4	0.5	0.4–8.2
Sampling year 2007	3.9	2.1	0.01	1.4–11.1
Sampling year 2008 (sampling year 2005)	1.8	0.8	0.2	0.8–4.2

<sup>a</sup> Centres for Economic Development, Transport, and the Environment.

**Table 4**

Effects of different factors on the seroprevalence of *Lawsonia intracellularis* in 231 European wild boars according to the random-effect logistic regression model.

Variable	Odds ratio	Standard error	P	95% Confidence interval
Herd size	1.04	0.02	0.01	1.01–1.08
Gender (female) (male)	3.1	1.4	0.01	1.3–7.5
Sampling year 2006	0.7	0.6	0.7	0.1–4.3
Sampling year 2007	1.4	0.9	0.6	0.4–4.7
Sampling year 2008 (sampling year 2005)	3.1	1.6	0.02	1.2–8.4

*intracellularis* were 46.5% (95% CI, 41–52%), 51.1% (95% CI, 45–57%), and 59.2% (95% CI, 54–65%), respectively. The apparent farm prevalences for these pathogens were 56.3% (95% CI, 39–73%), 21.9% (95% CI, 8–36%), and 78.1% (95% CI, 64–92%), respectively.

The results of statistical modelling are illustrated in Table 2. Two variables, 'number of animals/herd' and 'year of sampling', acted as risk factors for the seroprevalence of PPV. The odds ratio (OR) for seropositivity to PPV increased 12-fold for each additional 50 animals/herd. Increasing ORs for the presence of PPV antibodies were detected from samples collected earlier in the study period. The highest OR (3.9) for PCV2 antibodies was found in 2007, while the years 2006 and 2008 had similar ratios (1.8). Farms in Eastern Finland had lower ORs (0.2) in relation to seroprevalence to PCV2 (Table 3).

The prevalence of antibodies to *L intracellularis* was significantly influenced by gender, number of animals/herd, and sampling year. For each 50-animal increase in herd size, the OR increased by 8.8. Females were at greater risk of being seropositive for this pathogen (OR 3.1). Again, the last sampling year (2007) had the greatest OR for positive test results when compared with the first year of sampling (3.1) (Table 4).

## Discussion

The study highlights the significant overlap in the seroprevalence to viral and bacterial pathogens in farmed European wild boar and domestic pig populations (Anonymous, 2010). There was no evidence of exposure to a wide range of pathogens suggesting farmed European wild boar do not pose a significant risk of transmitting disease to domestic pigs. Our seroprevalence data for PPV and PCV2 are at the higher end of previously reported results for these pathogens. Vicente et al. (2004) compared PCV2 antibody prevalences among free-living, fenced, and intensively managed wild pigs and found an increasing seroprevalence with intensification. The fact that the boars in the current study were intensively managed may explain the higher prevalence of antibodies in this study compared to serological surveys of feral pigs.

The OR for the prevalence of PCV2 antibodies was lowest in regions where the domestic pig population density was low, a finding similar to that reported in the USA (Corn et al., 2009). Although there is little direct or indirect contact between domestic pigs and farmed wild boar in Finland, there remains the possibility of agents such as PCV2 spreading from domestic pig populations to farmed wild boar. Evidence of exposure to *L. intracellularis* was higher than that previously reported and may be due to differences in the methods of diagnosis: surveys reporting prevalences up to 19% used PCR analysis on tissue or faecal samples (Jacobson et al., 2005; Dezorzo-Tomanova et al., 2006; Phillips et al., 2009) and these were from free-living rather than farmed boars.

Farm size was identified as a risk factor for exposure to PPV and *L. intracellularis*. The OR for seropositivity increased 12- and 8.8-fold for every additional 50 animals in a herd for PPV and *L. intracellularis*, respectively. Our findings indicate that animal density increases with farm size (linear regression coefficient 0.54,  $P = 0.00$ ). Following statistical modelling, animal density was not found to be a significant variable in the exposure of boar to either PPV or *L. intracellularis*, indicating that the effect of farm size is not mediated through animal density. Greater numbers of animals/farm could result in more persistent herd infections and consequent higher seroprevalences to the causative pathogens.

No antibodies were found to SIV, *M. hyopneumoniae*, or *Leptospira* spp. The first clinical cases of swine influenza in domestic pigs in Finland were reported in 2009 and retrospective analysis of abattoir samples from 2007, 2008, and 2009 found seroprevalences of 2%, 5%, and 17%, respectively (Anonymous, 2010). Since the seroprevalence to SIV was low in domestic pigs and wild boars sampled prior to 2009, it was not surprising that we did not find antibodies to this virus. Our finding of little evidence of leptospiral infection in the farmed wild boar was anticipated given that few domestic pigs have been found seropositive for *Leptospira* spp. in the last 5 years in Finland (Anonymous, 2010).

## Conclusions

Seroprevalence to a range of viral and bacterial pathogens in farmed European wild boar populations in Finland was similar to that found in the domestic pig population. Our findings suggest that farmed wild boar do not pose a significant risk of disease transmission to domestic swine although increased farming of European wild boar may increase the risk of exposure of these animals to some viral infections.

## Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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