DOI: 10.7589/2017-05-120 *Journal of Wildlife Diseases*, 54(2), 2018, pp. 229–237

A SEROLOGIC SURVEY OF PATHOGENS IN WILD BOAR (*SUS SCROFA*) IN SWEDEN

Anna Malmsten,[1,4] Ulf Magnusson,[1] Francisco Ruiz-Fons,[2] David González-Barrio,[2,3] and Anne-Marie Dalin[1]

[1] Division of Reproduction, Department of Clinical Sciences, Swedish University of Agricultural Sciences, Box 7054, SE-750 07 Uppsala, Sweden

[2] Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM), Ronda de Toledo 12, 13071 Ciudad Real, Spain

[3] Departamento de Sanidad Animal, Facultad de Veterinaria, UCO, Campus Universitario de Rabanales, 14071 Córdoba, Spain

[4] Corresponding author (email: anna.malmsten@slu.se)

ABSTRACT: The wild boar (*Sus scrofa*) population has increased markedly during the last three decades in Sweden and in other parts of Europe. This population growth may lead to increased contact between the wild boar and the domestic pig (*Sus scrofa scrofa*), increasing the risk of transmission of pathogens. The objective of our study was to estimate the seroprevalence of selective pathogens, known to be shared between wild boars and domestic pigs in Europe, in three wild boar populations in Sweden. In total, 286 hunter-harvested female wild boars were included in this study. The sera were analyzed for antibodies against nine pathogens using different commercial or in-house enzyme-linked immunosorbent assays. Antibodies were detected against porcine parvovirus (78.0%), porcine circovirus type 2 (99.0%), swine influenza virus (3.8%), *Erysipelothrix rhusiopathiae* (17.5%), *Mycoplasma hyopneumoniae* (24.8%), and *Toxoplasma gondii* (28.6%). No antibodies were detected against porcine respiratory and reproductive syndrome virus, *Brucella suis*, or *Mycobacterium bovis*. Our results highlight the potential importance of the wild boar as a reservoir for pathogens potentially transmissible to domestic pigs and which also may affect human health.

Key words: ELISA, pathogens, seroprevalence, *Sus scrofa*, wild boar, wildlife.

INTRODUCTION

Wild boar (*Sus scrofa*) have been part of the Swedish fauna for thousands of years. The species became extinct in the 17th century but re-entered into wild habitats in the southern and middle parts of the country in the 1970–80s after wild boars had escaped from enclosures in which they were kept for recreational and hunting purposes (Naturvårdsverket 2010). The population has increased since the species reappeared, and now about 100,000 animals per year are culled by hunting (Svenska Jägareförbundet 2014). The wild boar is a popular game species and is hunted in Sweden primarily for meat but also for trophies. Besides hunting, the species is managed by supplementary feeding, more or less in every area in Sweden that holds wild boars today.

The wild boar is considered a reservoir of various pathogens that can infect other wildlife (Gortázar et al. 2007), domestic animals, and humans (Meng et al. 2009; Ruiz-Fons 2017). Thus, an increase of the wild boar population also implies an increased epizootic risk of transmission of pathogens to the domestic pig and other species.

In comparison to other European countries, Sweden has a favorable status in domestic and wild animals concerning epizootic diseases. For example, Sweden has attained and maintained a disease-free status for pathogens such as porcine reproductive and respiratory syndrome virus (PRRSV), classical and African swine fever viruses, and *Mycobacterium bovis* (Swedish National Veterinary Institute [SVA] 2015). In Sweden, there is an ongoing active and passive surveillance for many of these diseases in both domestic pigs and in wild boars (SVA 2015). Other pathogens of economic and animal welfare importance in the domestic pig are porcine parvovirus (PPV), porcine circovirus type 2 (PCV-2), swine influenza virus (SIV), *Erysipelothrix rhusiopathiae*, *Mycoplasma hyopneumoniae*,

and *Toxoplasma gondii*, of which *T. gondii* and *E. rhusiopathiae* are of zoonotic concern (SVA 2015). Antibodies against all of these pathogens have previously been detected in various wild boar populations in Europe (Table 1). However, the status of most of these diseases in Swedish wild boar is unknown.

We initiated our study because of the scarce information about prevalence and distribution of pathogens among wild boars in Sweden. Our objective was to estimate the seroprevalence of selective pathogens, known to be shared between wild boars and domestic pigs in Europe, in three wild boar populations in Sweden.

MATERIALS AND METHODS

Study areas and data collection

We collected samples between January 2013 and December 2015 as part of a larger study on the reproduction of female wild boar. Sampling was conducted during regular hunting at three estates located in three counties (Blekinge: 56°12′36″N, 015°16′34″E; Södermanland: 59°22′00″N, 017°2′00″E; Uppland: 59°33′59″N, 017°31′48″E) in southern Sweden (Fig. 1). Information on habitat composition, feeding practices, and estimated population sizes (wild boar and other ungulates) was provided by the wildlife manager at each estate. The area of the estates varied between 10–87 km^2 (nonfenced), and the density of wild boars was estimated at 5–40/km^2 based on hunting bags and counting at feeding stations. All estates used supplementary feeding throughout the year.

Samples were collected from hunter-harvested female wild boars weighing >30 kg. Harvest date was noted and the age of each individual was estimated using tooth eruption and tooth replacement according to Matschke (1967), and the animals were classified as juveniles (<1 yr old), yearlings (1–2 yr old), and adults (>2 yr old).

Blood samples were collected from freshly opened blood vessels near the jugular vein in sterile tubes (BD Vacutainer®, Becton, Dickinson and Co., Franklin Lakes, New Jersey, USA) within 2–4 h after culling, and serum was separated by centrifugation in the field and thereafter put in cryovials (Globe Scientific Inc., Paramus, New Jersey, USA) and stored at –20 C prior to analyses.

The epidemiologic software, EpiTools (Sergeant 2017), was used to calculate the sample size required for each study area and pathogen to provide accurate estimates of seroprevalence (Table 2). The analyses were performed separately by study area and pathogen. To run the tests, we entered lowest and highest pathogen seroprevalence values reported in European wild boar populations (Table 1) and the estimated wild boar sample size per study area based on the estimated populations size obtained from wildlife managers (Blekinge County=435, Södermanland County=560, Uppland County=125). The analyses were performed with a 95% confidence level and a 7% acceptable error.

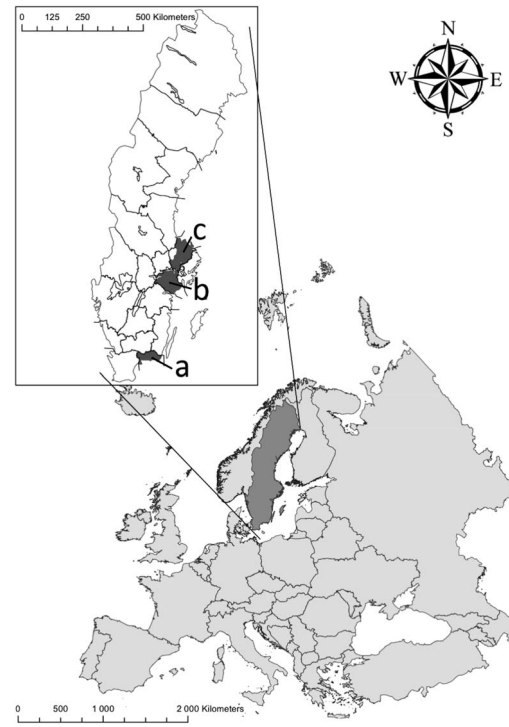

FIGURE 1. Map of Sweden highlighting the three counties (a=Blekinge, b=Södermanland, c=Uppland) where female wild boars (*Sus scrofa*) were serologically sampled for antibodies against pathogens: porcine parvovirus, porcine cirvovirus type 2, porcine respiratory and reproductive syndrome virus, Swine influenza virus, *Erisypelothrix rhusiopathiae*, *Brucella suis*, *Mycoplasma hyopneumoniae*, *Mycobacterium bovis*, and *Toxoplasma gondii*.

Serologic analyses

In total, 286 serum samples were tested for antibodies against PPV, PCV-2, PRRSV, SIV, *E. rhusiopathiae*, *M. hyopneumoniae*, and *M. bovis*. Also, 276 of these serum samples were tested for antibodies against *T. gondii*. A total of 92 wild boar

TABLE 1. Prevalence (percent) of antibodies against porcine parvovirus (PPV), porcine cirvovirus type 2 (PCV-2), porcine respiratory and reproductive syndrome virus (PRRSV), swine influenza virus (SIV), *Erisypelothrix rhusiopathiae*, *Brucella suis*, *Mycoplasma hyopneumoniae*, *Mycobacterium bovis*, and *Toxoplasma gondii* detected in wild boars from different countries and regions reported in the present study and others. NA = not applicable.

	Countries														
Pathogen	Sweden (current study)	Croatia (Cvetnic et al. 2003)	Croatia (Roic et al. 2012)	Czech Republic (Sedlak et al. 2008)	Finland (Hälli et al. 2012)	Germany (Gethöffer et al. 2007)	Greece (Touloudi et al. 2015)	Iberian Peninsula (Boadella et al. 2011b)	Italy (Montagnaro et al. 2010)	Slovenia (Vengust et al. 2006)	Northeast Spain (Closa-Sebastiá et al. 2011)	South-central Spain (Vicente et al. 2002)	South and central Spain (Barasona et al. 2016)	Spain (Gonzáles-Barrio et al. 2015)	Spain (Ruiz-Fons et al. 2006)
PPV	78	NA	56	NA	47	58	NA	NA	8	49	55	10	NA	58	57
PCV-2	99	NA	15	43	51	NA	19	48	NA	NA	64	NA	NA	75	52
PRRSV	0	NA	6	NA	0	2	13	2	38	0	3	0	NA	NA	0
SIV	4	NA	NA	NA	NA	NA	1	NA	NA	NA	6	NA	NA	NA	NA
E. rhusiopathiae	18	NA	NA	NA	NA	NA	NA	15	NA	NA	5	5	NA	NA	NA
B. suis	0	29	NA	NA	0	2	NA	NA	4	0	NA	0	NA	NA	30
M. hyopneumoniae	25	NA	NA	NA	0	NA	0	NA	NA	21	27	0	NA	NA	NA
M. bovis	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	78	NA	NA
T. gondii	29	NA	NA	NA	NA	NA	5	NA	NA	NA	44	NA	NA	NA	36

TABLE 2. Calculation of required sample size for pathogen prevalence estimates in the study populations of wild boar (*Sus* scrofa) according to pathogen: porcine parvovirus (PPV), porcine cirvovirus type 2 (PCV-2), porcine respiratory and reproductive syndrome virus (PRRSV), swine influenza virus (SIV), *Erisypelothrix rhusiopathiae*, *Brucella suis*, *Mycoplasma hyopneumoniae*, *Mycobacterium bovis*, and *Toxoplasma gondii* seroprevalence ranges reported in the scientific literature (Table 1) for European wild boar populations and wild boar population size. The tests were run with a 95% confidence level and an accepted error of 7% in EpiTools (Sergeant 2017).

County	Population size	Pathogen	Seroprevalence range (%)	Required sample size range	Study sample size
Blekinge	435	PPV	8–58	52–133	102
		PCV2	15–75	82–110	
		PRRSV	1.5–40	12–132	
		SIV	1–50	8–136	
		E. rhusiopathiae	5–35	35–127	
		B. suis	1.5–30	12–120	
		M. hyopneumoniae	1–25	8–110	
		M. bovis	6.8–70	45–120	
		T. gondii	5–45	35–135	
Södermanland	560	PPV	8–58	53–143	110
		PCV2	15–75	85–117	
		PRRSV	1.5–40	12–142	
		SIV	1–50	8–146	
		E. rhusiopathiae	5–35	36–136	
		B. suis	1.5–30	12–128	
		M. hyopneumoniae	1–25	8–117	
		M. bovis	6.8–70	46–128	
		T. gondii	5–45	36–145	
Uppland	125	PPV	8–58	40–76	74
		PCV2	15–75	56–68	
		PRRSV	1.5–40	11–76	
		SIV	1–50	8–77	
		E. rhusiopathiae	5–35	30–74	
		B. suis	1.5–30	11–72	
		M. hyopneumoniae	1–25	8–68	
		M. bovis	6.8–70	36–72	
		T. gondii	5–45	30–77	

sera were analyzed for antibodies against *B. suis*; this sample size allowed us to determine with 95% confidence that *B. suis* was not circulating in Swedish wild boar above a 5% prevalence. This prevalence threshold was set up according to expected mean values from wild boar populations at similar densities (Muñoz et al. 2010). The sera were analyzed by different commercial enzyme-linked immunosorbent assays (ELISA) in accordance with the manufacturer's instructions (INgezim PPV compact, Ingenasa, Madrid, Spain; INgezim CIRCO IgG, Ingenasa; Indirect ELISA PRRS, Ingenasa; INgezim Influenza Porcina, Ingenasa; INgezim Mal Rojo, Ingenasa; INgezim Brucella compact 2.0, Ingenasa; INgezim M. hyo Compac, Ingenasa). The presence of antibodies against *M. bovis* was tested using an in-house ELISA using bovine tuberculin purified protein derivative as previously reported (Boadella et al. 2011a). The presence of antibodies against *T. gondii* was tested by an in-house ELISA following the protocol described by Račka et al. (2015) using the IDEXX Toxotest Ab antigen and reagents (IDEXX Laboratories, Inc., Westbrook, Maine, USA).

Optical density was determined on a spectrophotometer (Multiskan Go Plate Reader, ThermoFisher Scientific, Waltham, Massachusetts, USA). The results were expressed as positive or negative based on the manufacturer's recommended cutoff values. One of the samples in the *M. bovis* ELISA and 68 in the *E. rhusiopathiae* ELISA were considered as borderline or doubtful and were retested.

Statistical analyses

The statistical analyses were carried out using the software "R" (R Core Team 2013). The prevalence and exact confidences intervals were calculated using the propCI() function from the prevalence package for R. The associations of age class, as well as of regions of sampling with the seroprevalence of antibodies, were tested using a chi-square test. The statistically significant level was set at alpha=0.05.

RESULTS

The number of samples obtained was appropriate to estimate pathogen seroprevalence rates within the ranges reported in the literature for European wild boar populations with a 95% confidence level and an allowed error of 7% (Table 2). The distributions of age classes, sampling regions, and seroprevalences are presented in Table 3. The number of tested animals in the different categories as well as the number of animals with the test result 'borderline' for *E. rhusiopathiae* are presented in Supplementary Material Table S1. Seventy-eight percent of the samples were positive for PPV, 99% for PCV-2, 4% for SIV, 18% for *E. rhusiopathiae*, 25% for *M. hyopneumoniae*, and 29% for *T. gondii*. No antibodies were detected in the sera against PRRSV, *B. suis*, or *M. bovis*.

Age classes were positively correlated to the prevalence of antibodies against PPV (χ^2=55.87, df=2, P<0.001), SIV (χ^2=13.27, df=2, P=0.001), *M. hyopneumoniae* (χ^2=6.67, df=2, P=0.036), *E. rhusiopathiae* (χ^2=6.14, df=2, P=0.046), and *T. gondii* (χ^2=29.57, df=2, P<0.001). The antibody prevalence increased with individual's age for PPV and *T. gondii* but, in *E. rhusiopathiae*, it was higher in adults only when compared to juveniles. In SIV the highest antibody prevalence was observed in juveniles whereas in *M. hyopneumoniae* yearlings displayed the highest risk of exposure. In contrast, animal age class was not associated with PCV-2 antibody prevalence.

The region of sampling was significantly associated with the prevalence of antibodies against PPV (P<0.001) and *M. hyopneumoniae* (P<0.001) only. The antibody prevalence of PPV was higher in Södermanland (Fig. 1) whereas the prevalence of antibodies against *M. hyopneumoniae* was highest in the County of Blekinge.

DISCUSSION

This is the first expanded serologic survey of Swedish wild boar populations for antibodies against pathogens also found in domestic pigs. The knowledge about pathogens circulating among wildlife, such as the wild boar, is of major importance not only for management of the wildlife species themselves but is also important knowledge for domestic species. We must know the reservoir species of the pathogens so that certain measures preventing transmission of the disease to domestic animals can be applied. In addition, as some pathogens also have zoonotic potential, the risk of transmission to humans should not be neglected (Ruiz-Fons 2017). We did not detect antibodies against PRRSV, *B. suis*, or *M. bovis* in the sera of tested wild boars, which was expected because Sweden is reported as free from these pathogens in domestic animals (SVA 2015). On the contrary, our study showed that wild boars in Sweden are seropositive to PPV, PCV-2, *E. rhusiopathiae*, and *M. hyopneumoniae*, highlighting the potential of the wild boar as a reservoir of different pathogens that can affect domestic pigs and, in some cases, be transmitted to humans.

The seroprevalence of pathogens detected in this study are within the same range (SIV, *E. rhusiopathiae*, and *M. hyopneumoniae*) or among the highest ever reported (PPV and PCV-2) in wild boar populations in Europe (Table 1). Previous studies have shown that intensive management of wild boar populations, such as fencing and feeding, can have an effect on the prevalence of pathogens (Vicente et al. 2004; Ruiz-Fons et al. 2006; Hälli et al. 2012). Even though the wild boar in Sweden are free-ranging, the population densities are considerably high and comparable to what can be seen in fenced hunting areas as found in Spain (Acevedo et al. 2007). Supplementary feeding, which is commonly practiced in Sweden including in all of the regions that

TABLE 3. Serologic test result (percent positive with 95% confidence interval) of female juvenile, yearling, and adult and wild boar (*Sus scrofa*) of unknown age in three counties in Sweden, for antibodies against pathogens: porcine parvovirus (PPV), porcine cirvovirus type 2 (PCV-2), porcine respiratory and reproductive syndrome virus (PRRSV), swine influenza virus (SIV), *Erisypelothrix rhusiopathiae*, *Brucella suis*, *Mycoplasma hyopneumoniae*, *Mycobacterium bovis*, and *Toxoplasma gondii*.[a]

County	Age class	Percent positive (95% confidence interval)								
		PPV	PCV2	PRRSV	SIV	*E. rhusiopathiae*	*B. suis*	*M. hyopneumoniae*	*M. bovis*	*T. gondii*
Blekinge	Juveniles	31 (16–48)	94 (81–99)	—	14 (5–30)	22 (10–39)	—	31 (16–48)	—	15 (5–32)
	Yearlings	88 (74–96)	98 (87–100)	0	0	17 (7–32)	0	56 (40–72)	0	42 (26–58)
	Adults	95 (74–100)	100 (82–100)	0	5 (0–26)	21 (6–46)	0	42 (20–67)	0	74 (49–91)
	Unknown	100 (54–100)	100 (54–100)	0	0	17 (0–64)	—	50 (12–88)	0	17 (0–64)
	Total	70 (60–78)	97 (92–99)	0	6 (2–12)	20 (12–29)	0	44 (34–54)	0	37 (28–48)
Södermanland	Juveniles	79 (60–92)	100 (88–100)	0	10 (2–27)	31 (15–51)	—	24 (10–44)	0	10 (2–27)
	Yearlings	96 (82–100)	100 (88–100)	0	0	25 (11–45)	0	14 (4–32)	0	18 (6–37)
	Adults	100 (82–100)	100 (8–100)	0	0	0	0	16 (3–40)	0	47 (24–71)
	Unknown	97 (85–100)	100 (90–100)	0	0	15 (5–31)	—	24 (11–41)	0	29 (15–48)
	Total	93 (86–97)	100 (97–100)	0	3 (1–8)	19 (12–28)	0	20 (13–29)	0	25 (17–34)
Uppland	Juveniles	29 (8–58)	100 (77–100)	0	7 (0–34)	7 (0–34)	—	7 (0–34)	0	14 (2–43)
	Adults	100 (90–100)	100 (90–100)	0	3 (0–15)	6 (1–19)	0	0	0	45 (26–64)
	Unknown	20 (0–72)	100 (48–100)	0	0	0	—	20 (0–72)	0	0
	Total	68 (56–78)	100 (95–100)	0	3 (0–9)	8 (3–17)	0	5 (2–13)	0	22 (13–34)
All locations	Juveniles	48 (37–60)	98 (91–100)	0	11 (5–21)	23 (14–34)	—	24 (15–35)	0	13 (7–23)
	Yearlings	82 (72–89)	99 (94–100)	0	0	19 (12–29)	0	33 (23–43)	0	25 (16–35)
	Adults	99 (93–100)	100 (95–100)	0	3 (0–10)	8 (3–17)	0	15 (8–25)	0	54 (41–66)
	Unknown	89 (76–96)	100 (92–100)	0	0	13 (5–27)	—	27 (15–42)	0	24 (13–40)
Total	All	78 (73–83)	99 (97–100)	0	4 (2–7)	18 (13–23)	0	25 (20-30)	0	29 (23-34)

[a] — = no samples.

we sampled, likely contributes to these high densities. High densities of wild boars are desirable for hunters for many reasons (e.g., economic, recreational, and access to game meat). But high densities of animals may contribute to the spread and maintenance of circulation of certain pathogens. The very-high seroprevalence of PPV and PCV-2 among the wild boar populations we studied could be a density-dependent consequence. In addition, feeding also results in aggregations of animals at the feeding sites, leading to increased contact between individuals and groups of animals, thus possibly increasing the rate of pathogen transmission. High wild boar densities could pose a risk of enhancing the spread of emerging diseases such as African swine fever virus if they were to enter Sweden from other countries (European Commission 2017).

The age of wild boars is associated with the prevalence of antibodies against Aujeszky's disease virus, PPV, PCV-2, and *T. gondii* (Ruiz-Fons et al. 2006). The same results (prevalence increasing with age) were found for PPV and *T. gondii* in our study, suggesting that exposure to these infections increases with age. The high prevalence of PCV-2 found in our study perhaps precluded finding statistically significant differences in prevalence between age classes.

In our study, only female wild boars were tested due to the aims of the main project of studying female reproduction (Malmsten and Dalin 2016; Malmsten et al. 2017a, b). This precludes analysis of results between sexes. However, previous studies found no sex differences in the prevalence of PCV-2 (Vicente et al. 2004), PPV (Roić et al. 2012), *Brucella* sp., PPV, PRRSV (Montagnaro et al. 2010), PCV-2 and PRRSV (Boadella 2011b), and PRRS, PCV-2, PPV, *M. hyopneumoniae*, *E. rhusiopathiae*, and *T. gondii* (Closa-Sebastià et al. 2011). Further studies including both sexes are necessary to confirm if this holds true in Sweden as well.

Currently, there is a gap of knowledge concerning the direct effect of the pathogens we detected in our study on wild boars, both at individual and population levels. Studies of clinical manifestation of diseases in free-ranging wild boars are few. However, clinical cases of PPV (Zhang et al. 2010), PCV-2, *T. gondii*, and acute septicemic erysipelas in farmed wild boar have been demonstrated (Yamamoto et al. 1999; Risco et al. 2011). In Sweden, sporadic cases of PCV-2 and PPV have been diagnosed at the National Wildlife Disease Surveillance Program at the SVA in Sweden (C. Bröjer pers. comm.). These findings show that wild boars are susceptible for the pathogens and can develop severe disease which, if widely spread in a population, may affect population dynamics and health. However, more studies on the direct effect of these pathogens on wild boars are needed.

The high seroprevalence of antibodies in present study, especially against PCV-2 and PPV but also against *E. rhusiopathiae*, *M. hyopneumoniae*, and *T. gondii* in the wild boar, implies that the wild boar could be a potential source of these pathogens for domestic pigs. The majority of commercial pig farms in Sweden use vaccination programs as the main preventative measure against PCV-2, PPV, and *E. rhusiopathiae* (Gård & Djurhälsan 2017). Moreover, biosecurity at the commercial farms may prevent direct contact between wild boars and commercial pigs. According to regulations for Swedish organic farming, the pigs must have access to outdoor living at least 4 mo/yr (KRAV 2017). This may expose them to a high risk of contact with wild boars that may be carriers of pathogens (Boqvist et al. 2012; Gavier-Widén et al. 2015). Further studies regarding transmission of the studied pathogens between wild boar and domestic pigs in Sweden are needed to fully understand the role of wild boar as a reservoir of pathogens. Such an approach should firstly include identification of areas in which transmission at the wild boar-domestic pig interface is most probable (e.g., organic pig farms, farms with no vaccination programs). Thereafter, improving biosecurity protocols for the domestic pigs and developing and evaluating different strategies to control the prevalence of shared pathogens in the wild boar (including population control,

testing and culling, and vaccination strategies) should be pursued.

Our findings of pathogens of zoonotic concerns, such as *E. rhusiopathiae* and *T. gondii*, among wild boars may suggest that wild boar could be a possible route of these pathogens to humans. In humans, *E. rhusiopathiae* is considered as an occupational disease, as most cases of the disease occur by infection of the bacteria via scratches or puncture wounds in the skin of people working in close contact with infected animals or their products (Opriessnig and Wood 2012). *Toxoplasma gondii* may be transmitted by infected food or water contaminated with sporulated *T. gondii* oocysts or by consuming meat containing tissue cysts. Prenatal infection may occur if the mother is infected during pregnancy (Lindsay et al. 2012). Thus, our results stress the importance of proper hand hygiene and the use of gloves when handling meat and offal from wild boar. Washing hands and tools that come in contact with uncooked meat with water and soap is effective to prevent infection by *T. gondii*. In addition, *T. gondii* organisms in meat can be killed by exposure to extreme heat (greater than 67 C) or cold (less than 13 C; Hill and Dubey 2002). Yet, the association of wild boar and human infection of these pathogens in Sweden is still to be investigated.

ACKNOWLEDGMENTS

Our work was supported by the Swedish Environmental Protection Agency (grants 13/257 and 14/86) and the Swedish Association for Hunting and Wildlife Management (grant 5950/ 2014). We thank all of the wildlife managers and hunters who assisted in data collection. Thanks to Linda Höglund for help with Figure 1 and to Bertil Malmsten for sampling. We are most grateful to the staff of the Instituto de Investigación en Recursos Cinegéticos for their assistance and support during the laboratory work. F.R.F. acknowledges funding from the Spanish Ministry of the Economy, Industry and Competitiveness through the "Ramón y Cajal" contract.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/ 2017-05-120.

LITERATURE CITED

Acevedo P, Vicente J, Höfle U, Cassinello J, Ruiz-Fons F, Gortazar C. 2007. Estimation of European wild boar relative abundance and aggregation: A novel method in epidemiological risk assessment. *Epidemiol Infect* 135:519–527.

Barasona JA, Acevedo P, Diez-Delgado I, Queiros J, Carrasco-García R, Gortazar C, Vicente J. 2016. Tuberculosis-associated death among adult wild boars, Spain, 2009–2014. *Emerg Infect Dis* 22:2178–2180.

Boadella M, Lyashchenko K, Greenwald R, Esfandiari J, Jaroso R, Carta T, Garrido JM, Vicente J, De La Fuente J, Gortázar C. 2011a. Serologic tests for detecting antibodies against *Mycobacterium bovis* and *Mycobacterium avium* subspecies paratuberculosis in Eurasian wild boar (*Sus scrofa scrofa*). *J Vet Diagn Invest* 23:77–83.

Boadella M, Ruiz-Fons JF, Vicente J, Martín M, Segalés J, Gortazar C. 2011b. Seroprevalence evolution of selected pathogens in Iberian wild boar. *Transbound Emerg Dis* 59:395–404.

Boqvist S, Eliasson-Selling L, Bergström K, Magnusson U. 2012. The association between rainfall and seropositivity to *Leptospira* in outdoor reared pigs. *Vet J* 19:135–139.

Closa-Sebastiá F, Casas-Díaz E, Cuenca R, Lavín S, Mentaberre G, Marco I. 2011. Antibodies to selected pathogens in wild boar (*Sus scrofa*) from Catalonia (NE Spain). *Eur J Wildl Res* 57:977–981.

Cvetnic Z, Mitak M, Ocepek M, Lojkic M, Terzic S, Jemersic L, Humski A, Habrun B, Sostaric B, Brstilo M, et al. 2003. Wild boars (*Sus scrofa*) as reservoirs of *Brucella suis* biovar 2 in Croatia. *Acta Vet Hung* 51: 465–473.

European Commission. 2017. *African swine fever–Latest developments*. http://ec.europa.eu/food/animals/animal-diseases/control-measures/asf/what-is-asf_en. Accessed April 2017.

Gavier-Widén D, Gortázar C, Ståhl C, Neimanis AS, Rossi S, Hård av Segerstad C, Kuiken T. 2015. African swine fever in wild boar in Europé: A notable challenge. *Vet Rec* 176:199–200.

Gethöffer F, Sodeikat G, Pohlmeyer K. 2007. Reproductive parameters of wild boar (*Sus scrofa*) in three different parts of Germany. *Eur J Wild Res* 53:287–297.

Gonzáles-Barrio D, Martín-Hernando MP, Ruiz-Fons F. 2015. Shedding patterns of endemic Eurasian wild boar (*Sus scrofa*) pathogens. *Res Vet Sci* 2:206–211.

Gortázar C, Ferroglio E, Höfle U, Frölich K, Vicente J. 2007. Diseases shared between wildlife and livestock: A European perspective. *Eur J Wildl Res* 53:241–256.

Gård & Djurhälsan. 2017. *Stalltips: Vaccinationsprogram*. http://www.gardochdjurhalsan.se/sv/gris/kunskapsbank/stalltips/. Accessed February 2018.

Hill D, Dubey JP. 2002. *Toxoplasma gondii*: Transmission, diagnosis and prevention. *Clin Microbiol Infect* 8:634–640.

Hälli O, Ala-Kurikka E, Nokireki T, Skrzypczak T, Raunio-Saarnisto M, Peltoniemi OAT, Heinonen M.

2012. Prevalence of and risk factors associated with viral and bacterial pathogens in farmed European wild boar. *Vet J* 194:98–101.

KRAV. 2017. *Grisar*. http://www.krav.se/regel/kravs-regler-2017/54-grisar. Accessed April 2017.

Lindsay DS, Dubey JP, Santín-Durán M, Fayer R. Coccidia and other protozoa. 2012. In: *Diseases of swine*, 10th Ed., Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, editors. John Wiley, West Sussex, UK, pp. 895–907.

Malmsten A, Dalin AM. 2016. Puberty in female wild boar (*Sus scrofa*) in Sweden. *Acta Vet Scand* 58:55.

Malmsten A, Jansson G, Dalin AM. 2017a. Postmortem examination of the reproductive organs of female wild boars (*Sus scrofa*) in Sweden. *Reprod Domest Anim* 52:570–578.

Malmsten A, Jansson G, Lundeheim N, Dalin A-M. 2017b. The reproductive pattern and potential of free ranging female wild boar (*Sus scrofa*) in Sweden. *Acta Vet Scand* 59:52.

Matschke GH. 1967. Aging European wild hogs by dentition. *J Wildl Manage* 31:109–113.

Meng XJ, Lindsay DS, Sriranganathan N. 2009. Wild boar as sources for infectious diseases in livestock and humans. *Philos Trans R Soc Lond B Biol Sci* 364: 2697–2707.

Montagnaro S, Sasso S, De Martino L, Longo M, Iovane V, Ghiurmino G, Pisanelli G, Nava D, Baldi L, Pagnini U. 2010. Prevalence of antibodies to selected viral and bacterial pathogens in wild boar (*Sus scrofa*) in Campania Region, Italy. *J Wildl Dis* 46:316–319.

Muñoz PM, Boadella M, Arnal M, de Miguel MJ, Revilla M, Martínez D, Vicente J, Acevedo P, Oleaga Á, Ruiz-Fons F, et al. 2010. Spatial distribution and risk factors of brucellosis in Iberian wild ungulates. *BMC Infect Dis* 10:46.

Naturvårdsverket. 2010. *Nationell förvaltningsplan för vildsvin (Sus scrofa)—Vägledning för regionala och lokala förvaltningsplaner*. https://www.naturvardsverket.se/upload/miljoarbete-i-samhallet/miljoarbete-i-sverige/vilt/planera-viltforvaltning/forvaltningsplan-vildsvin-2013.pdf. Accessed October 2017.

Opriessnig T, Wood, RL. 2012. Erysipelas. In: *Diseases of swine*, 10th Ed., Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, editors. John Wiley, West Sussex, UK, pp. 750–759

Račka K, Bártová E, Budíková M, Vodrážka P. 2015. Survey of *Toxoplasma gondii* antibodies in meat juice of wild boar (*Sus scrofa*) in several districts of the Czech Republic. *Annals Agri Environ Med* 22:231–235.

R Core Team. 2013. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Risco D, Llario PF, Velarde R, García WL, Benítez JM, García A, Bermejo F, Cortés M, Rey J, de Mendoza JH, et al. 2011. Outbreak of swine erysipelas in a semi-intensive wild boar farm in Spain. *Transbound Emerg Dis* 58:445–450.

Roić B, Jemersic L, Terzic S, Keros T, Balatinec J, Florijancic T. 2012. Prevalence of antibodies to selected viral pathogens in wild boars (*Sus scrofa*) in Croatia in 2005–06 and 2009–10. *J Wildl Dis* 48: 131–137.

Ruiz-Fons F. 2017. A review of the current status of relevant zoonotic pathogens in wild swine (*Sus scrofa*) populations: Changes modulating the risk of transmission to humans. *Transbound Emerg Dis* 64:68–88.

Ruiz-Fons F, Vicente J, Vidal D, Höfle U, Villanúa D, Gauss C, Segalés J, Almería S, Montoro V, Gortázar C. 2006. Seroprevalence of six reproductive pathogens in European wild boar (*Sus scrofa*) from Spain: The effect on wild boar female reproductive performance. *Theriogenology* 65:731–743.

Sedlak K, Bartova E, Machova J. 2008. Antibodies to selected viral disease agents in wild boars from the Czech Republic. *J Wildl Dis* 44:777–780.

Sergeant ESG. 2017. Epitools epidemiological calculators. http://epitools.ausvet.com.au. Accessed October 2017.

SVA (Swedish National Veterinary Institute). 2015. *Surveillance of infectious diseases in animals and humans in Sweden 2015. SVA: Rapportserie 34 ISSN 1654-7098*. National Veterinary Institute, Uppsala, Sweden, 132 pp.

Svenska Jägareförbundet. 2014. *Hur mycket vilt har vi egentligen?* http://svenskjakt.se/Start/Nyheter/2014/08/hur-mycket-vilt-har-vi-egentligen/. Accessed July 2016.

Touloudi A, Valiakos G, Athanasiou LV, Birtsas P, Giannakopoulos A, Papaspyropoulos K, Kalaitzis C, Sokos C, Tsokana CN, Spyrou V, et al. 2015. A serosurvey for selected pathogens in Greek European wild boar. *Vet Rec Open* 2:e000077.

Vengust G, Valencak Z, Bidovec A. 2006. A serological survey of selected pathogens in wild boar in Slovenia. *J Vet Med* 53:24–27.

Vicente J, León-Vizcaíno L, Gortázar C, Cubero MJ, Gonzáles M, Martín-Atance P. 2002. Antibodies to selected viral and bacterial pathogens in European wild boars from southcentral Spain. *J Wildl Dis* 38: 649–652.

Vicente J, Segalés J, Höfle U, Balasch M, Plana-Durán J, Domingo M, Gortázar C. 2004. Epidemiological study on porcine circovirus type 2 (PCV) infection in the European wild boar (*Sus scrofa*). *Vet Res* 35: 243–253.

Yamamoto K, Kijima M, Takahashi T, Yoshimura H, Tani O, Kojyou T, Yamawaki Y, Tanimoto T. 1999. Serovar, pathogenicity and antimicrobial susceptibility of *Erysipelothrix rhusiopathiae* isolates from farmed wild boars (*Sus scrofa*) affected with septicemic erysipelas in Japan. *Res Vet Sci* 67:301–303.

Zhang CF, Song CP, Chen CM, Cui SJ, Miao LF. 2010. Reproductive failure in wild boars associated to porcine parvovirus infection and in vivo and in vitro characterization of the causal isolate. *Trop Anim Health Prod* 42:1611–1613.

Submitted for publication 29 May 2017.
Accepted 12 October 2017.