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Antibodies to selected pathogens in wild boar (*Sus scrofa*) from Catalonia (NE Spain)

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Abstract From 2004 to 2007, blood samples from 273 healthy wild boars (*Sus scrofa*), culled during the hunting season, were obtained in three areas of Catalonia (NE Spain): Pyrenees, Sant Llorenç del Munt i l'Obac Natural Park (SLM), and Ports de Tortosa i Beseit National Hunting Reserve (PTB). We investigated the presence of antibodies against classical swine fever virus (CSFV), African swine fever virus (ASFV), porcine vesicular disease virus (PVDV), porcine respiratory and reproductive syndrome virus (PRRSV), Aujeszky's disease virus (ADV), porcine influenza A virus (PIV), porcine circovirus type 2 (PCV2), porcine parvovirus (PPV), *Mycoplasma hyopneumoniae*, *Erysipelothrix rhusiopathiae*, *Salmonella* spp., and *Toxoplasma gondii*. Four wild boars were suspicious for CSFV, but the infection was discarded with a virus neutralization test, and infection with a border disease virus was confirmed. Negative results were obtained against ASFV and PVDV. Antibodies were detected against PRRSV (3%), ADV (0.8%), PIV (6.4%), PCV2 (64.6%), PPV (54.7%), *M. hyopneumoniae* (26.6%), *E. rhusiopathiae* (5.3%), *Salmonella* spp. (11.3%), and *T. gondii* (43.5%). In SLM, we detected a higher seroprevalence for PIV and *M. hyopneumoniae* and a lower seroprevalence for *E. rhusiopathiae* than in the other two areas. In PTB, seroprevalence was higher for PPV, *Salmonella* spp., and PCV2. Adult wild boar displayed higher seroprevalence for PPV, PIV, and *M. hyopneumoniae*, whereas presence of antibodies for

Salmonella spp. was higher in juveniles compared with adults and piglets.

Keywords Seroprevalence · Spain · *Sus scrofa* · Wild boar

Introduction

Wild boar (*Sus scrofa*) is widely distributed in Europe, and it is expanding in most of the countries, including Spain (Acevedo et al. 2006). This increase may affect the circulation of pathogens between this species, domestic pigs, and humans (Artois et al. 2002; Acevedo et al. 2006). During the last years, the occurrence of wild boar disease agents in various parts of Spain has been documented (Vicente et al. 2002; Ruiz-Fons et al. 2006; Muñoz et al. 2010; Closa-Sebastià et al. 2010). However, there is little information on the presence of other important infectious diseases among wild boars in Catalonia (NE Spain). This region concentrates an important part of the Spanish pig industry and has very high densities of wild boar, which is one of the most important big game species in this area (the harvest during the 2008–2009 hunting season was approximately 29,000 animals). The objective of this study is to investigate the presence of antibodies against several pathogens in wild boar from Catalonia.

Materials and methods

From 2004 to 2007, we collected blood samples from 273 healthy wild boar hunted in three different study areas of Catalonia (NE Spain): Pyrenees (PYR; 42° 26'–42° 22' N; 1° 27'–2° 26' E), Sant Llorenç del Munt i l'Obac Natural Park (SLM; 41° 39'–41° 42' N; 1° 53'–2° 09' E), and Ports

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de Tortosa i Beseit National Hunting Reserve (PTB; 40° 43′–40° 51′ N; 0° 15′–0° 25′ E). Sex was determined (126 males and 147 females), as well as age class (47 piglets from 0 to 6 months, 49 juveniles from 6 months to 1 year, and 177 adults over 1 year). Blood was collected from the heart, centrifuged at 1,200×g for 15 min, serum removed, and stored at –20°C.

Serum samples were tested for antibodies against African swine fever virus (ASFV), classical swine fever virus (CSFV), porcine vesicular disease virus (PVDV), porcine respiratory and reproductive syndrome virus (PRRSV), Aujeszky's disease virus (ADV), porcine influenza A virus (PIV), porcine parvovirus (PPV), *Mycoplasma hyopneumoniae*, *Erysipelothrix rhusiopathiae*, and *Salmonella* spp. by using commercial enzyme-linked immunoassay (ELISA) tests. Antibodies against porcine circovirus type 2 (PCV2) were determined by means of an immunoperoxidase monolayer assay (Vicente et al. 2004), and *Toxoplasma gondii* antibodies were detected with a modified agglutination test (Dubey and Desmonts 1987).

In four CSFV-seropositive wild boars, a comparative virus neutralization test (VNT) for neutralizing antibodies against CSFV Alfort, bovine viral diarrhoea virus 1 NADL, and border disease virus (BDV) strain 137/4 was performed. VNT was carried out under procedure described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE 2004). Titres of 1:10 and higher were considered as positive. Viral replication was monitored by the immunoperoxidase monolayer assay with home-made polyclonal pestivirus-specific serum.

Variables related to study areas, sex, and age were subjected to multivariate analysis with a logistic regression procedure. Wald's test was used, and significance was assumed for $p < 0.05$.

Results

Serologic results are presented in Tables 1 and 2. Antibodies were detected against PCV2 (64.6%), PPV (56.3%), *T. gondii* (43.5%), *M. hyopneumoniae* (26.1%), *Salmonella* spp. (11.3%), PIV (6.4%), *E. rhusiopathiae* (5.3%), PRRSV (3%), and ADV (0.8%). No antibodies were detected against ASFV, PVDV, and CSFV (four wild boars were detected as CSFV positive, but a cross reaction of BDV antibodies was confirmed).

In SLM, we detected a higher seroprevalence for PIV and *M. hyopneumoniae* and a lower seroprevalence for *E. rhusiopathiae* than in the other two study areas. In PTB, seroprevalence was higher for PPV, *Salmonella* spp., and PCV2. Adult wild boar displayed higher seroprevalences of PPV, PIV, and *M. hyopneumoniae*, whereas it was higher in juveniles for *Salmonella* spp. when compared with adults and piglets. No differences between sexes were observed.

Discussion

Animal health surveillance is limited in wildlife but applied routinely in domestic animals. Wild boar can

Table 1 Seroprevalence (seropositive/total analyzed) against several pathogens in wild boar in the three different areas from Catalonia (NE Spain)

Pathogen	PYR	SLM	PTB	Total
CSFV	0.0% (0/74)	0.0% (0/97)	0.0% (0/59)	0.0% (0/230) ^a
ASFV	0.0% (0/74)	0.0% (0/92)	0.0% (0/55)	0.0% (0/221)
PVDV	0.0% (0/15)	0.0% (0/97)	0.0% (0/59)	0.0% (0/171)
PRRSV	3.9% (3/76)	2.7% (3/111)	2.6% (2/78)	3.0% (8/265)
ADV	0.0% (0/76)	0.0% (0/100)	2.6% (2/78)	0.8% (2/254)
PIV	2.6% b (2/76)	10.6% a (12/113)	3.8% b (3/78)	6.4% (17/267)
PCV2	36.4% a (28/77)	69.8% b (81/116)	84.6% b (66/78)	64.6% (175/271)
PPV	42.1% a (32/76)	58.4% b (66/113)	61.5% b (48/78)	54.7% (146/267)
<i>M. hyopneumoniae</i>	19.7% b (15/76)	46.0% a (52/113)	5.1% b (4/78)	26.6% (71/267)
<i>E. rhusiopathiae</i>	6.6% b (5/76)	0.9% a (1/109)	10.3% b (8/78)	5.3% (14/263)
<i>Salmonella</i> spp.	6.6% b (5/76)	7.2% b (8/111)	21.8% a (17/78)	11.3% (30/265)
<i>T. gondii</i>	45.3% (34/75)	42.2% (46/109)	43.6% (34/78)	43.5% (114/262)

Different letters indicate statistical ($p < 0.05$) differences

PYR Pyrenees, SLM Sant Llorenç del Munt i l'Obac Natural Park, PTB Ports de Tortosa i Beseit National Hunting Reserve, CSFV classical swine fever virus, ASFV African swine fever virus, PVDV porcine vesicular disease virus, PRRSV porcine respiratory and reproductive syndrome virus, ADV Aujeszky's disease virus, PIV porcine influenza A virus, PCV2 porcine circovirus type 2, PPV porcine parvovirus, *M. hyopneumoniae* *Mycoplasma hyopneumoniae*, *E. rhusiopathiae* *Erysipelothrix rhusiopathiae*, *T. gondii* *Toxoplasma gondii*

^a Four wild boars in PYR finally confirmed as positive for border disease virus by VNT

Table 2 Seroprevalence of antibodies against several pathogens according to three age classes: piglets (0–6 months), juveniles (6–12 months), and adults (more than 12 months)

Pathogen	Piglets	Juveniles	Adults	Total
CSFV	0.0% (0/44)	0.0% (0/41)	0.0% (0/145)	0.0% (0/230) ^a
ASFV	0.0% (0/42)	0.0% (0/41)	0.0% (0/138)	0.0% (0/221)
PVDV	0.0% (0/21)	0.0% (0/34)	0.0% (0/116)	0.0% (0/171)
PRRSV	4.3% (2/46)	4.2% (2/48)	2.3% (4/171)	3.0% (8/265)
ADV	2.2% (1/46)	0.0% (0/44)	0.6% (1/164)	0.8% (2/254)
PIV	0.0% b (0/47)	0.0% b (0/48)	9.9% a (17/172)	6.4% (17/267)
PCV2	27.7% (13/47)	58.3% (28/48)	76.1% (134/176)	64.2% (174/271)
PPV	17.0% a (8/47)	35.4% b (17/48)	70.3% c (121/172)	54.7% (146/267)
<i>M. hyopneumoniae</i>	8.5% a (4/47)	22.9% b (11/48)	32.6% b (56/172)	26.6% (71/267)
<i>E. rhusiopathiae</i>	2.1% (1/47)	6.3% (3/48)	6.0% (10/168)	5.3% (14/263)
<i>Salmonella</i> spp.	4.3% b (2/47)	20.8% a (10/48)	10.6% b (18/170)	11.3% (30/265)
<i>T. gondii</i>	48.9% (23/47)	34.0% (16/47)	44.6% (75/168)	43.5% (114/262)

Different letters indicate statistical ($p < 0.05$) differences

CSFV classical swine fever virus, ASFV African swine fever virus, PVDV porcine vesicular disease virus, PRRSV porcine respiratory and reproductive syndrome virus, ADV Aujeszky's disease virus, PIV porcine influenza A virus, PCV2 porcine circovirus type 2, PPV porcine parvovirus, *M. hyopneumoniae* *Mycoplasma hyopneumoniae*, *E. rhusiopathiae* *Erysipelothrix rhusiopathiae*, *T. gondii* *Toxoplasma gondii*

^a Two piglet and two adult wild boars finally confirmed as positive for border disease virus by VNT

serve as a potential source of infectious diseases for domestic animals, and this study contributes to know the sanitary status of an area of Spain where wild boar and domestic pig share habitat.

The four wild boars detected initially as positive to antibodies against CSFV but confirmed ultimately as positive for BDV were from an area in the Pyrenees where epidemics associated to a BDV have been reported in Pyrenean chamois (*Rupicapra pyrenaica*; Marco et al. 2007) and where a high seroprevalence of border disease has also been reported in sheep (Alba et al. 2008). Experimental studies in pigs infected with a BDV isolated from Pyrenean chamois confirmed this seroconversion and the diagnostic challenge due to the cross-reactivity of the BDV antibodies for CSFV ELISA-based tests (Cabezón et al. 2010).

The low prevalence of ADV antibodies found in the wild boars of our study suggests that this pathogen may not circulate in Catalonia. In this area, an eradication program was adapted and reinforced in 2003, leading to the almost eradication of the disease (only one positive farm in 2009). In other areas of Central and Southern Spain, seroprevalence in wild boar has been reported to be as high as 44% (Vicente et al. 2002; Ruiz-Fons et al. 2008) and outbreaks of the disease have also been described (Gortázar et al. 2002).

Antibody titres to PIV found in the wild boars of our study were low and similar to other reports in Spain (Vicente et al. 2002). Porcine influenza in wild boars is mainly density dependent in such a way that circulating rates of virus in

free-ranging populations are usually low when compared to semi-captive or farmed dense populations (Ruiz-Fons et al. 2008). PCV2 seroprevalence was higher than that described previously in Hungary (20.5%; Cságola et al. 2006), Slovenia (25%; Toplak et al. 2004), Spain (29.7–40%; Vicente et al. 2002; Ruiz-Fons et al. 2006), the Czech Republic (42.5%; Sedlak et al. 2008), Belgium (35.6%; Sanchez et al. 2001), and Germany (63.1%; Reiner et al. 2010). Antibodies to PRRSV were higher than those previously described in free-ranging wild boars (1.3–1.7%) (Ruiz-Fons et al. 2008). PPV seroprevalence was also high but similar to that previously described in other areas (Vicente et al. 2002; Roic et al. 2005; Ruiz-Fons et al. 2006; Vengust et al. 2006). The high infection rate in domestic pig herds, especially with PCV2 and PPV, suggests that they could be the source of infection for the wild boars (Ruiz-Fons et al. 2008). In addition, the low seroprevalence found in PYR against PPV and PCV2 supports this hypothesis because there are few domestic pig farms in this high mountain area, when compared to both the other study areas where this density is higher. But, recent investigations with non-serological studies show that wild boar is a host of pathogens like PCV2, therefore it could act as reservoir for these infections (Morandi et al. 2010, Reiner et al. 2010).

Antibodies against *M. hyopneumoniae* in the wild boar of our study were higher than those previously published in Spain (Vicente et al. 2002; Sibila et al. 2010; 0% and 21.5%, respectively), but lower to the prevalence observed in France (58%; Marois et al. 2006). Pig farms, which usually display high prevalence of this pathogen, could be a

reservoir for the wild boar. However, the wild boar, in which subclinical infection has been described, could also represent a potential threat for *M. hyopneumoniae* free farms (Sibila et al. 2010). Therefore, further research would be needed in the high seroprevalence areas to assess whether it has an impact on the wild boar populations. Seroprevalence found against *E. rhusiopathiae* and *Salmonella* spp. are similar to those previously described in other studies (Vicente et al. 2002, Vengust et al. 2006, Montagnaro et al. 2010). In PTB, a high prevalence of *Salmonella* spp. isolates from fecal samples was reported in the wild boar and associated with transmission from cattle (Mentaberre et al. 2009).

We found a high seroprevalence of *T. gondii* compared to other reports in wild boar in the same and in other areas of Spain (Gauss et al. 2005; Ruiz-Fons et al. 2006). However, it is similar to that described in other species in our study area, such as the red deer (*Cervus elaphus*; 44%; Gauss et al. 2006) and urban cats in the Barcelona area (45%; Gauss et al. 2003). This high seroprevalence of *T. gondii* among wild boars deserves further investigation and suggests that wild boars used for human consumption could represent a public health risk for persons handling or consuming raw or undercooked infected meat.

Sex and age are factors that can affect seroprevalence of several diseases in wild boar. Roic et al. (2005) found that adult wild boar had a higher seroprevalence to PPV (70%) than juveniles (31%), as observed in our study, together with PIV. Similar differences were found for *M. hyopneumoniae*, but adults and juveniles had higher seroprevalence. However, juveniles displayed higher seroprevalence of *Salmonella* sp. antibodies. This is similar to what is described for the domestic pig, in which antibodies are low from the first weeks of age to 14–15 weeks and increase until week 30, when these animals usually are sent to abattoir (Creus 2007). Except for *Salmonella* spp., adult wild boars have more probability of being exposed to pathogens and thus have higher titres of antibodies.

In conclusion, the seroprevalences to several pathogens found in our study are similar to other studies, except for PCV2, *M. hyopneumoniae*, and toxoplasmosis, which were higher. Source of the first two infections for the wild boar could be related to transmission from domestic pig farms. However, further studies are needed to confirm this assumption. The high rate of *T. gondii* infection of wild boar in Catalonia has important implications for public health since wild boar meat is consumed frequently.

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