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ORIGINAL PAPER

A serological survey of selected pathogens in wild boar (*Sus scrofa*) in northern Turkey

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Abstract During the hunting season in March 2012, a total of 93 blood samples were collected from wild boars (*Sus scrofa*) shot in the area of northern Turkey (Samsun and Gumushane provinces). These blood samples were examined by enzyme immunoassay (ELISA) for the presence of antibodies to classical swine fever virus (CSFV), Aujeszky's disease virus (ADV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine respiratory coronavirus (PRCV), swine influenza virus (SIV), porcine parvovirus (PPV), swine vesicular disease virus (SVDV), hepatitis E virus (HEV), African swine fever virus (ASFV), porcine rotavirus (PRV), transmissible gastroenteritis virus (TGEV) and bovine viral diarrhoea virus (BVDV). Out of 93 serum samples examined, 65 (69.9 %) were positive for PRV, 22 (23.7 %) were positive for ADV, 5 (5.4 %) were positive for BVDV, 4 (4.3 %) were positive for PPV and 2 (2.2 %) were positive for PRRSV. All sera were negative for ASFV, SVDV, HEV, SIV, PRCV, TGEV and CSFV. The results, recorded for the first time in Turkey, supported the hypothesis that wild boar act as a potential reservoir of selected viruses and thus have a role in the epidemiology of these diseases.

Keywords ELISA · Seroprevalence · Swine viral diseases · Turkey · Wild boar

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Introduction

Wildlife diseases may represent a potential threat not only to local wildlife populations but also to domestic animals and humans. Various studies have been carried out to analyse the prevalence of pathogens in wild boar populations and the role of these populations as reservoir for pathogens or a source of infection for domestic pigs (Kaden et al. 2009). Wild boar (*Sus scrofa*) populations are found in many regions worldwide. The role of wild boar as a reservoir of some viruses, and thus a possible source of infection for domestic swine and other domestic animals, is still unclear. However, there are reports on the epidemiological association between wild boar and domestic animals in some viral diseases and movement of these animals can potentially result in dissemination of these diseases (Župančič et al. 2002). Currently, there is no recorded data regarding prevalence and distribution of the most important infectious agents among wild boars in Turkey. Therefore, our objective was to determine the seroprevalence against selected infectious pathogens in wild boars from northern Turkey.

Materials and methods

Wild boar and domestic pigs are a similar species. In this study, serum samples were collected from 93 hunter-killed wild boars that were harvested during the 2012 hunting season in northern Turkey (Samsun and Gumushane provinces) which has the suitable geographical conditions, including agricultural area and forest for wildlife (Fig. 1). Sex was determined (38 males and 55 females) and also age was determined using tooth eruption patterns, and animals were grouped into two age classes: 13 to <24 months old (31 subadults) and >24 months old (62 adults). Blood was collected from the heart, centrifuged at 1,200×*g* for 15 min,

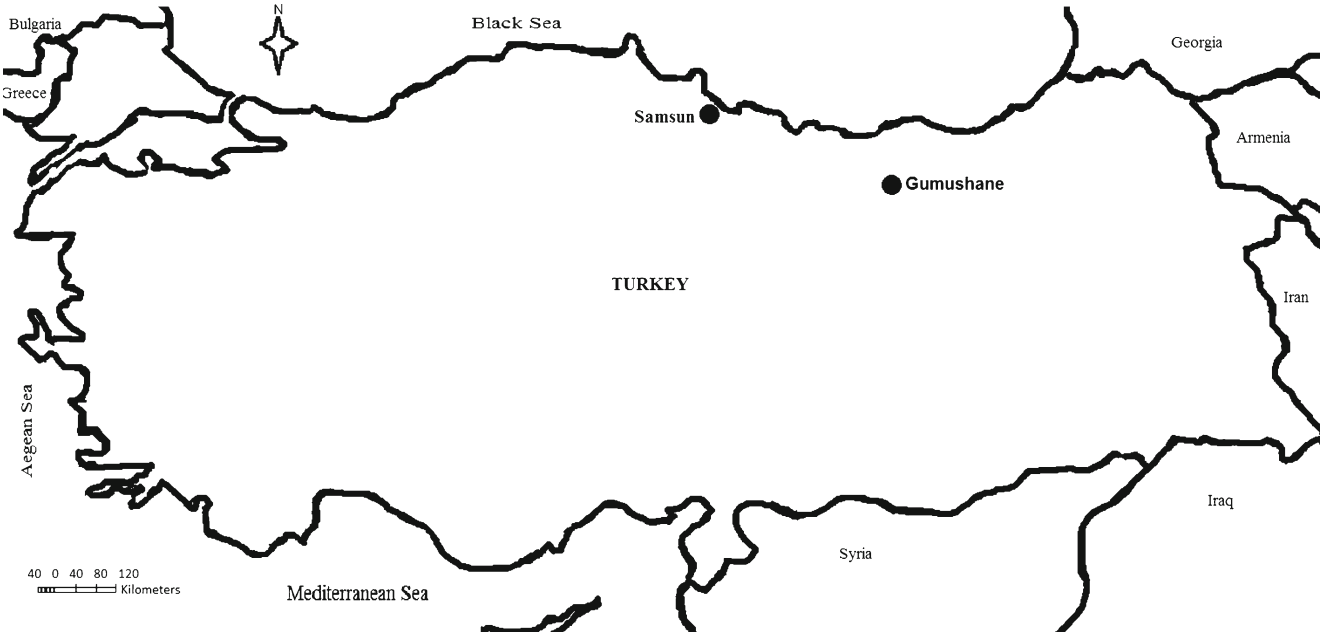

Fig. 1 Sampling areas

serum removed, and stored at −20 °C. The commercial enzyme-linked immunosorbent assay (ELISA) kits were obtained from ID.vet Innovative Diagnostics, Montpellier, France for swine vesicular disease virus (SVDV), hepatitis E virus (HEV), swine influenza virus (SIV), Aujeszky's disease virus (ADV) and African swine fever virus (ASFV), and from PRIONICS, Lelystad, Netherlands for classical swine fever virus (CSFV) and porcine parvovirus (PPV), and INGENASA, Madrid, Spain for porcine rotavirus (PRV), porcine respiratory coronavirus (PRCV) and transmissible gastroenteritis virus (TGEV), and IDEXX, Bern, Switzerland for bovine viral diarrhoea virus (BVDV) and porcine reproductive and respiratory syndrome virus (PRRSV) and the tests were performed according to the producer's description. Plates were read with an ELISA reader at 450 and 650 nm for ID. vet, PRIONICS, INGENASA and IDEXX kits, respectively. Determined OD values were calculated.

Results

Out of 93 serum samples examined, 65 (69.9 %) were positive for PRV, 22 (23.7 %) were positive for ADV, 5 (5.4 %) were positive for BVDV, 4 (4.3 %) were positive for PPV and 2 (2.2 %) were positive for PRRSV. All sera were negative for ASFV, SVDV, HEV, SIV, PRCV, TGEV and CSFV (Table 1). Most commonly, anti-agent antibodies were detected against one agent in the same animal. Out of serum samples tested, 58 animals were found to be seropositive against only one agent, 46 (49.5 %) were found to be positive for only PRV, 7 (7.5 %) were positive for only ADV, 3 (3.2 %) were positive for only PPV and 2 (2.2 %) were positive for only BVDV, while 15 animals were free of antibodies to those agents concerned. Multiple (triple, quartet and quintet agents) infections were not detected. Double infections seropositivity obtained at the end of the study varied and were as follows: 15.1 % (14/93) (ADV + PRV), 2.2 % (2/93) (PRRSV + PRV) and (BVDV + PRV), 1.1 % (1/93) (BVDV + ADV) and (PRV + PPV).

The prevalence of antibodies did not differ between male and female wild boar for any of the diseases (Fisher's exact test, $p>0.05$). In Samsun Province, we detected a higher seroprevalence for PRRSV and PRV and a lower seroprevalence for BVDV and PPV than Gumushane Province. Adult wild boar displayed higher seroprevalences of ADV, BVDV and PPV,

Table 1 Seroprevalence (seropositive/total analysed) against several pathogens in wild boar in the two different areas from northern Turkey

Pathogen	Samsun	Gumushane	Total
ADV	22.2 % (12/54)	25.6 % (10/39)	23.7 % (22/93)*
ASFV	0.0 % (0/54)	0.0 % (0/39)	0.0 % (0/93)
BVDV	3.7 % (2/54)	7.6 % (3/39)	5.4 % (5/93)
CSFV	0.0 % (0/54)	0.0 % (0/39)	0.0 % (0/93)
HEV	0.0 % (0/54)	0.0 % (0/39)	0.0 % (0/93)
PRRSV	3.7 % (2/54)	0.0 % (0/39)	2.2 % (2/93)
PRCV	0.0 % (0/54)	0.0 % (0/39)	0.0 % (0/93)
PPV	1.9 % (1/54)	7.6 % (3/39)	4.3 % (4/93)
PRV	85.2 % (46/54)	48.7 % (19/39)	69.9 % (65/93)*
SIV	0.0 % (0/54)	0.0 % (0/39)	0.0 % (0/93)
SVDV	0.0 % (0/54)	0.0 % (0/39)	0.0 % (0/93)
TGEV	0.0 % (0/54)	0.0 % (0/39)	0.0 % (0/93)

*$p<0.01$

whereas it was higher in subadults for PRRSV. The seroprevalence detected in this study for ADV is similar between Samsun and Gumushane provinces, and also for PRV it is similar between adults and subadults. No differences between sexes were observed. The use of the Chi-square test to determine significance of ADV and BVDV were more common in adults than subadults ($p<0.01$), (Table 2). The seropositivity rates for ADV and PRV were higher than other diseases ($p<0.01$) (Table 1).

Discussion

Consequent pathogen surveillance in wildlife may provide an effective epidemiological overview which allows to assess the risk of infection and of a spread of agents within the wild boar populations as well as from this wildlife species to domestic pigs and, in case of zoonotic agents, also to humans. Out of the 12 viral diseases, antibodies to five viruses were found in the investigated populations, however, with differences between the individual populations. It is not surprising that PRV is widespread in our wild boar populations (seroprevalence rate, 69.9 %), and that its seroprevalence rate differs significantly from those of the other viruses tested. Most animals tested positive for PRV were derived from Samsun (85.2 %). Although there has been no extensive report on the presence of porcine rotavirus in wild boars, Svensmark (1983) found antibodies against porcine rotavirus (64.2 %) in swine herds in Denmark.

The second highest seroprevalence rate (averaged 23.7 %) was found for ADV. Generally, the differences in the seroprevalence rates between the provinces were lower than for PRV. As the investigation areas were largely identical, the findings suggest a stable epidemiological situation. The seroprevalence rates for ADV generally were on the same level in Samsun (22.2 %) and Gumushane (25.6 %). Considerably higher percentages of seropositive wild boars were detected in other European wild boar populations, e.g. between 36 % (Vicente et al. 2002) and 60.6 % (Ruiz-Fons et al. 2006) in Spain, 26–31 % in Slovenia (Vengust et al. 2005, 2006), 54.5 % in Croatia (Župančič et al. 2002), 30.7 % in Italy (Montagnaro et al. 2010) and 30 % in the Czech Republic (Sedlak et al. 2008). In contrast, the proportion of wild boars seropositive to ADV seems to be relatively low in Catalonia part of Spain (0.8 %) (Closa-Sebastià et al. 2011) and Germany (11.6 %) (Kaden et al. 2009).

Table 2 Seroprevalence of antibodies against several pathogens according to two age classes: subadults (1–2 years) and adults (more than 2 years)

Pathogen	Subadults	Adults	Total
ADV	0.0 % (0/31)	35.5 % (22/62)*	23.7 % (22/93)
ASFV	0.0 % (0/31)	0.0 % (0/62)	0.0 % (0/93)
BVDV	0.0 % (0/31)	8.1 % (5/62)*	5.4 % (5/93)
CSFV	0.0 % (0/31)	0.0 % (0/62)	0.0 % (0/93)
HEV	0.0 % (0/31)	0.0 % (0/62)	0.0 % (0/93)
PRRSV	6.5 % (2/31)	0.0 % (0/62)	2.2 % (2/93)
PRCV	0.0 % (0/31)	0.0 % (0/62)	0.0 % (0/93)
PPV	3.2 % (1/31)	4.8 % (3/62)	4.3 % (4/93)
PRV	74.2 % (23/31)	67.7 % (42/62)	69.9 % (65/93)
SIV	0.0 % (0/31)	0.0 % (0/62)	0.0 % (0/93)
SVDV	0.0 % (0/31)	0.0 % (0/62)	0.0 % (0/93)
TGEV	0.0 % (0/31)	0.0 % (0/62)	0.0 % (0/93)

*$p<0.01$

Serologic evidence of PPV infection has been previously described in the European wild boar populations by several authors. In our study, antibodies were present in four (4.3 %) serum samples. Antibody seroprevalence was lower than that reported in Germany (ranging from 64.3 to 77 %) (Lutz and Wurm, 1996; Kaden et al. 2009), Italy (ranging from 56.7 % to 99 %; Cordioli et al. 1993; Mignone et al. 1995), Spain (ranging from 54.7 % to 56.6 %; Ruiz-Fons et al. 2006; Closa-Sebastià et al. 2011), Slovenia (49 %; Vengust et al. 2005), Italy (7.9 %; Montagnaro et al. 2010) and Croatia (41.6 %; Roic et al. 2005).

The low proportion of wild boars with antibodies against PRRSV in this study generally corresponds with previous serological investigations in Germany (3.8 %) (Kaden et al. 2009) and Catalonia part of Spain (3 %) (Closa-Sebastià et al. 2011). In contrast to our findings, wild boars were found to be free from antibodies to PRRSV in some European countries, e.g. in Spain (Vicente et al. 2002; Ruiz-Fons et al. 2006), in Croatia (Župančič et al. 2002) and in Slovenia (Vengust et al. 2006). Likewise, low seroprevalence rates were reported by Albina et al. (2000) in French wild boars. Infections with this *Arterivirus* also occur with high seroprevalence (37.7 %) in Italy (Montagnaro et al. 2010).

Although coronaviruses, especially TGEV and PRCV, are important pathogens in commercial pig farms worldwide, only little is known on the epidemiological situation in wild boars. No antibody response was detected against PRCV and TGEV in northern Turkey. In Slovenia, Vengust et al. (2006) found no antibodies against TGEV in wild boars; however, 3 % of the investigated samples were seropositive to PRCV. In the Czech Republic, seropositivity rate of TGEV in wild boars was detected as 0.7 % (Sedlak et al. 2008). In Germany, Kaden et al. (2009) detected antibodies against TGEV (1.6 %) and PRCV (7.9 %) in wild boars.

There was no evidence for exposure to SIV within this wild boar population in northern Turkey; however in Germany and Spain (Catalonia), antibodies against SIV in wild boars were detected as 7.8 and 6.4 %, respectively (Kaden et al. 2009; Closa-Sebastià et al. 2011).

Pigs and wild boars are natural reservoirs of CSFV. No antibody response was detected against CSFV in northern

Turkey. In Spain (Catalonia), Closa-Sebastià et al. (2011) and, in the Czech Republic, Sedlak et al. (2008), found no antibodies against CSFV in wild boars; however, the possibility that CSFV can persist in wild boar populations has been shown in France with 0.7 % antibody prevalence (Albina et al. 2000), in Croatia with 38.6 % antibody prevalence (Župančič et al. 2002), and also in the Netherlands, Germany and Italy (Laddomada 2000).

BVD is prevalent in cattle in Turkey (Albayrak and Ozan 2012; Albayrak et al. 2012). From our results, it is evident that wild boars in the northern Turkey are rarely exposed to this infection. Cross-reaction with CSFV can be excluded because all sera were negative for this virus. However, it is possible that antibodies relate to exposure to a related pestivirus other than BVDV (e.g. border disease virus). In Germany, the Czech Republic and Croatia, the reported prevalence of BVDV antibodies in wild boar is 0.8, 0.6 and 4.5 %, respectively (Dahle et al. 1993; Župančič et al. 2002; Sedlak et al. 2008). The seroprevalence (5.4 %) detected in this study is higher than the seroprevalence in Germany and the Czech Republic but is similar to Croatia.

No antibody response was detected against SVDV in northern Turkey. In Spain (Catalonia), Closa-Sebastià et al. (2011), in Italy, Montagnaro et al. (2010), in Slovenia, Vengust et al. (2006) and, in the Czech Republic, Sedlak et al. (2008) found no antibodies against SVDV in wild boars.

ASFV is able to infect both domestic and wild suids and can also replicate in soft ticks of the genus *Ornithodoros*. No antibody response was detected against ASFV in northern Turkey. Although, ASFV was notified in Spain, Portugal and Sardinia in the past, no ASF outbreaks have been reported in Spain since 1994, confirming the effectiveness of eradication efforts. Nevertheless, ASFV can persist for long periods of time in the environment, soft ticks and contaminated products. In particular, wild boars are a natural host for ASFV, as shown in studies in the Iberian Peninsula, Sardinia and in the currently affected areas of the Russian Federation and trans-Caucasus countries (Mur et al. 2012).

Hepatitis E is an important disease of public health concern due to its zoonotic character (Ruiz-Fons et al. 2008). There are no serologic evidence for HEV in wild boars, but HEV has been found in wild boar by molecular analyses in Japan (Takahashi et al. 2004; Nakamura et al. 2006).

Antibodies against the tested viruses were present in animals of all sex classes. As expected, the seroprevalences generally were higher in the older animals (>2 years old) except those for PRRSV. We can only speculate on the origin of antibodies in the subadults (≤2 years old) as we do not have any information on the precise age of these wild boar. Based on the experiences with other diseases, it must be assumed that the antibodies of serologically positive subadults are induced by natural infection.

In conclusion, it is commonplace knowledge that the result of the seroprevalence studies are influenced by many factors such as the number of sampled animals, the age of the animals, the time of sampling, the conditions of care and feeding, individual differences and so on. In this respect, when the result of these studies was evaluated extensively, similar and different findings were found about the existence/prevalence of infection in this area. Our data confirm that wild boar populations in the northern Turkey are free of CSFV, HEV, PRCV, SIV and SVDV infections. However, this situation can rapidly change with regard to changing ecology (especially the movement of wild boars and their increasing population density).

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