

Retrospective Serosurvey of Three Porcine Coronaviruses among the Wild Boar (*Sus scrofa*) Population in the Campania Region of Italy

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ABSTRACT: The growing interest in porcine coronaviruses (CoVs) is due to both their negative effect on the swine industry and their propensity to mutate and overcome host barriers. Since information on CoVs in wild boar (*Sus scrofa*) is limited, especially in Italy, a serosurvey was conducted to assess the epidemiologic situation in the Campania region and to clarify the role of wild boar as reservoirs for enteric (porcine epidemic diarrhea virus [PEDV], transmissible gastroenteritis virus [TGEV]) and respiratory (respiratory coronavirus [PRCV]) swine CoVs. During the 2016–17 hunting season, serum samples were collected from 444 wild boars and tested for antibodies to enteric (PEDV, TGEV) and respiratory (PRCV) porcine CoVs by enzyme-linked immunosorbent assay. The highest seroprevalence in wild boars was for PEDV, with a positivity of 3.83% (95% confidence interval [CI] 2.05–5.6), whereas very low seroprevalences were found for TGEV and PRCV (0.67% positivity; 95% CI 0–1.44 in both cases). There was no statistical association between seropositivity to CoVs, sex, and location, whereas the prevalence of seropositive animals was positively correlated with young age (0–12 mo old). Our data confirm the presence of CoVs in wild boars in the Campania region. Our data are in agreement with the results of similar studies from other European countries, which attribute a minor role to wild boar in the transmission of these infections to domestic pigs. Our results suggest that continuous serologic surveys are necessary to monitor wild animals and detect emerging threats to livestock and humans.

Key words: Coronavirus, serosurvey, *Sus scrofa*, wild boar.

Coronaviruses (CoVs) are a group of enveloped, single-stranded, positive-sense RNA viruses that cause respiratory, gastrointestinal, liver, and central nervous system diseases. The CoVs are the largest RNA viruses known to date and are found in an extremely wide range of vertebrate hosts

(Cheng et al. 2021). Six alpha, beta, and delta coronaviruses are known to infect pigs, mainly causing respiratory and intestinal diseases. Of these, porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), and porcine respiratory coronavirus (PRCV) are widely distributed and frequently reported in Europe (Wang et al. 2019).

An alpha coronavirus, PEDV causes an acute enteric syndrome transmissible via the orofecal route and is characterized by diarrhea, vomiting, and malabsorption secondary to villous atrophy after colonization of the intestinal villi. In the European Union, PEDV is not a notifiable disease and is not included in eradication plans, so the status of this disease is not fully known (Antas et al. 2021).

Another highly contagious and deadly alpha coronavirus is TGEV. There are several similarities between PEDV and TGEV, such as tropism, pathogenesis, and clinical signs (Turlewicz-Podbielska and Pomorska-Mól 2021). It affects pigs of all ages. Infection is characterized by gastrointestinal symptoms that cause significant economic losses due to high morbidity and mortality in infected piglets and stunted growth in surviving older pigs (Zhou et al. 2010). A TGEV mutant isolated in 1984, PRCV results from a deletion in the N-terminal region of the spike protein, leading to loss of viral binding to intestinal cells and acquisition of the ability to colonize respiratory tract epithelial cells, causing mainly subclinical respiratory tract infections (Wang et al. 2019). These viruses have a significant economic effect on the swine industry and carry the potential for cross-species transmission, including zoonotic potential (Lin et al. 2016). Several examples of spillover between CoVs have recently been

described in the literature, such as severe acute respiratory syndrome coronavirus, middle eastern respiratory syndrome coronavirus, and the recently pandemic severe acute respiratory syndrome coronavirus 2. For these reasons, improving knowledge of CoV and continuously updating its biology and distribution is a One Health (Islam et al. 2021) requirement.

The wild boar (*Sus scrofa*) is one of the most abundant wild animals in Europe and is a major problem as a natural reservoir of various diseases, both as a source of infection for livestock and humans (Meng et al. 2009). Recently, the wild boar population has increased significantly in many countries, including Italy, and frequent contacts with livestock and humans have been reported. In addition, synanthropic wild boar have been identified as an emerging public health threat that may affect nonhunters (Fredriksson-Ahomaa 2019). In the Campania region of southern Italy, the epidemiologic importance of wild boar in maintaining infections has already been demonstrated for various pathogens such as brucellosis, pseudorabies virus, and circovirus (Montagnaro et al. 2020; Ferrara et al. 2021; Amoroso et al. 2021).

In domestic pigs, CoV outbreaks have been described in several European countries (Lee 2015), including Italy (Pensaert and Martelli 2016; Boniotti et al. 2018; Pizzurro et al. 2018), but little is known about the spread of these viruses in wildlife and the role of wildlife as reservoirs of infection. The aim of our study was to determine the prevalence of CoVs in wild boar and to provide a snapshot of the distribution of these viruses in the Campania region.

During the 2016–17 hunting season, 444 blood samples were collected by cardiac puncture. All samples were provided by hunters with appropriate wild boar hunting licenses. All samples were matched to a form that included information on sex, age, and hunting area. Age was determined using a tooth eruption test, and animals examined were divided into three categories: adults (>36 mo), subadults (13–36 mo), and juve-

niles (0–12 mo). It was not possible to obtain age data for 12 animals.

The presence of specific immunoglobulin G antibodies to TGEV was determined by a blocking enzyme-linked immunosorbent assay (ELISA) using INgezim® TGEV (sensitivity [Sn] 94%; specificity [Sp] 98.2% [Ingenasa, Madrid, Spain]). The detection of specific PEDV antibodies was performed by indirect ELISA using INgezim PEDV (Sn 94.7%; Sp 98.8% [Ingenasa]). Finally, all INgezim TGEV ELISA block-positive samples were tested with INgezim differential coronavirus (Sn 94%; Sp 98.2% [Ingenasa]) to distinguish between TGEV and PRCV infections (which cross-react in serologic tests that are antigenically related) and to detect any coinfection (Magtoto et al. 2019).

The ELISA kits were used with 100 µL of diluted serum samples according to the manufacturer's instructions. Statistical analysis between the different classes was performed using a chi-square test from MedCalc statistical software version 16.4.3 (MedCalc Software 2022). For variables associated with higher seroprevalence, a binary logistic model was applied using JMP Pro version 15.0.0 (SAS Institute Inc. 2014), with $P < 0.05$ considered statistically significant. Analysis of the study area and creation of maps were performed using the geographic information system QGIS version 3.22 (QGIS Association 2018).

In total, we tested 444 wild boars from 119 hunting areas in four provinces of the Campania region (Fig. 1). The combined prevalence of PEDV, TGEV, and PRCV was 4.73% (21/444; 95% confidence interval [CI] 2.76–6.7; Table 1); the proportion of wild boar positive for one, two, or three pathogens was 4.3% (19/444; 95% CI 2.4–6.16), 0.45% (2/444; 95% CI 0–1.07), and 0% respectively. Only two feral pigs were coinfecting, the first with PEDV and TGEV, the second with TGEV and PRCV.

The most common CoV pathogen was PEDV, with a seroprevalence of 3.83% (95% CI 2.05–5.6; odds ratio 5.85 vs. TGEV and PRCV; Table 1). For each of TGEV and PRCV, 3/444 samples were seropositive, so

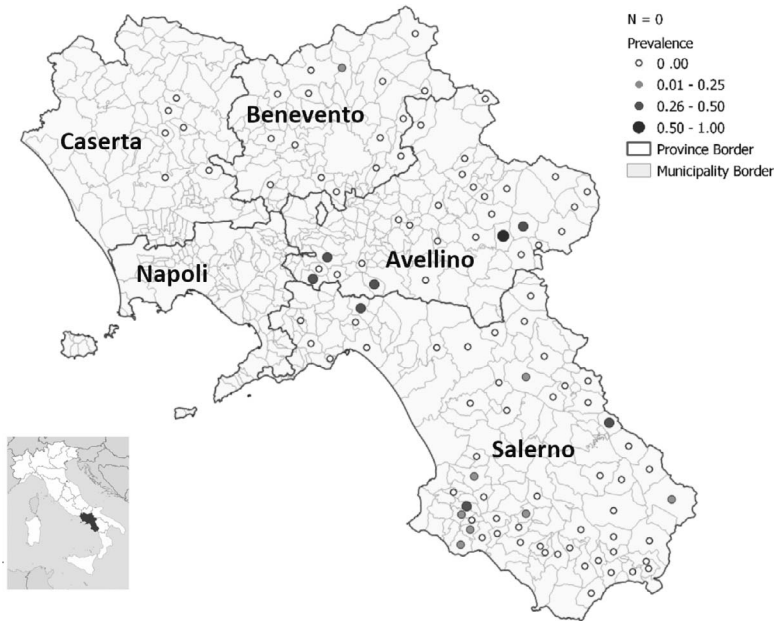


FIGURE 1. Map of the Campania region of Italy showing the distribution of samples of Eurasian wild boar (*Sus scrofa*) seropositive for porcine epidemic diarrhea virus.

the proportion of wild boar serum samples positive was 0.67% in both cases (95% CI 0–1.44; Table 1). Considering the very low seroprevalence observed in the study sample, TGEV and PCRV were not included in the statistical analysis of risk factors.

Surveys conducted in other European wild boar populations have found low prevalence values; for example, seroprevalences of 0.7% for PRCV and 0.4% for TGEV were found in four counties in mainland Croatia (Roic et al. 2012). In Germany, a survey of 1,221 blood

samples found 7.87% PRCV-positive and 1.59% TGEV-positive wild boar (Kaden et al. 2009). In Slovenia, a PRCV seroprevalence of 3% has been described, although a very high seroprevalence in domestic pigs (65%) has been reported (Vengust et al. 2006). In a study conducted on Bohemian wild boar in the Czech Republic, only 1% of the animals tested had antibodies to TGEV (Sedlak et al. 2008), whereas in a study conducted in Turkey, not a single positive animal was found (Albayrak et al. 2013). Information on PEDV

TABLE 1. Seroprevalence of the porcine coronavirus porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), and porcine respiratory coronavirus (PRCV) in hunted wild boar (*Sus scrofa*) in the Campania region of Italy during the 2016–17 hunting season.^a

Factor	<i>n</i>	Number positive	%	95% CI	χ^2	<i>P</i>	OR	SE % ^b	95% CI
Total	444	21	4.73	2.76–6.7					
Virus									
PEDV	444	17	3.83	2.05–5.61			Ref ^b		
TGEV	444	3	0.67	0–1.44	17.343	0.0002	5.85	1.7211	2.48–9.17
PRCV	444	3	0.67	0–1.44			5.85		2.48–9.17

^a CI = confidence interval; OR = odds ratio.

^b Reference category.

TABLE 2. Seroprevalence of porcine epidemic diarrhea virus and risk factor analysis by age, sex, and location in wild boars (*Sus scrofa*) in the Campania region of Italy as detected by indirect enzyme-linked immunosorbent assay.^a

Factor	n	Positive	%	SE %	95% CI	χ^2	P	OR	95% CI
Total	444	17	3.83	0.91	2.0–5.61				
Age (mo)									
0–12	139	10	7.2	2.19	2.89–11.49			Ref. ^b	
13–36	191	7	3.7	1.36	1–6.3	8.12	0.0172	2.03	0.75–5.49
>36	102	0	0	0	0				
n/a ^c	12	0	0	0	0				
Sex									
Male	284	10	3.5	1.09	1.38–5.66				
Female	160	7	4.4	1.62	1.2–7.55	0.037	0.8473	0.79	0.29–2.13
Location (province)									
Avellino	84	4	4.8	2.32	0.21–9.31			Ref.	
Salerno	287	12	4.2	1.18	1.86–6.5	1.56	0.66	1.14	0.35–3.65
Benevento	62	1	1.6	1.6	0–4.75			3.05	0.33–27.9
Caserta	11	0	0	0	0			—	—

^a CI = confidence interval; OR = odds ratio.

^b Reference category.

^c Not available.

in European wild boar is limited, with positive sera only described in a pilot study conducted in Poland, which showed a seroprevalence of 3.2%, whereas the virus was not detected by the reverse-transcription quantitative PCR assay (Antas et al. 2021). These data suggest that CoV infections are not widespread in European wild boar, although small differences could be due to the characteristics of the ELISA kit used and the number of samples collected.

Univariate analysis showed that there was no statistical correlation between location and PEDV seropositivity, even though the positive animals were mainly concentrated in the provinces of Avellino (4.8%) and Salerno (4.2%; Table 2). Similarly, although a higher prevalence was found in female wild boar (4.4%; 95% CI 1.21–7.55), there was no correlation between sex and PEDV positivity ($P=0.8473$). This is an unexpected result, as close contact between sows and piglets (which are the main vectors of the virus) should increase the risk of exposure for females (Marsan and Mattioli 2013). Perhaps a larger sample would provide more accurate statisti-

cal results, but this was not possible in our study. However, one possible explanation for this trend is changes in wild boar population composition due to hunting pressure, species dispersal, and wild-boar ethology (Montagnaro et al. 2020). Conversely, we observed a statistical correlation between age and PEDV seropositivity. The highest seroprevalence (7.2%) was observed in young animals (<12 mo), with a decreasing trend of seroprevalence in the three age groups tested (young animals 7.2%, subadults 3.7%, adults 0%). The lack of positive adult animals might be explained by the short duration of circulating detectable antibodies, which is typical for CoVs (Wesley 2002; Ouyang et al. 2015).

The low prevalence detected in our study suggests that these viruses are not widespread in the wild boar population in the Campania region and that these wild boar do not pose a real risk for the spread of CoVs to domestic pigs. Wild boar monitoring should continue. In addition, molecular diagnostic approaches would be beneficial to confirm the circulation of CoVs and characterize the circulating strains.

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