Molecular surveillance of selected pathogens in intestinal samples of wild boar from southern Italy

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A - Research concept and design, B - Collection and/or assembly of data, C - Data analysis and interpretation, D - Writing the article, E - Critical revision of the article, F - Final approval of the article

Abstract:
Monitoring disease among wildlife is critical to preserving health in both domestic animals and wildlife, and it becomes much more critical when the infections cause significant economic damage to the livestock industry or threaten public health. Given the continuous increase in populations and its role as a reservoir for several infections, wild boar (*Sus scrofa*) requires special attention regarding disease surveillance and monitoring. In this study, the molecular prevalence of selected pathogens was investigated in the wild boar population of Campania, southern Italy. A total of 59 wild boars hunted in the Campania region were sampled and rectum/colon fecal content was collected during slaughter operations. The extracted DNAs/RNAs were used as templates in real-time PCR or end-point PCR protocols described in the literature, obtaining the following molecular prevalences: HEV 6.8% (4/59), M. avium 2.4% (2/59), PCV-2 11.9% (7/59), PCV-3 3.4% (2/59), PPV 8.5% (5/59), and RVA 17% (10/59). A univariate risk factor analysis was carried out using location, sex, age, and weight as independent variables. HEV was shown to be significantly more prevalent in females, as occurred for PPV in young individuals and those weighing less than 50 kilograms. This study suggests that wild boars hunted in the Campania region harbor several enteric infections potentially transmissible to other mammals and/or humans. The observed prevalences also suggest the implementation of good hygiene practices during slaughter and compliance with external biosecurity requirements for pig farms to prevent direct and indirect contact between wild animals and domestic pigs.

Keywords: Surveillance, Reservoir, Pathogens, Wild boar, Molecular Prevalence.

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Short title
Detection of pathogens in fecal samples from wild boars

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Abstract:

Monitoring disease among wildlife is critical to preserving health in both domestic animals and wildlife, and it becomes much more critical when the infections cause significant economic damage to the livestock industry or threaten public health. Given the continuous increase in populations and its role as a reservoir for several infections, wild boar (*Sus scrofa*) requires special attention regarding disease surveillance and monitoring. In this study, the molecular prevalence of selected pathogens was investigated in the wild boar population of Campania, southern Italy. A total of 59 wild boars hunted in the Campania region were sampled and rectum/colon fecal content was collected during slaughter operations. The extracted DNAs/RNAs were used as templates in real-time PCR or end-point PCR protocols described in the literature, obtaining the following molecular prevalences: HEV 6.8% (4/59), *M. avium* 2.4% (2/59), PCV-2 11.9% (7/59), PCV-3 3.4% (2/59), PPV 8.5% (5/59), and RVA 17% (10/59). A univariate risk factor analysis was carried out using location, sex, age, and weight as independent variables. HEV was shown to be significantly more prevalent in females, as occurred for PPV in young individuals and those weighing less than 50 kilograms. This study suggests that wild boars hunted in the Campania region harbor several enteric infections potentially transmissible to other mammals and/or humans. The observed prevalences also suggest the implementation of good hygiene practices during slaughter and compliance with external biosecurity requirements for pig farms to prevent direct and indirect contact between wild animals and domestic pigs.

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The wild boar (*Sus scrofa*) is considered a widespread wild species that has spread throughout Europe, including Italy (von Essen et al. 2025). Despite hunting and population control measures, wild boars are constantly expanding. The impact of this increase is estimated to cause considerable economic losses due to road accidents and agricultural damage (Ficetola et al. 2014; Piscopo et al. 2023). This has also occurred in some Italian regions such as the Campania, which has adopted a management and control plan for wild boar to curb wild boar expansion (Piscopo et al., 2023; Herrero et al. 2006; Mazzatenta 2026). Among the factors that have contributed to making this species widespread is the easier access to food sources due to the increasing urbanization and landscape fragmentation that bring wildlife closer to human-dominated environments and food sources (Massei et al. 2015). Furthermore, the improved food availability resulted in an increase in sow fertility (Colomer et al. 2024). The expansion of wild boar populations promotes contact between wild boars and domestic animals, enhancing interactions and disease transmission, as proven for different infections, including *Brucella* spp., tuberculosis (TB), and African swine fever, for which wild boars serve as reservoirs (Jota Baptista et al. 2023). Pathogens of zootechnical interest that have been described in wild boars include porcine parvovirus (PPV) and porcine circovirus 2 and 3 (PCV-2 and PCV-3), which are responsible for abortion and/or systemic forms in piglets and are considered sources of significant economic losses (Yon et al. 2019). Furthermore, because wild boars are hunted and historically consumed, they represent a risk of spreading zoonotic pathogens, especially if appropriate hygiene measures are not followed during slaughter or raw/undercooked meat is ingested. Some examples of pathogens with zoonotic potential identified in wild boar are rotavirus A (RVA), hepatitis E virus (HEV, now classified as *Paslahepevirus balayani*), and *Mycobacterium avium* (*M. avium*) (Brown et al. 2018; Abrantes and Vieira-Pinto 2023). In recent years, numerous findings have been published globally demonstrating that wild boars host a variety of pathogens, including viruses, bacteria, and parasites (Fredriksson-Ahomaa et al., 2019; Jota Baptista et al., 2023; Meng et al., 2009).

This study aimed to obtain the relative molecular prevalence of a panel of pathogens (PCV-2, PCV-3, PPV, *M. avium*, HEV, RVA) in enteric tissues collected from wild boars hunted in the Campania region (southern Italy). The panel of pathogens was selected by taking based only fecal shedding and the scientific literature in the study area (Ferrara et al. 2024b; La Bella et al., 2023).

Since the number of wild boars in the study area was not established (the last census dates back to more than ten years ago with a population estimated between 60,000 and 80,000 specimens), and only hunted animals were accessible, convenience sampling was adopted (DGR n. 521 of 23/11/2021). In the study area there are numerous ruminants (about half a million, mainly water buffalo), but pig farming is not widely practiced (75,000 animals which corresponds to 2% of the national total) (Ferrara et al. 2024b). During the 2023/2024 hunting season, a total of 118 fecal samples were collected during slaughter operations from the colon and rectum of apparently healthy hunted wild boar (59). Wild boars are classified as young (<12 months) or adults (≥12 months) based on the eruption of their second permanent molar (which occurs at one year old) (Petruccelli et al. 2020). Information on gender, location and weight was also collected. The samples were transported to the Department of Veterinary Medicine laboratories from Naples under refrigeration and immediately subjected to DNA and RNA extraction using commercial kits (DNeasy® Blood & Tissue

Kit; RNeasy Plus Mini Kit; Qiagen). The RNA extracts were immediately reverse transcribed into cDNA using iScript™ cDNA Synthesis Kit (Biorad) and stored until processing. The DNA and cDNA samples were subjected to real-time PCR and endpoint PCR using protocols described in the literature (primers and protocols are described in Table 1) (Miller et al. 1999; Flores et al. 2021; Ferrara et al. 2024a, c). The kits used for the amplification of nucleic acids were iTaq Universal Probes Supermix (Biorad) for real-time PCR and HotStarTaq DNA Polymerase (Qiagen) for end-point PCR. The results obtained (positive or negative) were used as the dependent variable in a univariate statistical analysis to correlate higher prevalence with independent variables collected during the sampling (location, sex, age, and weight). A p-value of <0.05 using the Fisher exact test was considered significant.

Molecular analysis established that RVA was the most frequently detected pathogen in wild boar enteric tissues. It was detected in a total of 10 wild boars (10/59; 2 colon and 9 rectal samples) with a prevalence of 17% (Table 2). PCV-2 followed in frequency with a prevalence of 11.9% (8/59; 2 colon and 7 rectal samples), and PPV 8.5% (5/59; 2 colon and 4 rectal samples) (Table 3). Lower prevalences were observed for other pathogens, in particular, HEV 6.8% (4/59; 4 colon samples), PCV-3 3.4% (2/59; 2 rectal samples) and *M. avium* 2.4% (2/59; one colon and two rectal samples). Univariate analysis identified statistically higher prevalences in female animals for HEV (14.3%; $p=0.04$), and in young animals weighing less than 50 kilograms for PPV ($p=0.02$). For all other variables, no significant differences were observed.

The RVA prevalences obtained were similar to those described in other countries. A study performed in Japan revealed a prevalence of 10.8% and 20.7% respectively, from wild boar and domestic pig samples during 2017-2022 (Shizawa et al. 2024). However, other studies reported lower prevalences. For example, in other European countries, like Germany and the Czech Republic, lower prevalence has been described (ranging from 1 to 2.5%) (Moutelíková et al. 2016; Althof et al. 2023). The same studies also highlighted a high genomic homology between strains isolated in humans and pigs/wild boars, underlying the risk of potential interspecies transmission or reassortment during viral shedding carried out by wildlife (Flores et al. 2021; Kunić et al. 2025).

Regarding HEV, there is considerable evidence of the virus circulating in Italy and sporadically being responsible for human outbreaks due to game meat consumption, although such evidence was lacking in the present study area (Spada et al. 2022). For example, in Central Italy a large-scale investigation, based on hunted wild boar liver and diaphragm tissues, found a prevalence of 10.87% and 12% in two different periods (Ferri et al. 2023; Luca et al. 2024). In other regions of southern Italy (Apulia and Basilicata), a similar prevalence was obtained (10.4%) using liver and muscles (La Bella et al. 2023). Active circulation of HEV in wildlife was also confirmed by studies carried out in other countries, such as Portugal and Japan (Hara et al. 2014; Mesquita et al. 2016). Furthermore, the prevalence described by previous studies can vary based on the type of sampling (live or hunted animals), matrix (feces, liver, intestine, serum, etc. and their suitability), and approach (serological or molecular), as well as actual epidemiological differences due to geographical diversity (Ferrara et al. 2024c). Viral circulation also involves other animals, as suggested in previous investigations that focused on the detection of anti-HEV antibodies in pigs, wildlife and dogs, obtaining prevalences of 41.4, 4.8 and 8.2% respectively (Ferrara et al. 2024a, 2025; Minichino et al. 2025). In humans, a seroprevalence of 8.3% was described in the three-year period 2017-2019 in Italian blood donors (Spada et al. 2022).

Porcine circoviruses and PPV are very common infections in pigs, responsible for significant economic losses in intensive farming. The presence of these viruses in wild boars has been tested in several studies (Franzo et al. 2018). For example, evidence of circovirus infection has been described in Greece (Sofia et al. 2008). A Korean study found a prevalence of 5.6% testing 266 samples of varying nature (fecal swab, blood etc.) (Dhandapani et al. 2021). Some studies have also described the co-infection of PPV/porcine circovirus, like in Slovakia (19.1% positive for PPV3, 43.8% for PCV2 and a coinfection proportion of 11%), and in the Western Balkans (PCV2 in 50% and PPV in 28.8% of spleen samples) (Sliz et al. 2015; Glišić et al. 2025). A very high prevalence of PPV has been described in Russia, where more than 50% of tested wild boars were positive (Komina et al. 2025). Circulation of *M. avium* among wild boars has been described in Slovenia (11.8%) and Switzerland (Pate et al. 2016; Ghielmetti et al. 2021). Evidence of exposure had already been demonstrated in domestic pigs with a recent study that described a seroprevalence of 3.5% in the present study area (Ferrara et al. 2025).

It is crucial to evaluate the prevalence information obtained for some infections. Some of the examined agents, such as HEV and RVA, have well-established fecal excretion and fecal-oral transmission, therefore using fecal samples was appropriate. However, for some infections (PCV-2, PCV-3, PPV, and *M. avium*), feces are not the major or most informative matrix. Detection in stool samples may suggest temporary shedding, environmental contamination, or passive passage rather than active infection or epidemiologically significant transmission (Ghielmetti et al. 2021; de Souza et al. 2023). Furthermore, pathogen shedding is intermittent and this would explain the low prevalence.

Although this study focused on a limited number of samples, the risk analysis highlighted some correlations between the investigated variables and higher prevalence. In fact, higher HEV prevalence has been described for females (14.3% while 0% was found in males). This finding was also described in a study conducted in Central Italy, which found a prevalence of 16.9% for males and over 30% for females (Luca et al. 2024). The same trend was also observed in Spain

(Ruiz-Ponsell et al. 2024). The relationship between HEV infection and sex in suids varies per study, although generally there is no sex-related relationship observed. Ethological and social characteristics of the sow compared to the boar (such as, for example, living in close contact with piglets in the first months of life) could explain a different exposure (Gustafsson et al., 1999).
Furthermore, our study found higher prevalences for PPV in young animals and those weighing less than 50 kg. This outcome may indicate that PPV colonization of the intestinal mucosa is more consistent in young animals than in adults. This difference has not been described in the literature in similar studies or in a study looking for PPV in reproductive tissues. For the other pathogens, no other significant results were obtained. The present study, although small-scale and univariate analysis-based, provided important information for consideration regarding pathogens responsible for zoonoses and economic impacts in hunted wild boars. In fact, multiple comparisons could be associated with potential risk of type I error considering the small sample size and was not performed. Further studies involving larger sample sizes, as well as involving other pathogens (including those with significant impact such as PRRSV) are necessary to define a complete epidemiological picture of the study area.
. Given the reservoir/sentinel role that wildlife plays, continuous surveillance is necessary to establish potential containment measures for these infections, including in wild animals.
This study has explored the molecular prevalence of selected infections with zoonotic potential and those responsible for economic losses, in wild boars sampled in southern Italy. Despite using a small number of samples and without sequencing analysis, this study clearly shows the circulation of several pathogens, suggesting the necessity for regular surveillance in order to detect prevalence changes over time and implement further containment measures.

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Contributions: GF (corresponding author) was involved in work design, data acquisition, analysis, interpretation of data and writing. NP and LE were involved in the sampling, analysis and interpretation of data and reviewing the last version of the manuscript. UP, BO e DR were involved in writing and analysis and interpretation of data.

Competing interests: The authors declare they have no competing interests

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Table 1: Primers used in this study (target, length and reference).

Gene	Forward (5'-3')	Reverse (5'-3')	Size (bp)	Ref
16S rRNA (M. avium)	AGAGTTTGATCCTGGCTCAG	ACCAGAAGACATGCGTCTTG	193	Miller et al.
VP2 (PPV)	GGGCTTGGTTAGAATCAC	TGGTGGTGAGGTTGCTGAT	313	Ferrara et al.
ORF-2 (PCV-2)	ATGGCG GGAGGAGTAGTTT	CCCTTTGAATACTACAGCG	171	Ferrara et al.
Rep (PCV-3)	GCTACGAGTGTCCTGAAGATAAG	GCCTCCACACTCCACAATAG	138	Ferrara et al.
VP6 (RVA)	CTACDTGGTATTTYAAYCCAGT	GTCCAATTCATNCCTGGTGG	370	Rojas et al.
ORF3 (HEV)#	RGTRGTTTCTGGGGTGAC	AKGGRTTGGTTGGRTGA	70	Ferrara et al.

#In this real time-PCR also a Taq man probe was used 5'-FAM-TGAYTCYCARCCCTTCGC-TAMRA-3'

Table 2: Detection of pathogens with zoonotic potential in wild boars from Campania region, southern Italy.

	HEV				*M.avium*			RVA		
Factor	n	Positive	%	***p***	Positive	%	***p***	Positive	%	***p***
Total	59	4	6.8		2	3.4		10	17	
Location										
Greci	30	2	6.7		0	0		8	26.7	
Ariano	8	0	0	0.65	1	12.5	0.2	1	12.5	0.1
Montemarano	21	2	9.5		1	4.8		1	4.8	
Sex										
Male	31	0	0		1	3.3		7	22.6	
				0.04			1			0.3
Female	28	4	14.3		1	3.6		3	10.7	
Age										
Adult	42	3	7.1		1	2.4		8	19	
				1			1			0.7
Young	17	1	5.9		1	5.9		2	11.8	

Weight										
≤50 kg	18	1	5.6		1	5.6		2	11.1	
				1			1			0.7
>50 kg	41	3	7.3		1	2.4		8	19.5	

Table 3: Detection of pathogens of zootechnical interest in wild boars from Campania region, southern Italy.

	PCV-2				PCV-3			PPV		
Factor	n	Positive	%	***p***	Positive	%	***p***	Positive	%	***p***
Total	59	7	11.9		2	3.4		5	8.5	
Location										
Greci	30	5	16.7		1	3.3		1	3.3	
Ariano	8	0	0	0.4	1	12.5	0.25	0	0	0.09
Montemarano	21	2	9.5		0	0		4	19	
Sex										
Male	31	3	9.8		2	6.5		2	6.5	
				0.7			0.49			0.66
Female	28	4	14.3		0	0		3	10.7	
Age										
Adult	42	4	9.5		2	4.8		1	2.4	
				0.4			1			**0.02**
Young	17	3	17.6		0	0		4	23.5	
Weight										
≤50 kg	18	3	16.7		0	0		4	22.2	
				0.66			1			**0.02**
>50 kg	41	4	9.8		2	4.9		1	2.4	

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Dataset analyzed regarding the prevalence of selected pathogens in wild boar samples in the Campania region