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BRIEF REPORT

Porcine circovirus 3 subclinical infection in wild boar is not associated with systemic lesions

Àlex Cobos[1,2,3,4] · Eva Huerta[1,2,4] · Mónica Pérez[1,2,4] · Josep Estruch[5] · Roser Velarde[5] · Stefania Tampach[5] · Marina Sibila[1,2,4] · Joaquim Segalés[2,3,4]

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Abstract
Porcine circovirus 3 (PCV3) is known to endemically circulate in domestic pigs, both in intensive and extensive settings. This virus occasionally causes reproductive and/or postnatal disease, although its full impact is currently undetermined. The appearance of such diseases is most remarkably correlated with the presence of vascular lesions consisting of periarteritis in medium and small calibre arteries, which are found in many tissues, but are most consistently and abundantly detected within the mesenteric arterial plexus. Multiple studies demonstrated PCV3 high prevalence in wild boar (*Sus scrofa*) as well, which poses a risk as a potential wildlife reservoir. Nevertheless, no studies have specifically tackled the potential pathogenic role of PCV3 in wild boar populations. In this study, we evaluated the presence of periarteritis in a large number of tissues (mostly mesenteric plexus) from wild boar necropsied as part of a disease-monitoring program in Catalonia (northeastern Spain). The presence of PCV3 genome was evaluated by PCR (in serum samples) and in situ hybridization (ISH) (in tissues). While PCV3 presence was confirmed in 26 out of 87 (29.89%) serum samples, none of the tissue samples displayed histopathological lesions compatible with PCV3-associated disease (PCV3-AD). Moreover, ISH revealed PCV3 genome in lymphoid follicles in 4/6 studied cases (in which lymphoid tissue was available), and only one animal displayed mild arterial labelling despite absence of histological lesions. The lack of evidence of lesions associated to PCV3 in wild boar is probably related to ecological and epidemiological factors, which greatly differ from domesticated populations. Therefore, we hypothesize that the occurrence of PCV3-AD in wild boar is highly unlikely, with low or negligible impact on its populations.

Keywords Porcine circovirus 3 · PCV3 · Wild boar · Surveillance · PCV3-associated disease · *in situ* hybridization · Histopathology

✉ Marina Sibila
marina.sibila@irta.cat

✉ Joaquim Segalés
joaquim.segales@uab.cat

[1] IRTA, Animal Health, Centre de Recerca en Sanitat Animal (CReSA), Universitat Autònoma de Barcelona (UAB), Campus, Bellaterra 08193, Catalonia, Spain

[2] Unitat mixta d'investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra 08193, Catalonia, Spain

[3] Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Campus, Bellaterra 08193, Catalonia, Spain

[4] WOAH Collaborating Centre for the Research and Control of Emerging and Re-Emerging Swine Diseases in Europe (IRTA-CReSA), Bellaterra 08193, Catalonia, Spain

[5] Servei d'Ecopatologia de Fauna Salvatge (SEFaS) and Wildlife Ecology and Health group (WE&H), Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra 08193, Catalonia, Spain

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Introduction, methods, and results

Since the discovery of porcine circovirus 3 (PCV3) nearly a decade ago (Palinski et al. 2016; Phan et al. 2016), the pig industry has gained interest in defining its role as a swine pathogen. As an endemic virus, it remarkably circulates at high prevalence among pig farms without evidence of disease (Kwon et al., 2017; Saporiti et al. 2020; Stadejek et al. 2017; Zheng et al. 2017). However, it has been associated with clinical illnesses referred to as PCV3-associated disease (PCV3-AD). Specifically, this virus has been associated to reproductive disorders (PCV3 reproductive disease, PCV3-RD) characterized by mummified, stillborn and weak-born piglets (Cobos et al. 2022, 2024; Dal Santo et al., 2020; Palinski et al. 2016; Saporiti et al. 2021a, b), as well as disease in older pigs (PCV3 systemic disease, PCV3-SD), which is characterized by ill-thrift and poor growth (Alomar et al. 2021; Arruda et al. 2019). Although both diseases feature multisystemic inflammation evidenced under histologic evaluation, its most remarkable finding consists of systemic lymphohistiocytic inflammation of medium and small calibre arteries (namely lymphohistiocytic arteritis and periarteritis) (Cobos et al. 2022, 2025; de Conti et al. 2021; Molossi et al. 2022; Saporiti et al. 2021a, b). The detection of these lesions is highly dependent on the presence of this type of vessels within the evaluated tissues, which is often scarce. However, the mesenteric arterial plexus concentrates a high number of these arteries, hence its assessment is of great value when investigating this disease, and it is affected in around 95% of PCV3 diseased pigs (Cobos et al. 2022).

PCV3 related research is hindered by the few available tools to date. Most studies have focused on viral molecular detection, but for an endemic virus that often causes subclinical infection, its presence does not necessarily correlate with disease (Saporiti et al. 2021a, b). In situ hybridization (ISH) has been by far the most used technique due to its ability to correlate viral presence with histological lesions, although its high cost limits its usage for diagnostic purposes. Immunohistochemical techniques have been occasionally developed, although they are not commercially available (Molossi et al. 2023), and their results are not conclusive.

Multiple studies have assessed the presence of PCV3 in wild boar (*Sus scrofa*), mostly analysing their potential role as natural reservoirs (Klaumann et al. 2018). Its circulation in wild boar has been demonstrated with detection rates ranging from 4.20 to 71% (Table 1). Interestingly, persistent infection in wild boar has been documented (Klaumann et al. 2019), and prevalence in adults appears to be higher than in young wild boar (Kaneko et al., 2023; Klaumann et al. 2019). However, all of these studies relied exclusively on molecular detection techniques, without specifically evaluating the presence of PCV3-associated lesions. Notably, PCV2 (the most known porcine circovirus also infecting wild boar) has been demonstrated to have potential to induce the same histological lesions and disease in wild boar as those observed in commercial pigs (Ellis et al. 2003; Lipej et al. 2007; Sofia et al. 2008; Vicente et al. 2004). Whether this is also true for PCV3 remains undetermined. Therefore, the aim of the present study was to perform a histopathological assessment of the mesenteric arterial plexus and other tissues from wild boar specifically searching for systemic lymphohistiocytic periarteritis (hallmark lesion of PCV3-AD in domestic pigs) in order to investigate the potential occurrence of PCV3-AD.

In the present study, a total of 101 wild boars from various regions of Catalonia (northeastern Spain) were analysed between 2018 and 2024 (Fig. 1). Sixty-three individuals

Table 1 Reported PCV3 detection rates by molecular methods (PCR) in wild boar across different countries over the years

Country	Year	PCV3 Prevalence (%)	Nº Animals	Sample	Reference
Italy	2018	33.15	187	Blood/serum	Franzo et al. 2018
Germany	2019	29.20	89	Spleen	Prinz et al. 2019
Italy	2019	44.80	29	Blood/serum	Franzo et al. 2019
Spain	2019	42.66	518	Blood/serum	Klaumann et al. 2019
Brazil	2021	10.00	70	Blood/serum	de Souza et al. 2021
Italy	2021	49.00	148	Tissues	Amoroso et al. 2021
South Korea	2021	5.60	266	Blood/swabs	Dhandapani et al. 2021
Austria	2022	25.00	95	Lungs/spleen	Auer et al. 2022
China	2022	5.79	138	Tissues	Hu et al. 2022
Italy	2022	71.00	82	Liver	Fanelli et al. 2022
China	2023	10.90	247	Tissues/serum	Gong et al. 2023
Italy	2023	56.31	103	Tissues	Franzo et al. 2023
Japan	2023	16.80	256	Tonsils	Kaneko et al., 2023
South Korea	2024	4.30	167	Lungs	Park et al. 2024
		5.50	55	Lymph nodes	
Poland	2024	37.80	680	Tissues/blood	Frant et al. 2024
Russia	2024	6.60	30	Lung/lymph node	Krasnikov et al. 2024
		20.00	30	Spleen	

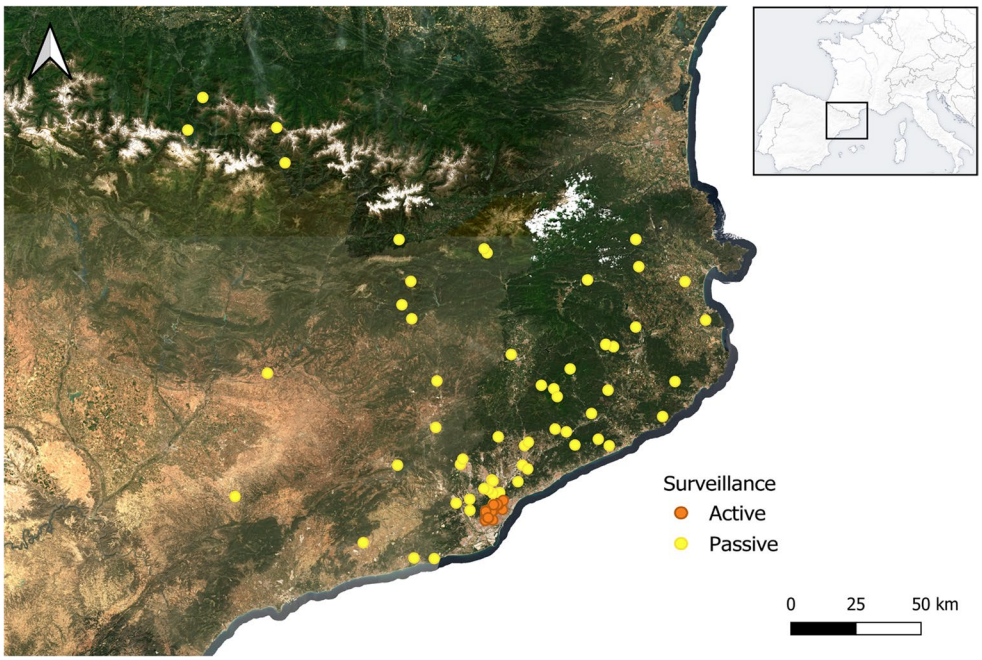

Fig. 1 Distribution of the animals included in this study. All wild boars included in the active surveillance program came from the periurban areas of Barcelona (orange dots), whilst the found dead animals were scattered throughout the region of Catalonia (yellow dots)

Table 2 Data regarding the studied animals

Parameter	*N* of animals
Male	51
Female	50
Young (< 12 months)	61
Subadult (12–24 months)	19
Adult (> 24 months)	21
Found dead	63
Active surveillance programme	38
Total animals	**101**

were found dead by hunters or rural agents (forestry rangers of the Catalan government) and transported refrigerated to the Veterinary Faculty of the *Universitat Autònoma de Barcelona*, within the region's Wildlife Health Passive Surveillance program. The remaining 38 wild boars were captured alive with no signs of disease in urban and periurban areas of Barcelona, and humanely euthanized via chemical methods as part of population control efforts and active surveillance (Fig. 1). All animals were sexed and assigned to one of three age categories (Table 2) — young (< 12 months), subadult (12–24 months), or adult (> 24 months) —based on criteria for tooth eruption, replacement, and wear patterns (Matschke 1967). Only fresh (non-autolytic) carcasses from wild boar passive surveillance program were sampled for the purpose of the present study. Samples from actively captured wild boar were fresh since they were taken just after euthanasia of animals.

On necropsy, a blood sample was obtained (when possible) from the heart, which was centrifugated and serum was kept frozen (−20 °C) until analysed. DNA extraction was performed on serum samples, followed by a quantitative PCR (qPCR) for PCV3 as previously described (Saporiti et al. 2020), with results below 10^3 PCV3 genome copies/mL of serum being considered positive but below quantification limit. Tissue samples were collected and fixed in 4% neutral-buffered formalin. Subsequently, they were trimmed, embedded in paraffin, and sections of 3 to 4 µm were stained with Mayer's haematoxylin and eosin (H&E) for microscopic evaluation in search for PCV3 associated lesions, regardless of their qPCR results. Additional sections of the tissues from the animals which tested over 10^3 PCV3 genome copies/mL in serum were obtained to perform an in situ hybridization (ISH) against PCV3 as previously described (Saporiti et al. 2021a, b). The main diagnoses of found-dead animals included pneumonia (n=41), trauma (n=10), sarcoptic mange (n=3), gastrointestinal disease (n=3), septicaemia (n=1), tuberculosis (n=1), and bacterial placentitis/metritis (n=1), head malformation (n=1) and not determined (n=2), for passive-monitoring studied individuals. Captured wild boar in the active surveillance program did not show any significant alteration on post-mortem exams.

A total of 87 serum samples and 394 tissue samples were collected from the 101 necropsied wild boars. Tissue samples consisted mainly of mesenteric arterial plexus (n=63), followed by lung (n=47), spleen (n=42), kidney (n=42), brain (n=39), liver (n=37), heart (n=32), lymph node (n=31), tonsil (n=26) and intestine (n=10). A detailed list of tissue samples per animal and their data (age, sex, origin) is available in supplementary Table 1.

From the serum samples, 26/87 (29.89%) yielded positive results for PCV3 through qPCR. From those, 10 of

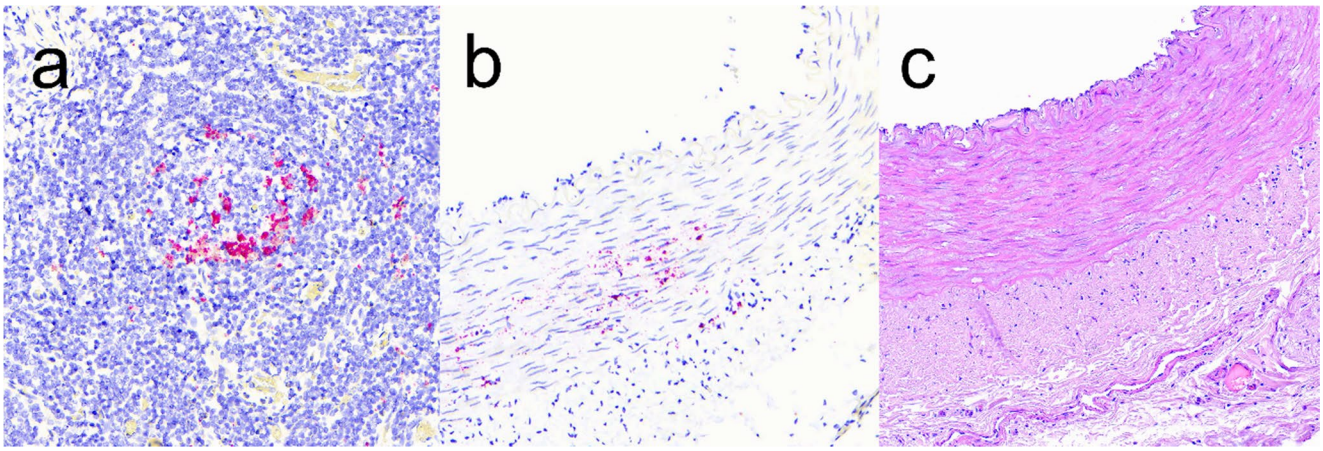

Fig. 2 Wild boar tissues. (**a**) Lymph node showing positive PCV3 labelling in the germinal centre of a lymphoid follicle. PCV3 ISH and haematoxylin counterstain. (**b**) Splenic artery displaying discrete labelling of smooth muscle cells. PCV3 ISH and haematoxylin counterstain. (**c**) The same splenic artery showing absence of histopathological alterations. Haematoxylin and eosin stain

them were quantifiable, ranging from $1.94 \cdot 10^3$ to $9.60 \cdot 10^4$ genome copies/mL of serum (mean $2.08 \cdot 10^4$ genome copies/mL of serum).

Histopathological assessment of H&E-stained sections of the 394 tissues (from the 101 animals) revealed absence of histological lesions attributable to PCV3. Systemic lymphohistiocytic arteritis and periarteritis was not observed in any of the assessed samples. Moreover, other lesions previously associated with PCV3-AD such as lymphohistiocytic myocarditis or non-suppurative encephalitis were not observed either.

ISH against PCV3 was performed in the 10 cases with quantifiable PCV3 levels, in different tissues upon availability. Labelling against PCV3 was observed in the follicular centres of lymphoid tissues in 4 of the 6 animals in which lymphoid tissues were available (total of 8 ISH positive tissues out of 11 samples). Specifically, positivity was observed in lymph nodes (3/4 samples, Fig. 2A), spleen (3/4 samples) and tonsil (2/3 samples). Additionally, one of the spleens revealed positive staining in the splenic artery (Fig. 2B), in absence of histological lesions (Figs. 2C). The remaining samples, consisting of mesenteric arteries ($n=5$), kidney ($n=3$), liver ($n=3$), heart ($n=2$), brain ($n=1$) and foetal heart and lung ($n=1$) yielded ISH negative results. qPCR and ISH results per animal are available in supplementary Table 2.

Discussion

PCV3 infection in wild boar has been thoroughly investigated using molecular techniques, but this is the first study to specifically address the presence of PCV3-associated lesions through histological evaluation in this species. The high prevalence of PCV3 in wild boar (Table 1), often surpassing that of domestic pigs (Franzo et al. 2023), has led to the hypothesis that wild boar may serve as a reservoir species (Klaumann et al. 2019). Interestingly, PCV3 has been also detected in ticks, which may act as vectors and aid in spreading its infection in the wild (Franzo et al. 2019). In fact, the current study further confirms previous data and aligns with already reported prevalences (Table 1), with detection of PCV3 genome in 29.89% wild boar serum samples.

Systemic lymphohistiocytic arteritis and periarteritis (the hallmark lesion of PCV3-AD) was not observed in our study, neither in animals coming from active nor passive surveillance, hence we did not find cases compatible with PCV3-AD in wild boar. As suggested by an experimental infection in pregnant gilts, the appearance of PCV3 associated lesions (including in animals at weaning) appears to be linked to the infection of the sows by early-mid gestation (Cobos et al. 2023). Although this may be common in commercial pig farms since the replacement sows are introduced in the herd around the insemination time, it may be an unlikely circumstance in wild boar populations, where the timing of infection of young animals is much more diverse (Klaumann et al. 2019).

Unlike PCV2, which is known to infect and cause similar histologic lesions in wild boar as in commercial pigs (Ellis et al. 2003; Lipej et al. 2007; Sofia et al. 2008), a similar situation for PCV3 has not been described. The results of this study (i.e. animals in which infection is confirmed in absence of histologic lesions, and presence of genome uniquely in germinal centres of lymphoid follicles) indicate subclinical infection, as it happens with this virus in domestic pig. Moreover, it has been suggested that development of disease and histological

lesions is linked to high viral loads (Cobos et al. 2023); in consequence, the low viral loads often detected in wild boar (such as in this study) reinforce the hypothesis that this species might act primarily as a subclinical carrier. Other cofactors influencing the development of PCV3-AD (such as host immune responses, genetic susceptibility, coinfections or environmental stressors) have not been demonstrated, yet they could play a role in preventing PCV3-AD development in wild boar. Only one case (from passive surveillance) displayed PCV3 genome within an arterial wall; however, it was to a low amount and in absence of histologic lesions. However, considering that PCV3-AD prevalence could be low, it is possible that the number of studied animals has not been enough. Overall, we presume that wild boar are mainly infected subclinically by PCV3 and, although we cannot rule out their potential susceptibility to suffer from disease following PCV3 infection, its likelihood of occurrence is probably very low given their epidemiological circumstances.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10344-025-01981-w.

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Author contributions AC performed histopathological assessment, in situ hybridization and manuscript writing. EH performed the quantitative PCR. MP performed the histopathological procedures. JE, RV and ST performed the wild boar necropsies and sample taking. JE prepared figure 1. MS and JS analysed and interpreted the data and reviewed the manuscript. All authors read and approved the final manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval The active and passive surveillance program of wild boar in Catalonia (Spain) is included within the "Programa de vigilància sanitària de la fauna salvatge a Catalunya", executed by the Department of Agriculture, Livestock, Fisheries and Food from the Autonomous Government of Catalonia (Spain). https://agricultura.gencat.cat/web/.content/07-ramaderia/sanitat-animal/vigilancia-sanitaria-fauna-salvatge/enllacos-documents/fitxers-binaris/pla-vigilancia-sanitaria-fauna-salvatge-catalunya.pdf (last checked December 2nd, 2024).

Competing interests The authors declare no competing interests.

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