

RESEARCH ARTICLE



Prevalence and viral load dynamics of PCV-2 and PCV-3 on large Hungarian swine farms

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ABSTRACT

Porcine Circovirus 3 (PCV-3) was first identified in 2016 and has since been detected in swine herds worldwide. The aim of this study was to survey the prevalence of PCV-2 and PCV-3 in large Hungarian swine herds. Between July 2020 and April 2022, a total of 1,408 blood samples were collected from 18 large herds and pooled sampling was applied by combining equal amounts from five individuals per pool. PCV-3 positive samples were detected in 94.4% (17/18) of the farms, with an overall apparent prevalence of 30.4% (89/293) in the tested pools. PCV-2 was found in 50.0% (9/18) of the farms, with 14.7% (43/293) of pools testing positive. After correction for imperfect PCR sensitivity and specificity (assumed to be 95%), the corrected prevalence of PCV-3 was 28.2% (83/293) and 10.7% (32/293) for PCV-2, farm-level prevalence estimates remained unchanged. Quantitative PCR analysis revealed that 24.2% of the PCV-3-positive pools had low ($C_q > 30$), 5.8% medium ($28 < C_q < 30$) and 1.0% high viral loads ($C_q < 28$). These results confirm that PCV-3 is widely present in large Hungarian swine farms. The relatively high prevalence, viral load and frequent co-infections raise the possibility of subclinical infections, although the clinical significance of PCV-3 requires further investigation.

KEYWORDS

porcine, circovirus, PCV-2, PCV-3, prevalence, virus load, co-infections, qPCR

INTRODUCTION

The porcine respiratory disease complex (PRDC) manifests due to numerous infectious pathogens, as well as environmental, housing, technological and management factors, markedly deteriorating production parameters such as average daily gain (ADG) and feed conversion ratio (FCR). It also increases the mortality, culling rates and veterinary treatment costs (Ózsvári and Búza, 2015). PRDC has a complex aetiology involving various viruses (e.g., *Porcine Circovirus 2* (PCV-2), *Swine Influenza Virus* (SIV)) and bacteria (e.g., *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*) (Búza, 2019). On large Hungarian swine farms, the most frequent PRDC pathogens are PCV-2 and *M. hyopneumoniae*, which appear in more than 90% of the cases (Ózsvári and Búza, 2015).

In the early 1970s, a German research team examined the PK-15 (permanent kidney) cell line (ATCC-CCL31) using electron microscopy and observed tiny, spherical virus-like particles (Tischer et al., 1974; Opriessnig et al., 2020). Later, these were characterised as

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icosahedral viruses of 17 nm diameter, containing a covalently closed single-stranded DNA genome and one main capsid protein. The newly identified virus was named 'porcine circovirus (PCV)' as the produced antibodies could only be detected in swine (Tischer et al., 1982; Opriessnig et al., 2020). Since then, several other circoviruses have been identified, and today four types are recognised: Porcine Circovirus type 1 (PCV-1), Porcine Circovirus type 2 (PCV-2), Porcine Circovirus type 3 (PCV-3) and the recently discovered Porcine Circovirus type 4 (PCV-4) (Opriessnig et al., 2020). Circoviruses belonging to the family *Circoviridae*, genus *Circovirus*, are small viruses with a circular, single-stranded DNA genome of approximately 2,000 nucleotides (Tischer et al., 1982; Meehan et al., 1997; Temeeyasen et al., 2020).

PCV-1 is not considered a pathogen in swine (Tischer et al., 1986; Temeeyasen et al., 2020). PCV-2 infection results in a disease with diverse clinical signs, collectively referred to as PCV-2-associated disease (PCVAD). This condition includes interstitial pneumonia, enteric disorders, reproductive failures and post-weaning multi-systemic wasting syndrome (PMWS) in piglets (Chen et al., 2019a). PCV-2 is frequently associated with enteric diseases during nursery and finishing periods (Baró et al., 2015). In addition, PCV-2 DNA is often detected in animals showing signs of porcine dermatitis and nephropathy syndrome (PDNS). However, PCV-2 antigen cannot be detected in some PDNS-affected animals, suggesting that other factors may contribute to the disease. Interestingly, most PCV-2-negative PDNS tissue samples have tested positive for PCV-3. These findings indicate that PCV-3, as a potential pathogen, warrants further investigation (Ouyang et al., 2019).

PCV-3 was first identified in 2016 in the USA in sows showing either PDNS-like clinical signs or multi-systemic inflammation (Phan et al., 2016; Palinski et al., 2017). Since then, the virus has been reported in many countries (e.g. Japan, South-Korea, China, Sweden, Russia, Thailand, Brazil, Denmark, Italy and Spain) demonstrating its global distribution (Ouyang et al., 2019). Although only recently discovered, retrospective studies suggest that PCV-3 had already been circulating in swine populations for decades prior to its first identification (Mora-Díaz et al., 2020).

In recent years, porcine circoviruses have also been investigated in several European regions, with increasing attention to their epidemiology and molecular diversity (Opriessnig et al., 2020; Saporiti et al., 2020; Almeida et al., 2025; Melegari et al., 2025). However, most available studies have focused on wild boar populations rather than commercial domestic pig herds (Prinz et al., 2019; Nišavić et al., 2024; Šolaja et al., 2025). For instance, a recent Serbian study detected both PCV-2 and PCV-3 in wild boars, with prevalence rates of 17.5 and 15.5%, respectively and a low level of co-infection, indicating that these viruses circulate widely among free-ranging populations in the Balkan region (Nišavić et al., 2024). Šolaja et al. (2025) also confirmed the presence of both viruses in wild boar, though their epidemiological impact on domestic pigs remains uncertain. In Portugal, PCV-3 showed a higher prevalence than PCV-2 in

domestic pigs (11.2% vs. 1.9%), whereas wild boars displayed considerably higher infection rates for both viruses (Almeida et al., 2025). Similarly, Melegari et al. (2025) found high levels of PCV-2 (47.1%) and PCV-3 (38%) infections in both domestic pigs and wild boars in Italy. Despite the growing number of international reports, data from Central and Eastern Europe remain scarce, particularly prevalence studies addressing both PCV-2 and PCV-3 dynamics in large commercial herds.

This study aims to investigate the prevalence and viral load patterns of PCV-2 and PCV-3 in large swine farms in Hungary.

MATERIALS AND METHODS

Sample collection

Altogether, 18 large commercial swine farms in Hungary were surveyed across six of the country's seven official regions between July 2020 and April 2022. The majority of the surveyed farms were located in the Southern Transdanubian region (55.6%, $n = 10$) and the Central Transdanubian region (22.2%, $n = 4$). In each of the other regions (Western Transdanubia, Northern Hungary, the Northern Great Plain and the Southern Great Plain) one large swine farm (5.6%, $n = 1$) was surveyed, except for Central Hungary, where no sampling was performed (Fig. 1).

A convenience sampling method was applied. The inclusion criteria for large commercial pig farms in the study were as follows: 1) a minimum herd size of 350 breeding sows and their progeny, 2) continuous population and performance recording (e.g. farrowing, mortality, culling, average daily gain, feed conversion ratio, animal movements), 3) detailed digital records of health issues, laboratory test results, morbidities, veterinary treatments and vaccinations, and 4) willingness to participate in the sampling process. Farms that did not meet these criteria or had incomplete or inconsistent veterinary health records were excluded from the study. Blood samples were collected from pigs aged 3–24 weeks. Altogether, 1,408 blood samples were collected from different age groups. Samples were obtained using an 18G needle from the jugular vein and placed into sterile serum blood collection tubes. From each farm, 14–15 samples were collected from pigs of the same age group every four weeks. After collection, the samples were stored at 5 °C until transport to the laboratory.

Laboratory methods

The blood samples were transported to Intervet International B.V. R&D Service Laboratory (Boxmeer, the Netherlands) under cooled (non-frozen) conditions, using a contracted courier partner via ground and air transport. Upon arrival at the laboratory, the samples were processed and pooled to accelerate quantitative PCR (qPCR) analysis. For the age distribution analysis, pooled sets were created for the age groups of 3–6, 7–10, 11–14, 15–18, 19–22 and 23–24 weeks, and the test results were categorized

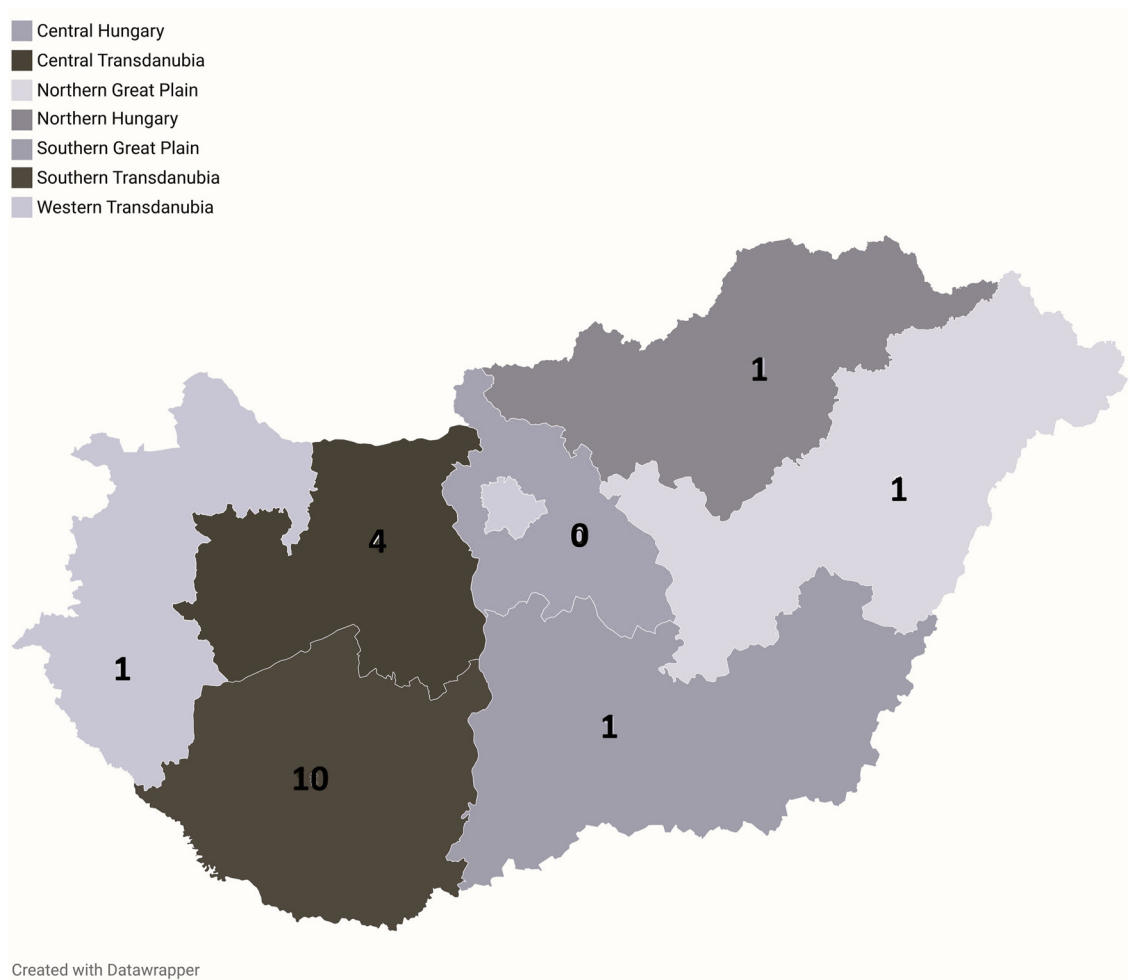


Fig. 1. Geographical distribution of the investigated swine farms in Hungary (*n* = 18)

accordingly (Fig. 2). Serum samples were tested in pools of five (5 sera per pool). Pooling was applied to increase testing efficiency but may have reduced detection sensitivity for

low-titre infections. DNA was purified using the MagNA Pure 96 system (Roche), an automated magnetic bead-based extraction method in which nucleic acids (NA) bind to

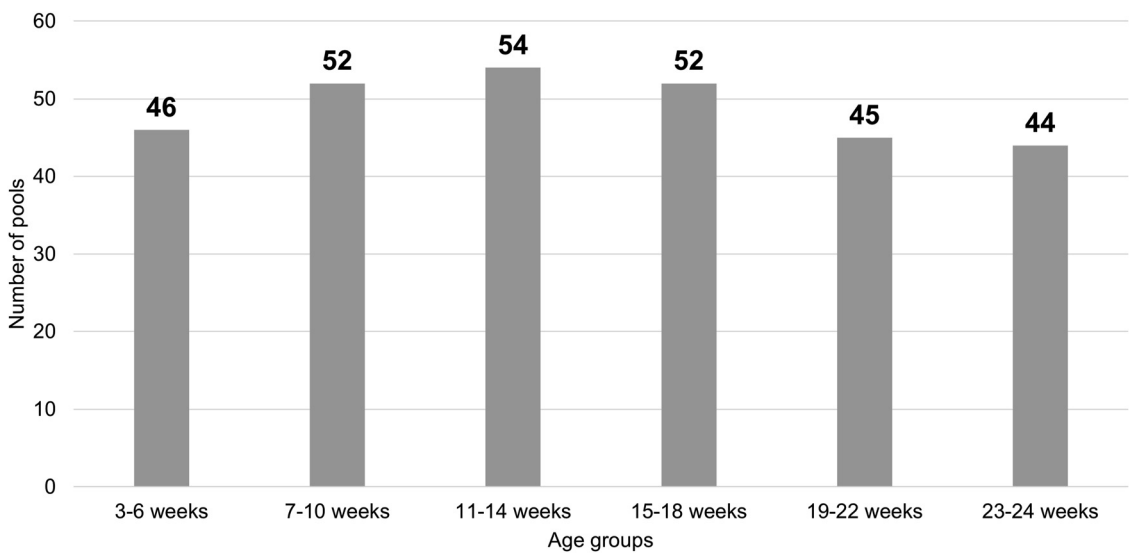


Fig. 2. Distribution of the examined pooled blood samples by age groups (*n* = 293)

magnetic glass particles (MGPs), are washed to remove contaminants, and are finally eluted in an elution buffer.

Cq values obtained from the quantitative PCR in positive cases were classified into three viral load categories. For laboratory processing, five serum samples were pooled by combining 200 µL from each individual sample and mixing thoroughly. From each pooled sample, 200 µL was subsequently used for DNA extraction. DNA extraction was performed using a bead-based method on the MagNA Pure 96 system (Roche Diagnostics, Mannheim, Germany). Thereafter, the resulting DNA extracts were tested using two PCR assays. PCV-2 was detected using an in-house real-time PCR based on a dual-labelled hybridisation probe (DLHP), employing a probe labelled with 6-carboxyfluorescein (FAM) at the 5' end and 6-carboxytetramethylrhodamine (TAMRA) at the 3' end. A PCV-2 DNA standard with known copy numbers per µL was run in parallel for quantification. The assay had a dynamic range of at least six logarithmic units (10^1 to 10^6 copies/µL DNA extract), with a limit of detection of 10^1 copies/µL DNA extract and a limit of quantification of 10^2 copies/µL DNA extract. PCV3 was tested using the commercial EXOone PCV-3 oneMIX kit (EXOPOL S.L., San Mateo de Gállego, Zaragoza, Spain), following the manufacturer's instructions. A lower Cq value indicates that fewer amplification cycles were required to detect the genomic copy signal in the positive sample.

Based on this, in the absence of quantified plasmid dilution series, positive samples were stratified into within-assay, semi-quantitative (Cq-based) viral load categories: Cq < 28 (high viral load), Cq 28–30 (medium viral load) and Cq > 30 (low viral load). This pragmatic stratification is consistent with previous PCV-3 qPCR studies (Saporiti et al., 2021b; Igriczi et al., 2022) in which Cq ≤ 30 has been used to indicate high viral loads, supporting the use of 30 as a pragmatic boundary between higher and lower viral burdens. As Cq values are assay-dependent, these categories are intended for relative comparisons within the present method and are not absolute copy-number thresholds. A PCV-3 interpretative Cq cutoff of 26.7 has been proposed for tissue diagnosis confirmation, providing additional context for classifying lower Cq values as high viral load (Cezar et al., 2024). A result was considered negative if no amplification was detected. For the commercial EXOone PCV-3 oneMIX kit (Exopol, Zaragoza, Spain), samples were interpreted as positive when Cq ≤ 38, in accordance with the manufacturer's instructions. For harmonised reporting across targets, the same positivity threshold (Cq ≤ 38) was applied to the in-house PCV-2 assay. Similar high-Cq positivity cut-offs have been used in multiplex PCV-2/PCV-3 qPCR protocols (e.g., Cq 37) (Wang et al., 2019).

The commercially available EXOone PCV-3 oneMIX kit (Exopol, Zaragoza, Spain) was used for the detection of PCV-3. As a first step, the kits were thawed and then filled with 15 µL of oneMIX reagent. Subsequently, 5 µL of nucleic acid sample was added to each tube, and both a positive control (PRS4) and a negative control (water) were included in each run. After centrifugation, the tubes were placed in the thermocycler. The qPCR protocol consisted of an initial

denaturation at 95 °C for 5 min, followed by 42 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 60 s. Following completion of the cycles, fluorescence data were analysed and results interpreted according to the manufacturer's instructions.

Detection of PCV-2 was performed at the Intervet R&D Service Laboratory using an in-house quantitative PCR (qPCR) method based on the procedures described by Brunborg et al. (2004). Both assays applied in this study were derived from previously validated protocols. PCV-3 was tested using the commercial EXOone PCV-3 oneMIX kit (Exopol, Zaragoza, Spain) according to the manufacturer's instructions. The manufacturer reports a diagnostic sensitivity and specificity of >95%. The kit manual specifies a maximum quantification limit of $\geq 10^{10}$ copies per reaction and a minimum quantification limit of at least 50 copies per reaction (kit manual). The in-house PCV-2 qPCR followed the same validated protocol described by Brunborg et al. (2004) using a dual-labelled hybridisation probe (FAM/TAMRA). Both the in-house PCV-2 assay and the commercial PCV-3 assay were run on a Bio-Rad CFX96 real-time PCR system. However, probe dyes and internal amplification controls are not specified in the available kit documentation, precluding direct comparison with the in-house assay. DNA yield was not measured by NanoDrop as part of the routine workflow and results were interpreted without normalisation to input-DNA.

Statistical analysis

Statistical analyses were performed using R statistical software (version 4.4.2; 2024-10-31, ucrt). Descriptive statistics were calculated to summarise prevalence estimates and viral load categories. Apparent prevalence was calculated directly from the raw PCR results. True prevalence was estimated by correcting for imperfect test performance using assumed PCR sensitivity and specificity values of 95% for both PCV-2 and PCV-3.

According to the manufacturer's information and published studies (Cao et al., 2024; Li et al., 2025), the sensitivity and specificity of the PCR assay exceed 95% for individual samples. However, because pooling samples may reduce diagnostic sensitivity and performance parameters for pooled samples had not been experimentally determined, a conservative value of 95% was applied to avoid both underestimation and overestimation of prevalence. The Rogan-Gladen correction formula (Rogan and Gladen, 1978) was used to adjust apparent prevalence estimates accordingly.

For individual virus prevalence, PCV-2 and PCV-3 were corrected separately, each treated as a binary outcome (positive/negative), using sensitivity and specificity values of 0.95. These estimates represent infection with the respective virus regardless of co-infection status.

Overall PCV prevalence was defined as positivity for either PCV-2 or PCV-3. Because this combined outcome is based on two diagnostic tests, its effective diagnostic performance differs from that of the individual assays. Assuming independent test errors, the specificity of the

combined outcome was calculated as 0.9025 and sensitivity was conservatively assumed to be 0.9525. These values were used in the Rogan–Gladen correction for overall prevalence.

To estimate the true prevalence of exclusive infection categories (PCV-2 only, PCV-3 only and co-infection), a joint misclassification model was applied using a matrix-based correction. This approach accounts for the combined performance of both PCR assays and adjusts the observed four-category test results (negative, PCV-2 only, PCV-3 only, both) to estimate the corresponding true infection states.

The relation between PCV-2 and PCV-3 infection status at the farm level was evaluated using Fisher’s exact test, implemented with the `fisher.test()` function in R. A significance level of 0.05 was applied.

RESULTS

Prevalence of PCV-2 and PCV-3 in the surveyed herds

The prevalence of PCV-2 and PCV-3 at the farm level and in pooled serum samples is shown in Fig. 3. Porcine circovirus type 2 and/or type 3 was detected in 94.4% of the 18 large commercial swine farms surveyed in Hungary. No farms were found to be infected solely with PCV-2 in the absence of PCV-3. One herd tested negative for both members of the *Circoviridae* family, based on PCR results. PCV-3 was detected in 94.4% (17/18) of the farms, while co-infection with PCV-2 was observed in 50% (9/18) of the herds.

Based on the pooled samples, which served as the analytical units, 61.1% (179/293) tested negative for both pathogens. The apparent prevalence of PCV-2 and/or PCV-3 was therefore 38.9% (114/293). After correction for imperfect test performance, accounting for the combined interpretation of two assays (effective specificity 0.9025 and conservative sensitivity 0.9525), the estimated true prevalence was 34.1% (approximately 99/293 pooled samples).

The apparent prevalence of PCV-2 in pooled samples was 14.7% (43/293). After correction, the estimated true prevalence was 10.7% (approximately 32/293).

The apparent prevalence of PCV-3 was 30.4% (89/293), corresponding to a corrected prevalence of 28.2% (approximately 83/293). The co-infection rate among the examined pooled samples was 6.8% (20/293).

At the farm level, application of the same correction did not change the classification of farms as positive or negative; therefore, the farm-level prevalence estimates remained unchanged.

Prevalence of PCV-2 and PCV-3 in different age groups

The observed and corrected prevalence of PCV-2 and PCV-3 across different age groups is shown in Fig. 4. PCV-2 was detected in 2.2% (1/46) of the pooled samples from the 3–6-week-old age group during the post-weaning period. The prevalence increased steadily across age groups, except for the 11–14 week-old age group in which the PCV-2 prevalence was 0%, reaching 31.1% in the 19–22-week-old group.

PCV-3 positivity was already detected in the youngest age group (3–6-week-old piglets, 10.9% of all pooled samples). The number of positive samples increased steadily up to the 15–18-week group, after which the prevalence fluctuated. The highest prevalence was observed during the second part of the finishing phase, at 23–24 weeks of age.

Relation between herd-level prevalence of PCV-2 and PCV-3

The relation between PCV-2 and PCV-3 infection status was evaluated using Fisher’s exact test. No statistically significant difference was detected ($P = 1.0$). Although all PCV-2-positive farms were also positive for PCV-3, most farms were PCV-3 positive regardless of their PCV-2 status (Table 1). These results suggest that while PCV-2 infection occurred exclusively in PCV-3-positive farms, PCV-3 infection was widespread and not dependent on PCV-2 status.

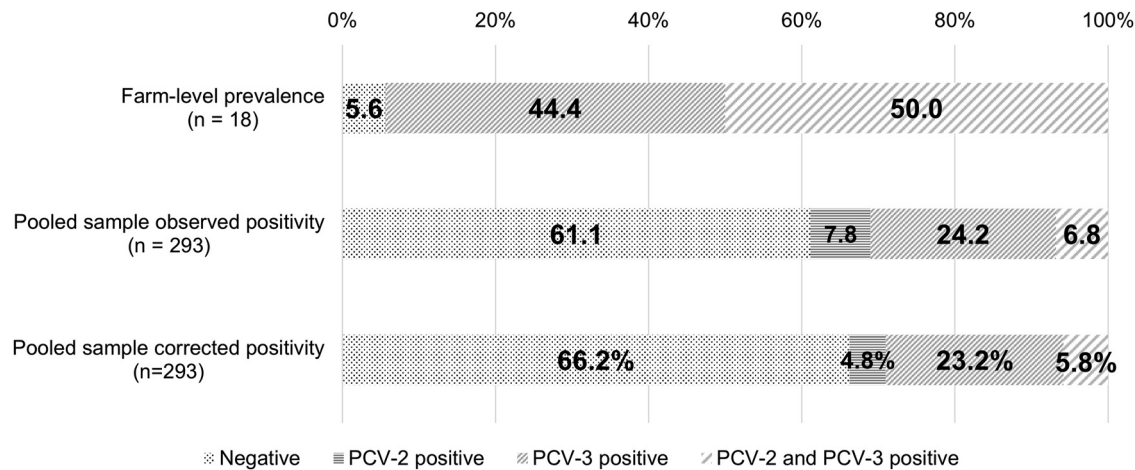


Fig. 3. Observed and corrected prevalence of PCV-2 and PCV-3 at the farm level ($n = 18$) and in pooled serum samples ($n = 293$)

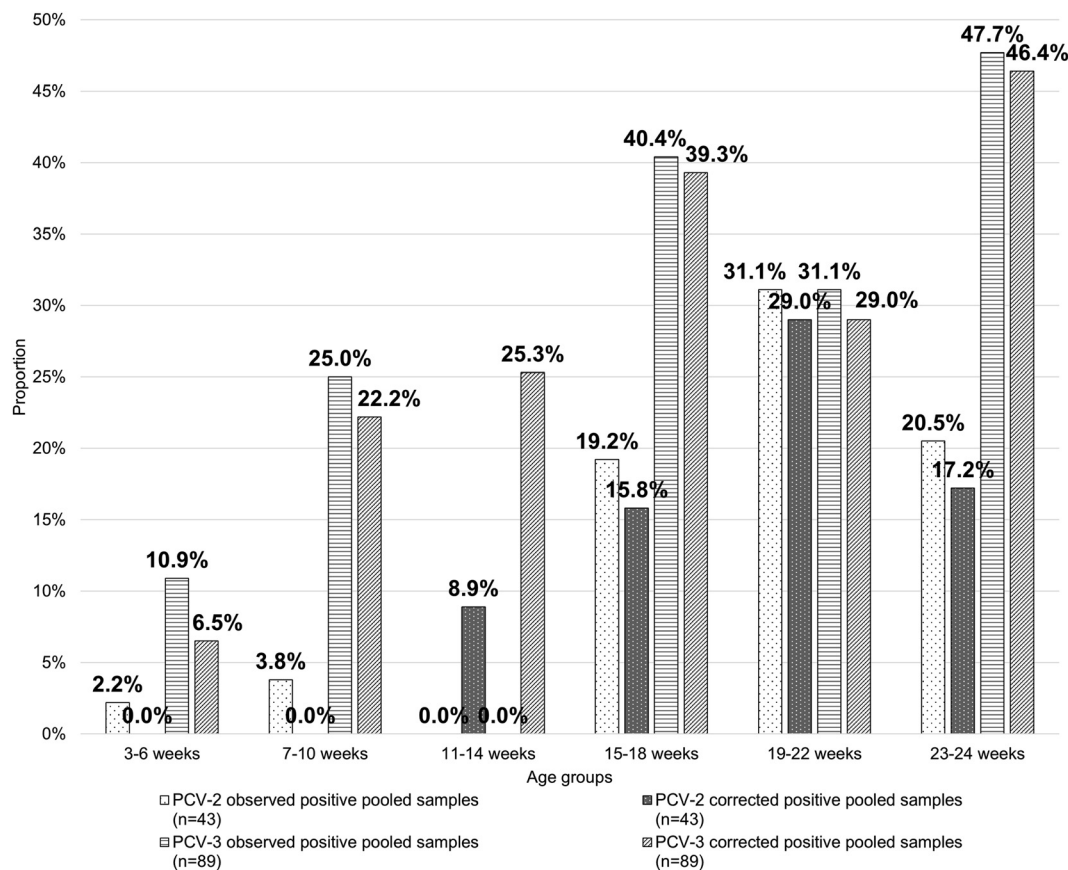


Fig. 4. Proportion of PCV-2 (*n* = 43) and PCV-3 (*n* = 89) positive pooled samples across age groups

Table 1. Four-fold contingency table used in the statistical analysis (*n* = 18)

	PCV-3 infected	PCV-3 not infected
PCV-2 infected	9	0
PCV-2 not infected	8	1

Table 2. Comparison of PCV-2 and PCV-3 prevalence in farms vaccinated or not vaccinated against PCV-2 (*n* = 18)

Swine farm	PCV-2		PCV-3	
	Positive	Negative	Positive	Negative
Vaccinated	8	9	16	1
Not vaccinated	1	0	1	0

Effects of PCV-2 vaccination and PCV-3 infection

A comparison was made between farms using vaccines against PCV-2 to assess a possible correlation with PCV-3 infection. Of the 18 swine farms surveyed, 17 included a PCV-2 vaccine in their routine vaccination programs. The applied PCV-2 vaccines represented five different types (Vaccine A-E), which differed in both duration of immunity and mode of administration. Among the vaccinated farms, 47.1% (8/17) still tested positive for PCV-2 and 94.1% (16/17) were positive for PCV-3. The single farm that did not apply a PCV-2 vaccine tested positive for both PCV-2 and PCV-3 (Table 2).

Detection of PCV-3 by quantitative PCR assay

Serum samples were examined by quantitative PCR to detect the genetic material of PCV-3, allowing estimation of the viral infection rate in positive cases. Cq values obtained by quantitative PCR were also analysed according to three age groups in order to assess PCV-3 viral load levels across

different production phases: (1) nursery, 3-10-week-old animals; (2) first finishing phase, 11-18-week-old animals; and (3) second finishing phase, 19-24-week-old animals. The distribution of PCV-3 viral load categories in pooled samples is shown in Fig. 5.

The majority of positive cases belonged to the low viral-load group ($C_q > 30$; 24.2%). A medium viral load ($28 < C_q < 30$) was observed in 5.8% of the pooled samples, while a high viral load ($C_q < 28$) was detected in 1.0% of the pools. Among the pooled samples collected during the nursery phase, 16.3% belonged to the low viral-load group and 3.1% to the medium viral-load group. In total, 80.6% of the pooled samples tested negative and no high viral load was detected during this phase. Animals typically enter the finisher barn at around 70 days of age and the subsequent fattening period was divided into two phases. In the first finishing phase, animals with a high viral load were already present (1.9%) and 65.1% of the total pooled samples tested negative for PCV-3. Compared to the nursery phase,

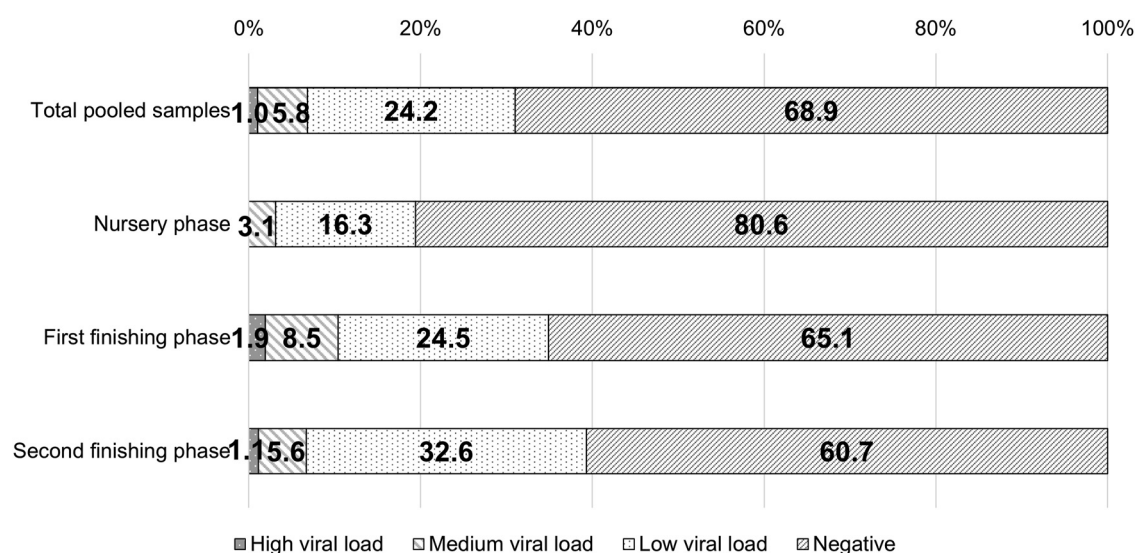


Fig. 5. Distribution of PCV-3 viral load categories in pooled samples ($n = 293$), overall and by production phase

the proportions of animals with low and medium viral loads increased. In the second finishing phase, the trend changed compared to the previous results: the proportions of animals with high and medium viral loads decreased slightly. However, the proportion of swine with a low viral load continued to increase, reaching 32.6%, while only 60.7% of the total pooled samples were negative.

DISCUSSION

This study presents a comprehensive dataset on the prevalence of PCV-2 and PCV-3 in large commercial pig farms in Hungary. Our results reveal high co-infection rates (50.0%) and the presence of PCV-3 across all age groups, suggesting continuous within-herd circulation (Igriczi et al., 2022; Klaumann et al., 2018). The detection of PCV-3 in clinically healthy pigs from multiple regions of the country supports the hypothesis of persistent subclinical infections (Igriczi et al., 2022; Saporiti et al., 2021a). These findings broaden the current understanding of PCV epidemiology in Central and Eastern Europe, where large-scale surveillance data remain scarce (Deim et al., 2019; Igriczi et al., 2022; Šolaja et al., 2025).

Prevalence of PCV-2 and PCV-3

In our survey, PCV-3 was detected in 94.4% of the examined Hungarian swine farms, and co-infection with PCV-2 was observed in 50.0% of the herds. However, no correlation was found between the herd-level prevalence of the two viruses. When considering the corrected pooled-sample prevalences, PCV-3 was 28.2% and PCV-2 was 10.7%. These results show a similar PCV-3 prevalence, a slightly lower PCV-2 prevalence and a substantially higher herd-level co-infection proportion than those reported in international studies, for example in the USA, where between 2016 and 2018, PCV-3

prevalence was 25.0%, PCV-2 prevalence was 12.3% and co-infection was 2.9% (Wang et al., 2020). Co-infection is known to markedly reduce resistance to other pathogens through the immunosuppressive effects of circoviruses (Ouyang et al., 2019). Importantly, in both our study and the cited US study, no clinical signs were observed at sampling, supporting the possibility that PCV-3 may often occur as a subclinical infection (Stadejek et al., 2017; Wang et al., 2020).

In our results, PCV-2 prevalence increased with age, reaching its highest level in the 19–22-week-old group. Improper vaccination timing – either before the decline of maternal antibodies or without considering the duration of vaccine protection – may explain this increase. Among the vaccinated farms, 47.1% (8/17) still tested positive for PCV-2. Because detailed information on vaccination timing, vaccine types and coverage was unavailable, this finding should not be interpreted as evidence of reduced vaccine efficacy. Rather, it is consistent with previous findings that PCV-2 vaccines are effective in preventing clinical disease but typically do not fully eliminate viral circulation within herds. Moreover, PCV-2 vaccines do not provide cross-protection against PCV-3 due to marked antigenic differences (Beach and Meng, 2012; Kristensen et al., 2015; Palinski et al., 2017; Woźniak et al., 2019; Bandrick et al., 2022).

The detection of PCV-3 across all age groups in Hungary is consistent with global findings of high homology and a slow evolutionary rate (Chen et al., 2019b; Ouyang et al., 2019; Wang et al., 2020). PCV-3 genomes are highly conserved, with 94.4–100% nucleotide identity across strains (Chen et al., 2019b; Ouyang et al., 2019; Wang et al., 2020). Phylogenetic analyses indicate a close relationship with bat circoviruses and retrospective investigations have confirmed PCV-3 circulation in swine populations since at least 1966 (Ouyang et al., 2019). Despite 35–40% capsid similarity to PCV-2, no serological cross-reactivity has been demonstrated (Bi et al., 2020).

Our results demonstrated PCV-3 positivity in nursery, grower and finisher phases, which may suggest ongoing within-herd transmission. PCV-3 has also been detected in sows with reproductive disorders, as well as in mummified and stillborn fetuses, suggesting vertical transmission (Kedkovid et al., 2018; Deim et al., 2019). Shedding through oral fluids and colostrum has also been described, with virus titers correlating between serum and colostrum levels (Kedkovid et al., 2018). Experimental and field studies have also identified PCV-3 in vectors such as mosquitoes, implying potential additional mechanical transmission routes (Ha et al., 2020). The relatively high prevalence we observed in early nursery pigs compared to younger piglets suggests that maternal immunity may temporarily suppress PCV-3 replication in early life, with post-weaning transmission being more prominent (Stadejek et al., 2017; Klaumann et al., 2019).

The widespread detection in Hungarian farms without overt clinical signs may indicate persistent subclinical circulation and possible adaptation of PCV-3 to swine populations. This aligns with international reports of PCV-3 in wild boars in Germany, Italy, Portugal, Serbia and Spain (Prinz et al., 2019; Nišavić et al., 2024; Almeida et al., 2025; Melegari et al., 2025), as well as in dogs and cattle (Hou et al., 2020; Vargas-Bermúdez et al., 2021), supporting its broad host range and potential for cross-species transmission.

Clinical manifestations

The pathogenesis of PCV-3 has not yet been fully clarified. In our study, pigs appeared clinically healthy despite high PCV-3 prevalence, which is consistent with the hypothesis that the virus often persists in a subclinical form. However, the high prevalence observed in pooled serum samples suggests that PCV-3 might contribute to latent immune modulation, similar to PCV-2. In foetal samples from Hungarian farms with reproductive problems, PCV-3 was detected without other pathogens, raising the possibility of a causal role in reproductive disorders (Deim et al., 2019). Saporiti et al. (2021a) demonstrated that PCV-2 co-infections with other pathogens result in more severe disease outcomes under both field and experimental conditions. The impact of PCV-3 co-infections remains poorly understood, but their frequent detection suggests that PCV-3 may exacerbate clinical signs or immune suppression, albeit this remains unproven. Although our study did not include clinical or pathological data, the observed high co-infection rate of PCV-2 and PCV-3 aligns with previous reports suggesting potential interactions between the two viruses.

Most of the positive pooled samples in our study belonged to the low viral load group (24.2%), while medium and high viral loads were rare (5.8% and 1.0%, respectively). No high viral load was observed in the nursery phase, while low and medium loads increased during the first phase of finishing. In the second phase, high and medium loads slightly decreased, but the prevalence of low viral loads further increased to 32.6%. This distribution suggests that

PCV-3 circulates persistently in herds, mostly at low levels, in line with observations from Spain and Poland (Stadejek et al., 2017; Klaumann et al., 2019).

Although the role of PCV-3 in disease remains debated, replication in vitro has already been achieved, albeit with relatively low efficiency compared to PCV-1 and PCV-2 (Mora-Díaz et al., 2020; Oh et al., 2020). Experimental studies have associated PCV-3 with reproductive failures, myocarditis, encephalitis and PDNS-like lesions (Ouyang et al., 2019; Faccini et al., 2017; Jiang et al., 2019) and in some cases reproduced fever, poor growth, diarrhoea and multisystemic inflammation (Jiang et al., 2019; Alomar et al., 2021). However, the virus does not consistently induce clinical disease under experimental settings. These findings highlight the need for further pathogenesis studies to clarify the clinical relevance of PCV-3.

The high proportion of subclinical infections in Hungary suggests that PCV-3 may impair growth or immune responses without overt clinical disease, especially during the stressful peri-weaning period. This interpretation is supported by studies highlighting the importance of stress, immune suppression and microbiome alterations in PCV-3-infected piglets (Campbell et al., 2013; Hou et al., 2020). Infected animals showed decreased microbial diversity and altered *Firmicutes/Bacteroidetes* ratios, correlating with weight loss and inflammation (Jiang et al., 2019; Hou et al., 2020). Although the microbiome was not investigated here, the increased prevalence of low viral load infections in the finisher phases may indicate long-term subclinical effects on productivity.

Diagnostics and control strategies

Quantitative PCR was successfully applied in our study to determine the viral load and prevalence of PCV-3, with most positive pools showing low viral loads. This pattern is consistent with European qPCR-based studies, which reported Cq values corresponding to low to moderate viral copy numbers (Yang et al., 2019; Alomar et al., 2021). Various molecular diagnostic methods, including multiplex PCR, digital droplet PCR (ddPCR) and quantitative PCR (qPCR), have been developed to enable sensitive detection and differentiation of PCV-1, PCV-2 and PCV-3 in clinical samples (Saporiti et al., 2020; Yang et al., 2022).

Our findings also showed that PCV-2 vaccination, applied in almost all surveyed farms, did not fully prevent PCV-2 detection and had no protective effect against PCV-3. This aligns with international findings showing that current PCV-2 vaccines do not provide cross-protection due to antigenic differences between PCV-2 and PCV-3 (Woźniak et al., 2019; Yang et al., 2019). However, as the present study was not designed to assess vaccine efficacy, these results should be interpreted with caution.

Considering the widespread detection of PCV-3 and its unclear clinical significance, the development of new control strategies, such as combined PCV-2/PCV-3 vaccines, might be considered in the future. Access to reliable and standardised diagnostic tools remains crucial for clarifying the epidemiological and clinical relevance of PCV-3 in swine

health and production. Recent advances in assay development (Shen et al., 2012; Woźniak et al., 2019) and preliminary experimental vaccine studies (Chen et al., 2019a; Woźniak et al., 2020; Fan et al., 2025) reflect the growing recognition of PCV-3 as a pathogen of potential relevance to swine health, highlighting the need for continued monitoring and further research into effective control strategies.

Limitations

Sampling was based on voluntary participation of large commercial farms, resulting in a convenience sample rather than a random national selection. Consequently, selection bias cannot be excluded and the findings may not fully represent the entire Hungarian swine population. Regional coverage was also uneven, further limiting geographical representativeness.

Serum samples were tested in pools of five to increase testing efficiency, which likely reduced the sensitivity for detecting low-titer infections. Therefore, both apparent and corrected prevalence estimates were reported to account for imperfect diagnostic sensitivity and specificity. As a single high-shedding animal could influence the pool's Cq value, viral load categories should be interpreted with caution. Moreover, the analytical cutoff for PCR positivity ($Cq \approx 37$ – 38) does not necessarily indicate clinical relevance, as Cezar et al. (2024) reported that only much higher viral loads ($Cq \approx 22$) were associated with histopathological evidence of PCV-2 systemic disease.

Although most participating farms applied PCV-2 vaccination, the study was not designed to assess vaccine efficacy or cross-protection. Hence, the persistence of PCV-2 and lack of apparent protection against PCV-3 should not be overinterpreted. The limited sample size ($n = 18$) reduced the statistical power of herd-level analyses and potential confounders (e.g., age, vaccination status, region) could not be accounted for. Finally, the cross-sectional design provided only a single time point per age group and farm, precluding assessment of temporal trends. Longitudinal studies with broader geographic coverage and individual-level testing are needed to confirm and extend these findings.

CONCLUSIONS

This large-scale study demonstrates that PCV-3 is widespread in Hungarian commercial swine herds across production phases, typically at low viral loads without overt clinical signs, consistent with persistent subclinical infection. Despite widespread PCV-2 vaccination, PCV-2/PCV-3 co-infection was common, suggesting limited cross-protection and a potential impact on herd productivity. Overall, the findings support continued monitoring alongside strong management and biosecurity, while future work should clarify PCV-3 pathogenesis and evaluate improved diagnostics and potential combined PCV-2/PCV-3 vaccines.

Ethical approval: According to the Hungarian legal regulations (Act CXXVII of 2012 on the Hungarian Veterinary

Chamber and the provision of veterinary services. Available at: <https://njt.hu/jogszabaly/2012-127-00-00>, accessed on 12 March 2025), no ethics committee permission was necessary for blood collection from pigs. All procedures were carried out by licensed veterinarians in accordance with national legislation and the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes.

Competing interest: There are no competing interests.

Author contributions: PM: conceptualisation, methodology, validation, investigation, data curation, writing - original draft preparation, writing - review and editing, supervision, project administration, funding acquisition. LM: formal analysis, investigation, data curation, writing - original draft preparation, writing - review and editing, visualisation. IM: investigation, data curation, writing - review and editing. MM: writing - original draft preparation, writing - review and editing, visualisation. BP: formal analysis, writing - review and editing. HS: methodology, validation, investigation, writing - review and editing; LÓ: conceptualisation, methodology, validation, resources, writing - original draft preparation, writing - review and editing, supervision, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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