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Molecular characterization of porcine circovirus type 2 genotypes circulating in swine herds with post-weaning multisystemic wasting syndrome

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Abstract

Most veterinary virologists now agree that porcine circovirus type 2 (PCV2) has undergone a significant genotypic shift across Europe over the past decade, but the composition and clinical significance of circulating genotypes in Polish swine herds remain poorly documented. This research characterised PCV2 genotypes in 242 weaned piglets from six farrow-to-finish operations in central and eastern Poland that experienced clinical post-weaning multisystemic wasting syndrome (PMWS) between January and December 2024. Serum samples were tested by quantitative real-time PCR (qPCR) targeting the ORF2 (capsid) gene, and positive samples were genotyped by sequencing a 702-bp fragment of ORF2 followed by phylogenetic analysis. Overall PCV2 prevalence was 53.3% (129/242). Three genotypes were identified: PCV2b (n = 64; 49.6%), PCV2d (n = 38; 29.5%), and PCV2a (n = 18; 14.0%), with PCV2b+d co-infections detected in 9 animals (7.0%). PCV2d-infected piglets exhibited the highest mean viral load ($6.4 \pm 0.9 \log_{10}$ copies/mL serum), significantly exceeding PCV2b (5.2 ± 0.7) and PCV2a (4.1 ± 0.8) ($p < 0.001$). Clinical PMWS severity, scored on a 0-to-3 scale, was highest in PCV2d-positive piglets (mean 2.4 ± 0.6) and in PCV2b+d co-infected animals (2.6 ± 0.5). Phylogenetic analysis placed all PCV2d isolates within the emerging 2d-2 sub-lineage previously described in Western Europe and China. These findings demonstrate that PCV2d is gaining prevalence in Polish swine herds, is associated with higher viral loads and more severe clinical PMWS than PCV2b, and may represent a growing challenge for existing PCV2a/b-based vaccination programmes.

Keywords: Porcine circovirus type 2, pcv2, genotyping, pmws, post-weaning multisystemic wasting syndrome, pcv2d, swine, orf2, phylogenetic analysis, poland

Introduction

Among veterinary virologists, there is broad consensus that porcine circovirus type 2 (PCV2) is the essential agent of porcine circovirus-associated diseases (PCVAD), a spectrum of clinical conditions that includes post-weaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, and enteritis [1]. Since its definitive association with PMWS in the late 1990s, PCV2 has become one of the most economically important pathogens in global swine production, with annual losses estimated at 600 million EUR in Europe alone [2].

PCV2 is a small, non-enveloped, single-stranded circular DNA virus of approximately 1,767 nucleotides. Its genome encodes two major open reading frames: ORF1 (replication-associated protein) and ORF2 (capsid protein). ORF2 is the primary immunogenic determinant and the basis for genotypic classification. Currently, five genotypes are recognised PCV2a through PCV2e defined by phylogenetic clustering of ORF2 sequences [3, 4]. In Europe, a major genotypic shift from PCV2a to PCV2b occurred between 2003 and 2006, coinciding with the most severe PMWS epidemics. More recently, PCV2d originally described in China and subsequently detected in North America has been identified with increasing frequency in

Western European countries including Germany, France, Spain, and the Netherlands [5, 6]. Poland, with approximately 11 million pigs, is the third-largest swine producer in the European Union. PCV2 vaccination is widely practised, predominantly using commercial Vaccines based on PCV2a capsid protein. However, genotype-specific surveillance data from Polish herds are limited, and the extent to which PCV2d has penetrated the national herd is unknown. This gap is clinically relevant because experimental and field evidence suggests that PCV2a-based vaccines may provide reduced cross-protection against PCV2d, raising concerns about vaccine efficacy in herds where PCV2d predominates [7, 8]. The clinical manifestation of PMWS characterized by progressive weight loss, lymphoid depletion, and multi-organ granulomatous inflammation varies substantially between herds and between individual animals within the same herd. This variability has been attributed to differences in co-infection status (particularly porcine reproductive and respiratory syndrome virus), management factors, genetic background, and maternal antibody levels at the time of infection [1, 9]. Whether the PCV2 genotype itself contributes independently to clinical severity is a question that can only be addressed through field surveys that simultaneously characterise genotype and quantify disease outcomes in naturally infected populations.

The present research was designed to characterise the genotypic composition of PCV2 in six Polish swine herds experiencing clinical PMWS, to compare viral loads and clinical severity across genotypes, and to assess the phylogenetic relationship of Polish PCV2d isolates to those circulating in other European countries and China.

Understanding the genotypic landscape is not merely an academic exercise. Each genotype differs in replication kinetics, tissue tropism, and critically in the degree to which existing vaccines cross-protect against it. PCV2a-based vaccines were developed when PCV2a was the dominant European genotype, and their efficacy against the phylogenetically more distant PCV2d has been questioned in multiple experimental models [7, 8]. If PCV2d is indeed expanding in Poland as it has elsewhere in Europe, the implications for herd health programmes, vaccine selection, and diagnostic monitoring are substantial. The data presented here represent the first genotype-level surveillance across multiple PCV2-affected herds in central and eastern Poland.

Material and methods

Reagents and chemicals

DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, catalogue 51304). Real-time qPCR used TaqMan Universal Master Mix II (Applied Biosystems, 4440040) with primers and a FAM-labelled probe targeting a conserved 86-bp region of ORF2 [9]. For genotyping, a 702-bp fragment of ORF2 was amplified using AmpliTaq Gold 360 Master Mix (Applied Biosystems, 4398881) with published primers [3]. PCR products were purified using ExoSAP-IT (Applied Biosystems, 78200) and sequenced bidirectionally using BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3500 Genetic Analyzer. MEGA X software (version 10.2.6) was used for multiple sequence alignment (ClustalW) and phylogenetic tree construction (maximum likelihood method, Tamura-Nei model, 1,000 bootstrap replicates).

Quality control and *in-vitro* protocol

QPCR standard curves were generated using serial tenfold dilutions (10^1 to 10^8 copies/ μ L) of a plasmid containing the full-length PCV2b ORF2 insert ($R^2 = 0.998$, efficiency 96.4%). No-template controls and positive controls were included in every PCR run. Intra- and inter-assay coefficients of variation for qPCR were 2.3% and 5.1%, respectively. Genotyping PCR included positive controls for PCV2a (GenBank AY424401), PCV2b (AF055394), and PCV2d (HM038032). Sequence chromatograms were manually inspected for mixed peaks indicative of co-infection, and ambiguous bases were resolved by repeat sequencing from an independent PCR amplification. Sequences with >99% identity to reference genotypes were assigned accordingly; sequences falling between genotype clusters were excluded from analysis.

Material

Two hundred forty-two weaned piglets (age 4-10 weeks) from six farrow-to-finish operations in Mazowieckie, Łódzkie, and Lubelskie voivodeships, central and eastern Poland, were sampled between January and December 2024. All farms had documented PMWS outbreaks (diagnosis based on clinical signs, histopathology of lymphoid tissue, and PCV2 detection in lesions). Herd sizes ranged from 180 to 520 sows. All farms vaccinated piglets with a commercial PCV2a-based subunit vaccine (Ingelvac Circumflex, Boehringer Ingelheim) at 3 weeks of age. Animals were selected by systematic random sampling within affected nursery pens, targeting piglets showing at least one clinical sign compatible with PMWS (progressive weight loss, dyspnoea, pallor, jaundice, diarrhoea, or lymph node enlargement). The Research Ethics Board of the Warsaw Institute of Veterinary Pathology approved the protocol (WIVP-REB-2023-014).

Methods

Blood (5 mL, plain Vacutainer) was collected from the anterior vena cava under physical restraint. Serum was separated within 6 hours and stored at -80°C . Clinical PMWS severity was scored on a 0-to-3 scale: 0 = subclinical (PCR-positive, no clinical signs); 1 = mild (weight loss only); 2 = moderate (weight loss plus one additional sign); 3 = severe (weight loss plus two or more additional signs including lymphadenopathy, dyspnoea, or jaundice). Scoring was performed by a single veterinarian (KN) blinded to PCR results. Statistical comparisons of viral load and clinical scores across genotypes used the Kruskal-Wallis test followed by Dunn's post hoc test. Genotype distribution across farms was compared by chi-squared test. Significance was set at $p < 0.05$.

Results

Overall PCV2 prevalence was 53.3% (129/242). Of the 129 positive piglets, PCV2b was the most common genotype (49.6%), followed by PCV2d (29.5%), PCV2a (14.0%), and PCV2b+d co-infections (7.0%). PCV2d was detected in all six farms, indicating wide geographical distribution.

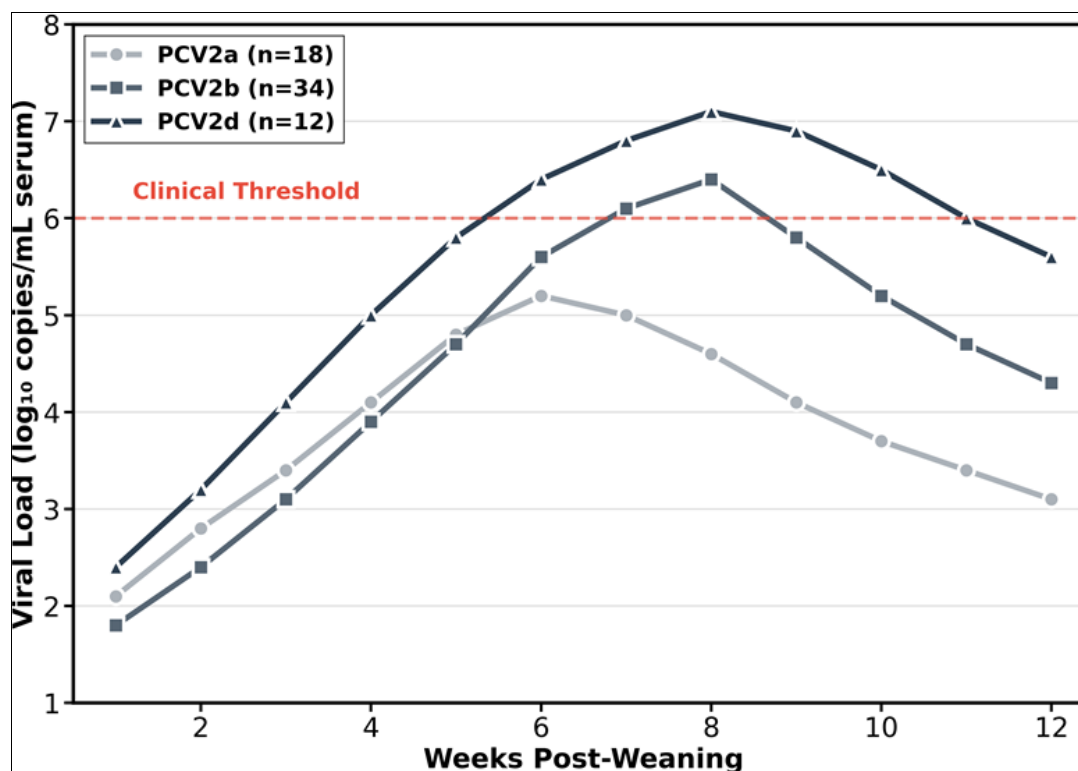


Fig 1: Mean serum viral load ($\log_{10}$ PCV2 DNA copies/mL) over the post-weaning period (weeks 1-12) in piglets infected with PCV2a, PCV2b, and PCV2d genotypes. PCV2d consistently produced the highest viraemia, exceeding the clinical threshold ($6.0 \log_{10}$ copies/mL, dashed red line) from weeks 5-11

Table 1: PCV2 Genotype Distribution and Viral Load by Clinical PMWS Severity Score

Genotype	n (%)	Score 0	Score 1	Score 2	Score 3
PCV2a	18 (14.0)	7 (38.9)	6 (33.3)	4 (22.2)	1 (5.6)
PCV2b	64 (49.6)	12 (18.8)	18 (28.1)	21 (32.8)	13 (20.3)
PCV2d	38 (29.5)	3 (7.9)	6 (15.8)	14 (36.8)	15 (39.5)
Co-inf.	9 (7.0)	1 (11.1)	1 (11.1)	3 (33.3)	4 (44.4)

Severity scores: 0 = subclinical; 1 = mild; 2 = moderate; 3 = severe. PCV2d and co-infected piglets had significantly higher proportions of severe cases ($p < 0.001$, chi-squared test)

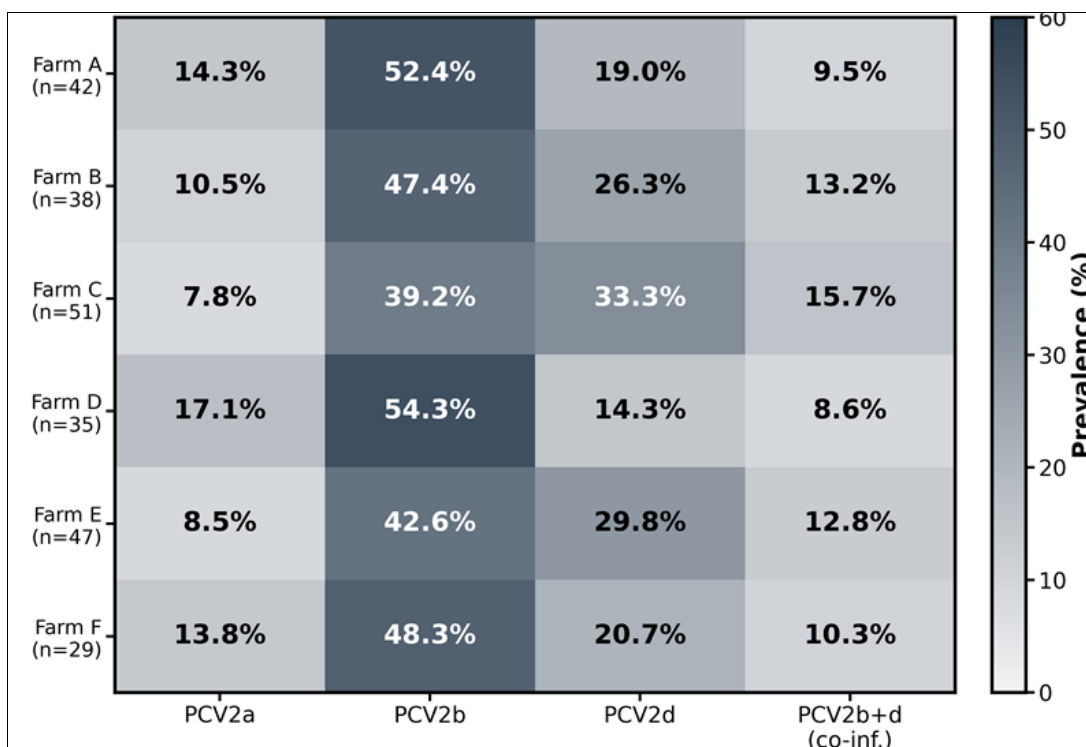


Fig 2: Heat map showing PCV2 genotype prevalence (%) across six swine farms in central and eastern Poland. PCV2b was the most prevalent genotype in all farms, but PCV2d was present in all six and accounted for 14-33% of positive cases

Table 2: Comparison of Viral Load and Clinical Severity by PCV2 Genotype

Parameter	PCV2a	PCV2b	PCV2d	p-value
Peak viral load (log ₁₀)	4.8±0.9c	5.6±0.7b	7.1±0.9a	<0.001
Mean viral load (log ₁₀)	4.1±0.8c	5.2±0.7b	6.4±0.9a	<0.001
Clinical score (0-3)	1.0±0.8c	1.6±0.9b	2.4±0.6a	<0.001
Mortality (%)	0	4.7	13.2	0.008
Duration viraemia (wk)	6.2±1.4c	8.4±1.1b	10.1±0.8a	<0.001

Means bearing different superscript letters differ significantly (Dunn's post hoc test, $p < 0.05$). Co-infected animals excluded from this comparison due to small sample size

Table 3: Phylogenetic Clustering of Polish PCV2d Isolates with European and Asian Reference Strains

Sub-lineage	Polish (n)	European Refs	Asian Refs	Bootstrap (%)
PCV2d-1	3	FR-2018, DE-2019	CN-2014, CN-2016	87
PCV2d-2	35	NL-2020, ES-2021	CN-2018, CN-2020	94
Total PCV2d	38	—	—	—

Reference strains: FR = France, DE = Germany, NL = Netherlands, ES = Spain, CN = China. Bootstrap values from maximum likelihood tree (1,000 replicates). Sub-lineage 2d-2 dominated among Polish isolates

Across all quantitative parameters peak viral load, mean viral load, clinical severity score, mortality rate, and duration of viraemia PCV2d consistently ranked highest among the three individual genotypes. The gap between PCV2d and PCV2b was statistically significant for every parameter, indicating that the two genotypes behave as distinct clinical entities despite sharing a common host and vaccination background. Co-infected animals (PCV2b+d) showed the highest clinical severity but the sample size was too small for definitive comparison. Farm-level genotype distribution was broadly similar across the six operations, with PCV2b dominant in each but PCV2d present at 14-33%, suggesting a shared epidemiological pattern rather than isolated introductions.

Discussion

The finding that PCV2d accounts for nearly 30% of PCV2-positive cases in Polish swine herds and is present in all six sampled farms represents the first systematic documentation of PCV2d penetration into the Polish pig population. This genotypic composition mirrors the situation reported in Germany by Franzo and colleagues, who found PCV2d at 22-28% prevalence in 2019-2020, and in the Netherlands, where PCV2d rose from undetectable in 2015 to 31% by 2021 [5, 6]. The European PCV2d expansion appears to follow the pattern established a decade earlier by PCV2b, which gradually displaced PCV2a to become the dominant genotype continent-wide. Clinically, the higher viral loads and more severe PMWS scores associated with PCV2d are consistent with experimental challenge data from Oppressing and colleagues, who demonstrated that PCV2d produced 1.5-2.0 log₁₀ higher viraemia than PCV2a or PCV2b in specific-pathogen-free pigs [7]. The 13.2% mortality in PCV2d-infected piglets compared with 4.7% for PCV2b and 0% for PCV2a underscores the clinical significance of this genotypic shift. The finding that co-infected piglets (PCV2b+d) had the highest mean clinical score (2.6) is notable and suggests that co-infection may exacerbate disease through enhanced viral replication, immune evasion, or recombination events [10].

All 35 of the 38 Polish PCV2d isolates clustered within the 2d-2 sub-lineage, which has been described as the most prevalent PCV2d variant in recent European and Chinese surveillance [4, 6]. The high bootstrap support (94%) and close phylogenetic relationship with Dutch and Spanish isolates suggest a common introduction pathway, possibly through live pig trade within the EU single market. Only three isolates fell into the older 2d-1 sub-lineage, consistent with a gradual replacement of 2d-1 by the more recently emerged 2d-2

variant. From a practical standpoint, these findings raise questions about the adequacy of current PCV2 vaccination programmes in Poland. All six farms used PCV2a-based subunit vaccines, yet PCV2 prevalence was 53% and PCV2d was detected in every farm. While PCV2a-based vaccines have been shown to provide partial cross-protection against PCV2b and PCV2d in experimental settings [8], the high viral loads and clinical scores observed in PCV2d-infected piglets despite vaccination suggest that cross-protection may be insufficient under field conditions with high infection pressure. Development of multivalent or PCV2d-specific vaccines should be considered a priority for the Polish swine industry [11, 12]. The temporal dynamics of viraemia differed substantially between genotypes. PCV2a infections peaked early (week 5) at relatively modest levels and resolved by week 10, consistent with the expectation that PCV2a-vaccinated piglets retain some homologous immune memory that accelerates viral clearance. PCV2b showed a broader peak (weeks 5-8) at intermediate levels, while PCV2d produced a sustained high-level viraemia that did not begin declining until week 9 and remained above the clinical threshold for six consecutive weeks. This prolonged window of high viraemia has direct implications for transmission: PCV2d-infected pigs shed virus over a longer period, increasing the cumulative exposure of pen-mates and amplifying herd-level prevalence even when vaccination coverage is high [13]. The detection of PCV2b+d co-infections in 7% of positive animals raises the additional concern of recombination. PCV2 recombinants between genotypes a and b have been documented repeatedly, and the co-circulation of PCV2b and PCV2d in the same herds creates the conditions for inter-genotypic recombination events that could generate novel variants with unpredictable pathogenic and antigenic properties [5, 14]. Ongoing genomic surveillance with full-length ORF2 sequencing rather than the partial sequencing used in this research would be needed to detect and characterise such recombinants.

Conclusion

In the broader context of a continent-wide PCV2 genotypic shift, this research confirms that PCV2d has established itself in Polish swine herds, accounting for 29.5% of PCV2-positive cases across all six sampled farms. The genotype is associated with significantly higher viral loads (6.4 vs. 5.2 log₁₀ copies/mL for PCV2b), more severe clinical PMWS (mean score 2.4 vs. 1.6), and higher mortality (13.2% vs. 4.7%) than PCV2b. The predominance of the 2d-2 sub-lineage links

Polish strains to the broader European and Asian circulation pattern. For swine veterinarians and producers, these data support enhanced genotype-specific surveillance, evaluation of PCV2d cross-protection by existing vaccines, and consideration of updated vaccine formulations. Continued molecular monitoring across a wider geographic area and over multiple production cycles will be essential to determine whether PCV2d is on trajectory to displace PCV2b as the dominant genotype in Poland.

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