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Novel Porcine Parvoviruses (PPV2–PPV7) in Colombian Gilts: Coinfection with PCV2–PCV4, PRRSV, and Link to Enhanced Farrowing Rate

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ABSTRACT

Seven novel porcine parvoviruses (nPPVs), designated PPV2 through PPV8, have been identified, though their pathogenic potential and contribution to porcine reproductive failure (PRF) remain unclear. The present investigation examined the occurrence of these nPPVs in gilts from Colombia, explored their coinfections with PPV1, PCV2, PCV3, PCV4, and PRRSV, and assessed possible links between nPPV detection and reproductive performance parameters (RPPs) in sows. A total of 234 serum samples were obtained from healthy gilts across 40 herds distributed in five Colombian regions, and real-time PCR was used for viral detection. Circulation of PPV2 through PPV7 was confirmed in Colombia, with PPV3 (40%), PPV5 (20%), and PPV6 (17%) being the most common. Neither PCV4 nor PPV8 was found. Multiple concurrent infections were observed among PPV2–PPV7 and the primary PRF-associated viruses, ranging from dual to sextuple coinfections. Statistical analysis revealed significant associations: PPV6 was more frequent in PCV3-positive gilts ($p < 0.01$) and PPV5 in PRRSV-positive gilts ($p < 0.05$), whereas PPV3 was notably present in PCV2-negative ($p < 0.01$) and PRRSV-negative ($p < 0.05$) animals. Considering RPPs, only PCV3 showed a significant correlation with the farrowing rate (FR)—the likelihood of a low FR was 94% lower in herds harboring PCV3-positive gilts (OR 0.06, $p = 0.0043$), suggesting that detection of PCV3 by PCR correlates with improved reproductive outcomes. Furthermore, PPV4 and PPV5 showed a tendency to associate with reduced FR ($p < 0.20$). These findings contribute to clarifying the potential involvement of nPPVs in PRF within Colombian swine herds.

Keywords: Porcine parvoviruses (PPVs), Novel porcine parvoviruses (nPPVs), PCV2, PCV3, PRRSV, Porcine reproductive failure (PRF)

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Introduction

Parvoviruses (PVs) are non-enveloped, single-stranded DNA (ssDNA) viruses belonging to the Parvoviridae family, known to infect both vertebrates and invertebrates [1]. Advances in metagenomics and next-generation sequencing have led to the discovery of numerous novel PVs in various animal hosts [2]. In pigs, eight porcine parvoviruses (PPV1–PPV8) have been identified [3–5]. PPV1, endemic in global swine populations since the 1960s, is the major etiologic agent of SMEDI syndrome, causing stillbirths, mummified fetuses, embryonic death, and infertility in sows [6]. Although the complete DNA sequences of the newer nPPV species (PPV2–PPV8) have been determined, their pathogenic roles remain uncharacterized [2].

Reports from various regions have demonstrated that these nPPVs are widely distributed [7]. PPV2, first described in Myanmar (2001) from serum samples [3], has since been reported globally with differing prevalence levels and sample sources [8–15]. Its detection via in situ hybridization (ISH) in alveolar macrophages with interstitial pneumonia, alongside high viral loads, suggests a role in the porcine respiratory disease complex (PRDC) [16].

PPV3, initially identified in 2008 in tissue collected from slaughtered pigs in Hong Kong [17], has been detected worldwide with variable frequencies [8, 13, 18] and is highly prevalent in German wild boars [19, 20].
PPV4 was first described in 2010, independently in the United States (in pigs with porcine circovirus-associated diseases, PCVADs) [21] and China (in pigs with PRDC and abortions) [22]. Although less common than other PPVs, its detection in semen, aborted fetuses, ovaries, and uteruses [8, 23] indicates a potential connection with PRF. PPV5, identified in 2013 in lungs from pigs with multiple syndromes [13, 24], and PPV6, discovered in 2014 in China in samples from aborted fetuses and piglets [25], have both been documented in the USA, Mexico, China, Korea, Poland, and Brazil, across diverse sample types such as serum, lungs, feces, oral fluids, chops, and fetal hearts [9, 15, 26–28]. PPV7, described in 2016 in the USA, was found in serum, stool, and nasal swabs and later reported in Korea [29], China [30], several European nations [31], and Colombia [32]. Most recently, PPV8 was detected for the first time in September 2022 in China from lung tissues [5].
Determining the pathogenic relevance of these nPPVs has proven difficult, as Koch's postulates have yet to be fulfilled. These viruses are found in both diseased and clinically healthy pigs and often occur alongside other swine pathogens, including porcine circovirus type 2 (PCV2), porcine circovirus type 3 (PCV3), and porcine reproductive and respiratory syndrome virus (PRRSV) [7, 13–15, 33, 34]. The pathological impact of such coinfections in field conditions remains uncertain.
In Colombia, both PCV2 and PRRSV circulate endemically, causing recurrent outbreaks. PCV2 is controlled primarily through vaccination, typically administered to piglets (three weeks old) and gilts, while some farms also immunize sows [35]. Conversely, PRRSV, which has been present since 1996, is managed through gilt acclimation and temporary herd closures, as licensed vaccines are not employed in Colombia [36, 37]. Furthermore, PCV3, first reported locally in 2019, has been associated with PRF [38, 39].
Given the coexistence of PPV1, PCV2, PRRSV, PCV3, and multiple nPPVs, their combined contribution to reproductive failure cannot be ruled out. Therefore, the aims of this study were:
(i) to detect the presence of nPPVs (PPV2–PPV8) in Colombian gilts by real-time PCR,
(ii) to analyze their coinfection patterns with PPV1, PCV2, PCV3, PCV4, and PRRSV, and
(iii) to evaluate potential associations between these viruses and reproductive performance parameters (RPPs) in sow herds.

The study targeted gilts aged 180–200 days, recognizing that the reproductive health of this group is critical for overall herd productivity, as reproductive disorders in gilts directly influence sow herd performance.

Materials and Methods

Sampling strategy and reproductive performance data
Blood sera were obtained from 234 gilts, aged 180–200 days, belonging to 40 herds distributed across five main swine-producing regions of Colombia. Herds containing more than 70 gilts (based on the national sow population census; https://www.ica.gov.co/.../censo-2018.aspx, accessed June 1, 2020) were chosen as primary sampling units and assigned into five strata according to geographic region. Following random selection of these herds, gilts within each selected unit were considered secondary samples.
From each region, eight herds were chosen at random, and five to six serum samples were taken from each. Sample sizes for farms and animals were estimated according to the total number of herds with more than 70 gilts in each region. The regional distribution and number of samples were as follows: Atlántico (n = 45), Antioquia (n = 47), Cundinamarca (n = 41), Eje Cafetero (n = 52), and Valle del Cauca (n = 49). Six gilts per herd were examined to determine viral status, assuming 66% prevalence among herds, 40% within herds, 96% sensitivity, 99% specificity, and 5% allowable error. Sample size estimation followed a hypergeometric model using the epiR package of R software [40].
Data concerning gilt management—including origin, acclimation (exposure to oral fluids, feces, or mummified materials), vaccination routines, replacement frequency, and herd size—were gathered via structured questionnaires **(Table 1)**. In addition, several reproductive performance parameters (RPPs) were obtained, namely farrowing rate (FR), percentages of stillbirths (SBs) and mummified fetuses (MMs), and piglets weaned per sow (PS) and per sow per year (PSY) for the last six-month period. Any incomplete survey entries were recorded as non-responses and removed from the analysis.

Table 1. Overview of management practices and reproductive performance indicators (RPPs).

Variable	Variable Categories	Frequency
Herd size	Small (100 to 300 breeding females)	16 (40%)
	Medium (301 to 1000 breeding females)	12 (30%)
	Large (≥1000 breeding females)	5 (12.5%)
	No response *	7 (17.5%)
Acclimatization practice	Yes	9 (22.5%)
	No	25 (62.5%)
	No response *	6 (15%)
Replacement rate	≥50%	9 (22.5%)
	30–50	16 (40%)
	≤30	2 (5%)
	No response *	13 (32.5%)
Source of the gilts	External (from other farms)	8 (20%)
	Internal (from the same farm)	19 (47.5%)
	Internal and external	7 (17.5%)
	No response *	6 (15%)
Farrowing rate (mean 86.6%)	Herds with a mean <86.6	11 (27.5)
	Herds with a mean >86.6	18 (45%)
	No response *	11 (27.5%)
Percentage of stillbirths (mean 5.4%)	Herds with a mean >5.4	15 (37.5%)
	Herds with a mean <5.4	16 (40%)
	No response *	9 (22.5%)
Percentage of mummies (mean 5.1%)	Herds with a mean >5.1	10 (25%)
	Herds with a mean <5.1	21 (52.5%)
	No response *	9 (22.5%)
Number of pigs weaned per sow (mean 11.2)	Herds with a mean >11.2	17 (42.5%)
	Herds with a mean <11.2	13 (32.5%)
	No response *	10 (25%)
Number of pigs weaned per year (mean 27)	Herds with a mean >27	15 (37.5%)
	Herds with a mean <27	9 (22.5%)
	No response *	16 (40%)

* Blank responses were categorized as non-responses and excluded.

Identification of PPVs, PCV2, PCV3, PCV4, and PRRSV

Blood samples were centrifuged at 3500 rpm for 10 minutes, and the sera were preserved at −80 °C until analysis. Nucleic acid extraction was performed individually from 200 µL of serum (n = 234) using the High Pure Viral Nucleic Acid Kit® (Roche, Mannheim, Germany) in line with the manufacturer's guidance. Extracts were divided into two aliquots, one for DNA virus and another for PRRSV detection, and stored again at −80 °C.

Detection of novel porcine parvoviruses (PPV2–PPV7) was carried out by real-time PCR, using published primer sets: PPV2 [10], PPV3 [41], PPV4–PPV5 [24], PPV6 [42], and PPV7 [43], with SYBR Green as the detection dye. The porcine β-actin gene served as a reference to confirm DNA integrity [44].

To produce positive controls, previously identified positive tissue samples (confirmed by conventional PCR) were used to generate amplicons, which were inserted into plasmids via the TOPO TA® Cloning Kit (Invitrogen, Carlsbad, CA, USA) and introduced into One Shot® Chemically Competent E. coli. Insert verification and orientation were confirmed by SSiGMol (Servicio de Secuenciación y Análisis Molecular, Instituto de Genética, Universidad Nacional de Colombia, Bogotá) through sequencing. Recombinant plasmids were purified with the Plasmid Maxi Kit® (Qiagen, Hilden, Germany), quantified via Nano200® spectrophotometer (Thermo Scientific, Wilmington, DE, USA) at OD260, and adjusted in RNase-free water to 10^8 plasmid copies/µL. Tenfold serial

dilutions (Log_{10}) were prepared to construct standard curves, with Ct < 35 set as the positivity threshold for each nPPV.

Specificity of each qPCR system was verified using both constructed plasmids and a panel of porcine DNA pathogens, including PPV1–PPV7, PCV2, PCV3, and Mycoplasma hyopneumoniae. Reactions were run in duplicate, each in 20 µL volumes (SsoAdvanced Universal SYBR Green Supermix®, Bio-Rad) containing 0.4 µM primers, on a LightCycler 480® (Roche, Burgess Hill, UK). The amplification profile was: 95 °C for 10 min, followed by 45 cycles of 95 °C for 1 min, 60 °C for 24 s, and 72 °C for 2 s. A melting curve analysis was performed from 65 °C to 95 °C.

Analytical validation was carried out with tenfold serial dilutions to calculate the limit of detection (LoD), regression coefficient (R^2), and amplification efficiency (E = 10^(−1/slope) − 1). The slope was obtained by regressing Cq values against log-transformed concentrations, and R^2 indicated linear fit quality. The LoD corresponded to the smallest detectable viral load in ≥50% of replicates. Repeatability was assessed using three dilutions (10^{-1}, 10^{-4}, and 10^{-8}) tested by two technicians over two days [45].

To validate qPCR-positive results, end-point PCRs were conducted for each nPPV, amplifying larger genomic fragments using specific primers [14]: PPV2 (563 bp), PPV3 (514 bp), PPV4 (416 bp), PPV5 (959 bp), PPV6 (650 bp), and PPV7 (241 bp) (as per [43]). Each reaction contained 0.25 µL Taq polymerase (5 U/mL) (GoTaq Flexi, Promega®), 2.5 µL of 5× buffer, 1 µL of each primer (20 µmol/L), and 2 µL of extracted DNA. The PCR cycles included 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min.

Amplicons from these reactions were purified and sequenced bidirectionally using the same primers at SSiGMol [14, 43]. Positive control DNA was obtained from archived tissue samples previously verified as positive for the corresponding virus.

PRRSV Analysis

For the detection of PRRSV, the aliquot allocated for this purpose was used to synthesize complementary DNA (cDNA) through the High-Capacity RNA-to-cDNA Kit® (Thermo Scientific). The resulting cDNA served as a template for real-time PCR assays, conducted with specific oligonucleotide primers and a TaqMan probe, as described in [46]. The same approach was used to test for PPV1 [47], PCV2 [48], and PCV3 [49], applying TaqMan chemistry.

This diagnostic assay, standardized for routine use in the laboratory, employed a cut-off cycle threshold (Ct) of less than 37 to classify samples as positive. Each virus was verified against plasmid-based positive controls carrying the target insert. The PCR reactions (20 µL total volume) included 0.25 µM of probe and 0.4 µM of each primer, using the LightCycler 480 Probes Master® (Roche). All reactions were performed in duplicate on a LightCycler 480® thermocycler (Roche, Burgess Hill, UK). The program consisted of an initial activation at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 45 s, and extension at 72 °C for 2 s.

In addition, two recently reported viruses, PCV4 and PPV8, were screened. Up to December 2020, neither had been identified in Colombia. PCV4 was examined using conventional PCR following the method outlined in [50], whereas PPV8 was searched by nested PCR (nPCR) using the protocol in [5]. Due to the lack of confirmed cases locally, positive control material was unavailable for these assays.

Statistical evaluation

The PCR outputs were coded as binary variables (presence/absence) for each viral agent. Initial associations among PPVs, PCV2, PCV3, and PRRSV were explored through univariate analyses using chi-square or Fisher's exact test, considering $p < 0.05$ as statistically significant.

To classify herds by reproductive outcomes, the mean values were calculated: farrowing rate (FR = 86.63), mummified fetuses (MMs = 5.08), stillbirths (SBs = 5.37), and piglets per sow per year (PSY = 27.03). Each herd was then categorized as high or low for each reproductive performance parameter (RPP). Associations between the presence of viruses (positive or negative for PCV2, PCV3, PPV1–PPV7, and PRRSV) and RPP categories were assessed again by univariate tests, keeping $p < 0.05$ as the significance level.

For significant results, crude odds ratios (ORs) were computed. Multivariable logistic regression was subsequently applied to adjust the odds, incorporating all pathogens showing $p < 0.16$ in the preliminary analysis. Only variables that remained significant ($p < 0.05$) or that modified coefficients by ≥10% were retained, in accordance with [51] (Chapters 3, 6, and 7).

A multiple correspondence analysis (MCA) was finally performed to visualize relationships among categorical predictors, including herd size, replacement rate, acclimatization practices, origin of gilts, and viral detection status. The "FactoMineR" and "Factoextra" packages in RStudio (version 2022.12) were used for this purpose [52, 53].

Results and Discussion

Management practices and reproductive indicators

Of the 40 herds assessed, 34 (85%) completed the management survey, and 30 (75%) provided reproductive performance records. Sampling and data acquisition occurred between June and December 2020. A summary of herd-level data, including management routines and reproductive metrics, appears in **Table 1**.

In terms of herd size, 16 herds (40%) had 100–300 sows, 12 (30%) kept up to 1000 sows, and 5 herds (13%) maintained more than 1000 animals. Information was not available for 7 herds (17%).

Detection of porcine parvoviruses in gilts

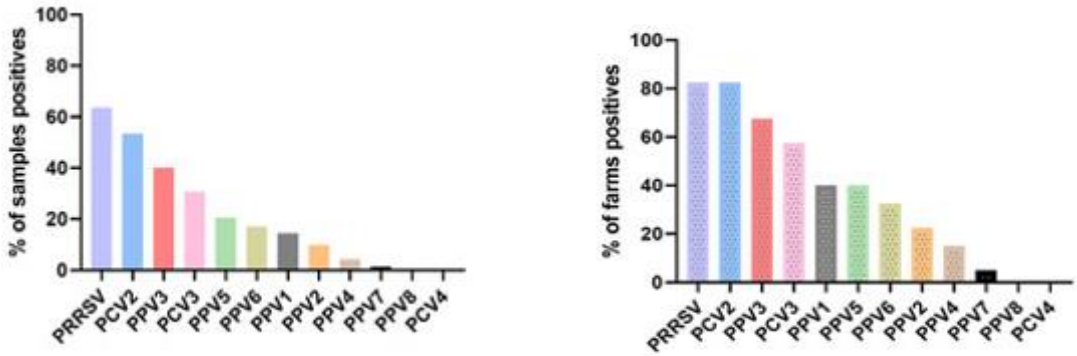
The study confirmed the presence of several novel PPV types (PPV2–PPV7) circulating in Colombian gilts **(Figure 1a)**. None of the samples tested positive for PPV8.

At the herd level (n = 40), PPV3 was the most frequent genotype, identified in 27 herds (67.5%), followed by PPV5 (40%, n = 16), PPV6 (32.5%, n = 13), PPV2 (22.5%, n = 9), PPV1 (17.5%, n = 7), PPV4 (15%, n = 6), and PPV7 (5%, n = 2) **(Figure 1b)**.

At the individual level (n = 234 serum samples), PPV3 again dominated (40.1%, n = 94), followed by PPV5 (20.5%, n = 48), PPV6 (17%, n = 40), PPV2 (9.8%, n = 23), PPV4 (4.2%, n = 10), and PPV7 (1.3%, n = 3).

Screening for PRF viruses (PPV1, PRRSV, PCV2, PCV3) revealed PRRSV as most prevalent (63.6%, 149/234), then PCV2 (53.4%, 125/234), PCV3 (30.7%, 72/234), and PPV1 (14.5%, 34/234) **(Figure 1a)**.

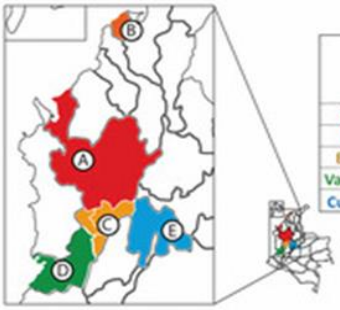
Across herds, PCV2 showed the highest detection frequency (85%), with PRRSV (82.5%) and PCV3 (57%) following. No PCV4 positives were recorded in any herd **(Figure 1b)**.



a) b)

Figure 1. Distribution of porcine parvoviruses (PPV1–PPV8), porcine circoviruses (PCV2–PCV4), and PRRSV among gilts from 40 farms located in the five largest pig-producing regions of Colombia. (a) Viral presence in individual samples and (b) viral occurrence per herd.

Figure 2 illustrates how viral detections were spread across Colombia's five evaluated regions. Core reproductive pathogens—PPV1, PCV2, and PRRSV—were present in all territories, as was PPV3. Other PPVs (PPV2, PPV4, PPV5, and PPV6) appeared in four regions, while PPV7 was restricted to Atlántico and Valle del Cauca.



Region	Swine population/ Km²	Percentage of virus in each region									
		PRRSV	PCV2	PCV3	PPV1	PPV2	PPV3	PPV4	PPV5	PPV6	PPV7
Antioquia (A)	104,269	74.4	27.6	74.4	14.9	14.9	68	2.1	25.5	27.5	0
Atlantico (B)	79,308	57.7	55.5	22.2	20	0	68.9	4.4	33.3	26.6	2.2
Eje cafetero (C)	45,849	65.3	36.5	21.1	23	26.2	21.1	0	0	0	0
Valle del cauca (D)	32,053	65.3	83.3	0	4	2	26.5	12.2	24.5	22.4	4
Cundinamarca (E)	29,312	48.7	65.8	39	9.7	2.4	17	2.4	21.9	9.7	0

Figure 2. Map showing Colombia's five primary swine-producing regions analyzed in this work: (A) Antioquia, (B) Atlántico, (C) Eje Cafetero, (D) Valle del Cauca, and (E) Cundinamarca. The detection percentage for each virus in each zone is presented. The base map was generated using the National Administrative Department of Statistics (DANE) database (https://geoportal.dane.gov.co/acerca-del-geoportal/acerca/#gsc.tab=0, accessed 12 Jan 2024) and modified in QGIS v3.34.5 (https://qgis.org/es/site/, accessed 12 Jan 2024).

Detection of viral agents at the farm and individual levels

Every herd tested carried at least two different viral species. Among these, 7.5% (3/40) had two viruses, 22.5% (9/40) had three, 25% (10/40) harbored four, 22.5% (9/40) had five, and 20% (8/40) contained six or more viral types.

At the gilt level, 4.2% (10/234) were negative for all agents. A single viral infection occurred in 16.6% (39/234) of the animals, most frequently involving PRRSV (35.8%, 14/39), PPV3 (23%, 9/39), or PCV2 (17.9%, 7/39). Dual infections affected 29.4% (69/234) of gilts, most commonly PCV2/PRRSV (30.43%, 21/69). PPV coinfections with PCV2 and PRRSV accounted for 20% (14/69) and 21.7% (15/69), respectively. The predominant combinations were PCV2/PPV6 (10.1%, 7/69), PRRSV/PPV3 (8.7%, 6/69), and PRRSV/PPV1 (8.7%, 6/69).

Triple coinfections were identified in 27.3% (64/234) of gilts, quadruple in 15.3% (36/234), quintuple in 5.1% (12/234), and sextuple or higher in 1.7% (4/234).

Figure 3 visualizes single and combined PPV infections (PPV1–PPV6) along with PCV2, PCV3, or PRRSV. No serum contained more than five PPVs simultaneously. Mixed infections occurred in all herds and regions analyzed.

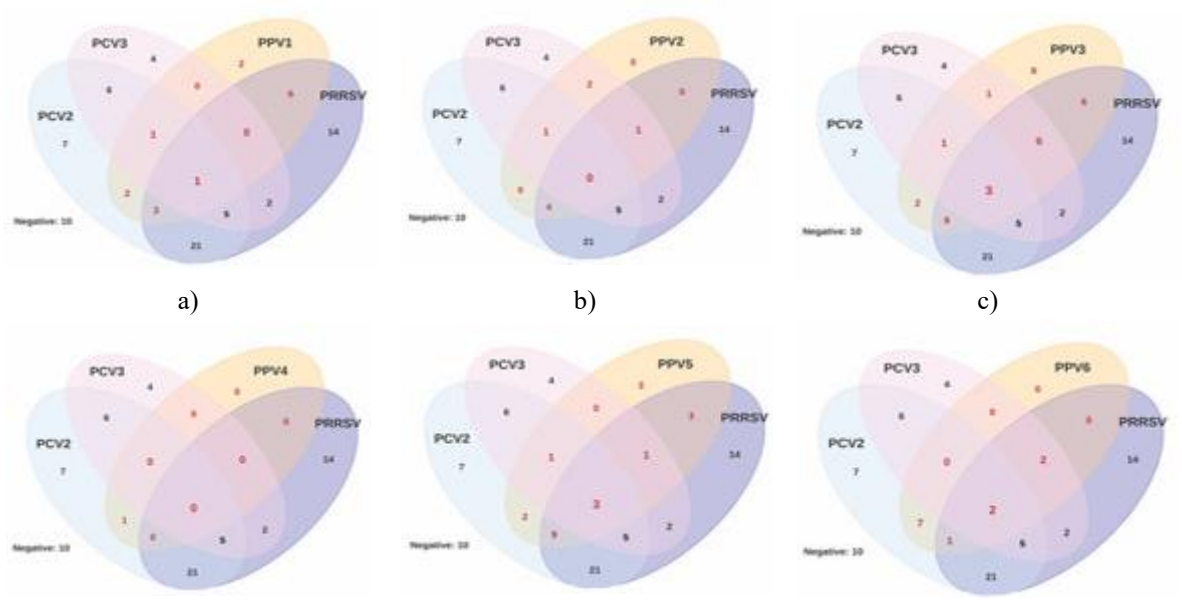


Figure 3. Coinfection profiles of PPVs (PPV1–PPV7) and other major swine viruses (PCV2, PCV3, PRRSV) detected in 40 Colombian herds. Venn diagrams depict the number of single (solid color) and combined

(overlapping) detections for PPVs [PPV1 (a), PPV2 (b), PPV3 (c), PPV4 (d), PPV5 (e), and PPV6 (f)] together with PCV2, PCV3, and PRRSV. PPV7 was omitted due to its low detection frequency.

Given the complexity of mixed infections (ranging from double to eightfold), only dual combinations including PPVs and the key reproductive viruses (PCV2, PCV3, PRRSV) were summarized. The dominant coinfections in gilts were PRRSV/PPV3 (27%), PCV2/PPV3 (16%), PRRSV/PPV6 (15.8%), and PRRSV/PPV5 (15.4%) **(Table 2)**.

Table 2. Rate of detection of PPVs (PPV1–PPV7) in sera from 234 gilts across 40 herds when co-occurring with PCV2, PCV3, or PRRSV.

PPVs	PCV2 n * (%)	PCV3 n (%)	PRRSV n (%)
PPV1	18 (7.7)	10 (4.3)	20 (8.5)
PPV2	10 (4.3)	9 (3.9)	15 (6.4)
PPV3	38 (16.2)	35 (15)	64 (27.3)
PPV4	8 (3.4)	0 (0.0)	7 (3)
PPV5	28 (12)	14 (6)	36 (15.4)
PPV6	22 (9.4)	21 (9)	37 (15.8)
PPV7	3 (1.3)	1 (0.4)	3 (1.3)

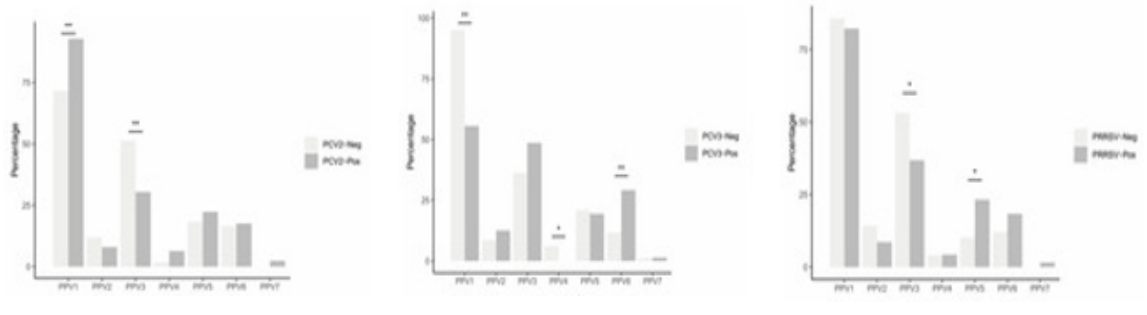
Values show number of positives among 234 analyzed samples.

Relationships between PPVs (PPV1–PPV7) and PCV2, PCV3, PCV4, or PRRSV

This section evaluates correlations between PPV infections (PPV1–PPV7) and the presence of PCV2, PCV3, or PRRSV. PPV1 infection occurred significantly more often in PCV2-positive gilts ($p < 0.01$), whereas PPV3 appeared mainly in PCV2-negative ones ($p < 0.01$) **(Figure 4a)**.

In PCV3-positive animals, PPV6 was notably more frequent ($p < 0.01$). Conversely, PPV1 ($p < 0.01$) and PPV4 ($p < 0.05$) were more common in PCV3-negative individuals **(Figure 4b)**.

For PRRSV, PPV5 prevalence was higher among positives ($p < 0.05$), while PPV3 showed the opposite pattern, being more frequent in PRRSV-negative gilts ($p < 0.05$) **(Figure 4c)**. Although not reaching significance, PPV3 tended to appear more in PCV3-positive animals ($p = 0.12$), and PPV6 in PRRSV-positive ones ($p = 0.07$).



a) b) c)

Figure 4. Comparison between PPV detection (PPV1–PPV7) and the presence of PCV2 (A), PCV3 (B), and PRRSV (C) in gilts from 40 Colombian herds. Bars show percentages (y-axis) of positive and negative samples per virus (x-axis). Significant (*) and highly significant (**) associations are indicated.

Relationship between PPVs, PCV2, PCV3, and PRRSV detection in gilts and reproductive outcomes in sows

The initial (crude) statistical assessment of virus presence (positive or negative) for PPVs, PCV2, PCV3, and PRRSV in gilts, compared with high or low reproductive performance indicators (RPPs)—namely farrowing rate (FR), proportion of mummified fetuses (MMs), proportion of stillbirths (SBs), and piglets weaned per sow per year (PSY)—revealed a significant association only between PCV3 status and FR ($p = 0.0043$). Specifically, herds with PCV3-positive gilts had a 94% lower probability of exhibiting a low FR (below the sample mean of 86%) compared with herds testing negative for PCV3 (OR: 0.06; 95% CI: 0.007–0.36).

In addition, low FRs (below the sample mean of 86.6%) appeared to show a tendency toward association with PPV4 (OR: 4.3, p = 0.16) and PPV5 (OR: 4.0, p = 0.13) detections in gilts. However, after running the multivariable logistic regression (complete model) to adjust the relationship between FR and PCV3, PPV4, and PPV5, the influence of PPV5 became statistically non-significant (p = 0.11) and was therefore excluded from the final model.

The simplified (reduced) model indicated that herds harboring PCV3-positive gilts had a 96% lower likelihood of a reduced FR (below 86%) compared to PCV3-negative herds after controlling for PPV4 presence (OR: 0.04; 95% CI: 0.002–0.30) **(Table 3)**. Conversely, herds with PPV4-positive gilts showed 9.6 times higher odds of experiencing a low FR compared to PPV4-negative ones after adjusting for PCV3. A comparison of PCV3 coefficients between the crude (−2.76) and adjusted (−3.18) models revealed that PPV4 acted as a confounding variable, as the coefficient changed by more than 10%.

Although not reaching statistical significance, elevated MMs tended to occur more frequently in herds with PCV3-positive (p = 0.07), PPV5-positive (p = 0.13), or PRRSV-positive (p = 0.14) gilts. Likewise, higher SB values were observed in association with PPV1-positive gilts (p = 0.07), and lower PSY values tended to be linked to PCV3-positive gilts (p = 0.12). Other associations were not considered meaningful (p > 0.20).

Table 3. Multivariable logistic regression evaluating the relationship between farrowing rate (below vs. above average) and PCV3/PPV4 status in gilts (positive or negative).

Variable	Regression Coefficient	p Value	OR *	95% CI
Intercept	0.4813	0.4487		
Positive PCV3	−3.1804	0.0071	0.04	0.002–0.30
Positive PPV4	2.2666	0.0993	9.64	0.84–266.18

Adjusted odds ratios represent farms with FRs below the sample average and positive viral detection compared with those above the average and negative for the virus.

Multiple correspondence analysis (MCA)

A multiple correspondence analysis (MCA) was employed to explore associations between gilt management practices **(Table 1)** and viral detection in serum samples. **Figure 5** presents the MCA projection of these variables. The first dimension was predominantly characterized by large herds (>1000 breeding females), high replacement rates (>50%), the implementation of acclimatization routines, and herds hosting six or more viral agents. The second dimension was mainly defined by medium-sized herds (100–500 sows), lower replacement rates (<50%), absence of acclimatization, and herds carrying fewer than six viruses. PPV4 and PPV7 were detected mainly in farms with seven or eight concurrent viral infections, typically exceeding 1000 sows and exhibiting replacement rates above 50%.

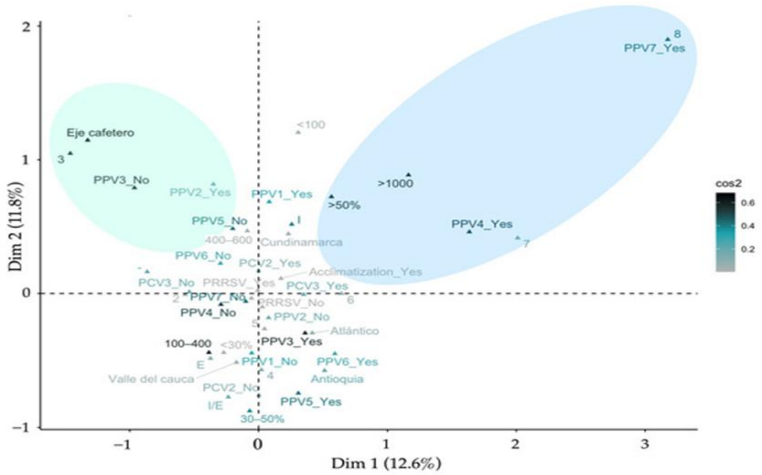


Figure 5. Relationship between gilt management strategies and detection of PPVs (PPV1–PPV7), PCV2, PCV3, and PRRSV in Colombian pig farms. The two-dimensional MCA map shows variable scores and a two-step cluster grouping (40 herds included in the analysis; dimension 1 = x-axis, dimension 2 = y-axis).

New porcine parvoviruses (PPV2–PPV7) have been identified in swine populations globally [9, 15, 52]. Evidence from earlier research suggests possible interactions between these newly described PPVs and other reproductive pathogens (PPV1, PCV2, PCV3, PRRSV) in reproductive failure (PRF) events [15, 54]; however, conclusive proof remains scarce. The influence of viral interplay on herd productivity and animal health under field conditions also remains unclear.

Our findings indicate that several nPPVs may coexist within both herd populations and individual gilts. Moreover, these viruses often appear concurrently with PRF-associated agents (PPV1, PCV2, PCV3, PRRSV) and could potentially correlate with key reproductive traits such as the farrowing rate. Clarifying the synergistic or antagonistic interactions among these endemic viruses is critical for developing preventive and control strategies aimed at minimizing spread and mitigating production losses.

Since their discovery, nPPV genotypes have been identified in multiple countries, host populations, and biological sample types [9, 13, 28]. Detection frequencies vary substantially across studies depending on the animal category (domestic vs. wild), sample matrix, and diagnostic protocols employed [12, 23, 34]. Most population-level reports have analyzed sera, feces, or oral fluids collected from nursery or finishing pigs [9, 27].

In the current work, PCR analysis of serum samples was used to investigate viremia by nPPVs, assuming that blood would provide a representative matrix for detection. Nonetheless, the exact tissue tropism of these viruses remains undetermined, meaning that optimal sample types for diagnostic purposes are still unknown. The limited understanding of nPPVs' biological properties represents a constraint in selecting the most reliable specimens for PCR-based identification.

The available data concerning the prevalence and significance of viral co-detection (at the herd level) or coinfection (within individual animals) in gilts remains limited and warrants further exploration. Viral coinfections may alter disease manifestation and modulate immune reactions observed in single infections [55]. In this study, several factors appeared to facilitate coinfection: (i) PCV2 and PRRSV were the viruses most frequently identified alongside nPPVs, and previous reports have demonstrated that both can suppress the immune system, thereby influencing the host's defense mechanisms [56–58]; (ii) the gilts used as the study population likely had immature immune systems and were less capable of counteracting infection compared with multiparous sows [59]; and (iii) the varying biosecurity levels across farms.

This investigation analyzed individual serum samples from gilts belonging to 40 swine herds across Colombia. Detection rates for nPPV species (PPV2–PPV7) ranged from 1.8% to 40%, illustrating their broad circulation among swine in five Colombian regions. Our findings offer new perspectives on the simultaneous presence and coinfection patterns between nPPVs and other reproductive failure-associated viruses such as PCV2, PCV3, and PRRSV. Studies examining concurrent infections of PCV2, PRRSV, PPV1, PCV3, and nPPVs in breeding females remain scarce, although two prior investigations reported PPV presence [27, 60]. The first evaluated PPV1–PPV4 in adult pigs (over one year old), detecting PPV3 in 17.5% of samples [60]. The second assessed PPV1–PPV7 in pooled sera from breeding females and found PPV3 and PPV6 to be most frequent (10.5%) [27]. Our results align with these earlier studies in identifying PPV3 as the predominant PPV in gilts; however, the proportion was higher (40%), possibly due to sampling a specific age group (180–210 days) rather than older sows [27] or growing pigs [60]. This highlights the epidemiological importance of gilts in PPV3 transmission. Other reports have also shown that gilt populations contribute significantly to the spread of viruses such as SIV [61] and play a crucial role in PRRSV persistence in endemic settings [36]. Additional research is required to clarify the epidemiology of PPV3 within swine herds and to determine the role of gilts in virus maintenance and dissemination. Moreover, variations in regional prevalence of nPPVs and their implications for herd health and productivity need further assessment.

Three prior studies—conducted in Mexico [15], South Korea [62], and Italy [54]—have examined nPPVs in connection with PRF, particularly in aborted fetuses, and reported the occurrence of PPV2–PPV6 at varying prevalence levels. These studies consistently found PPV4 to be the least common. The prevalence of PPV5 and PPV6 ranged from 18% to 56%, PPV3 occurred in 3%–38% of cases, and PPV2 showed the widest variation (2%–95%). Only the Italian research [54] included PPV7, which was detected at 14.5%. For PPV7, most reports document its detection independently of other nPPVs, predominantly in aborted fetuses. Comparing our findings with those three studies, PPV4 remained rare, while PPV5 and PPV6 frequencies were similar. PPV3 was again the most prevalent, comparable to results obtained from serum samples of sows [27, 60]. In contrast, PPV7 exhibited the lowest prevalence (1%), which differs from [54] and may relate to the sample type—our study analyzed sera, whereas the highest prevalence (up to 50%) was noted in aborted fetuses [29]. Collectively, our

data and those of previous works support the notion that nPPVs may contribute to PRF outbreaks, particularly through infections in gilts at the onset of their reproductive life.

Earlier studies have shown that primary PRF-related viruses—PRRSV [63], PCV2 [64], and PPV1 [65]—as well as PCV3 [38, 66], negatively impact reproductive outcomes in gilts and sows. However, most focus on single-virus effects rather than coinfections. The three investigations cited [15, 54, 62] also analyzed the relationship between nPPVs and PRF-associated viruses (PCV2, PCV3, PRRSV). The Mexican and South Korean studies evaluated double coinfections involving PCV2 and nPPVs (PPV2–PPV6), detecting all possible combinations, with PCV2/PPV2, PCV2/PPV5, and PCV2/PPV6 being the most frequent. The Mexican report identified significant correlations between PCV2-positive samples and both PPV5 and PPV6, while no such link was found in South Korea. The Italian study assessed associations between PCV2, PCV3, PRRSV, and nPPVs, revealing coinfections such as PCV2/PPV3–PPV7, PCV3/PPV3–PPV7, and PRRSV/PPV2–PPV7, with the most frequent being PCV2/PPV3, PCV3/PPV3 and PPV6, and PRRSV/PPV2 and PPV3. No statistical correlation with PRF was observed.

In our work, we detected coinfections involving PCV2/PPV2–PPV7, PRRSV/PPV2–PPV7, and combinations such as PCV2/PPV3, PCV3/PPV3, PCV3/PPV6, PRRSV/PPV3, and PRRSV/PPV5. Notably, we found strong correlations between PPV6 and PCV3-positive gilts and between PPV5 and PRRSV-positive gilts. Additionally, higher detection rates of PPV3 occurred in PCV2-negative gilts, PPV4 in PCV3-negative gilts, and PPV3 in PRRSV-negative gilts. These findings represent the first documentation of such associations and serve as preliminary evidence for potential synergistic interactions, possibly linked to the immunomodulatory properties of PRRSV [58]. Some previous research has proposed a relationship between PCV3 and PPV7 [67], which was not observed in our study.

Regarding PPV1, detection of its genome continues to be reported in numerous studies, even though vaccination against it is routinely practiced. Typically, its occurrence rate remains below 30%, although higher frequencies, reaching 44%, have been observed in boars from Italy [34]. Interestingly, several nPPVs—namely PPV2, PPV3, and PPV6—tend to display a greater average prevalence than PPV1. Coinfections involving PPV1 have also been documented, including PPV1/PCV2 (under 10%), PPV1/PCV3 (25%), and PPV1/PRRSV (9%). The data from this study align with these findings, showing PPV1/PCV2 (7.5%), PPV1/PCV3 (4.3%), and PPV1/PRRSV (8.5%). Moreover, we observed an increased PPV1 load in PCV2-positive gilts, consistent with earlier results reported in serum samples from pigs at other production stages and associated with PCVADs [68, 69]. In the context of our research, the interaction between PPV1 and PCV2 among gilts might play an important role in the onset of PRF when these animals enter the breeding cycle.

Due to their intrinsic biological stability, PPVs are challenging to eradicate, as they can tolerate environmental stress, remain viable for extended periods, and resist many disinfectants depending on the viral species [70]. Current control measures primarily involve vaccination and gilt acclimatization through feedback exposure (e.g., feces, fetuses, or tissues), both of which have proven most effective for PPV1 control [71]. Nevertheless, how feedback influences the epidemiology of nPPVs remains uncertain and requires further investigation. Additionally, new PPV1 variants have appeared in recent years, showing capsid protein (VP2) mutations that alter antigenicity and reduce cross-protection by available vaccines [72]. The nucleotide similarity between PPV1 and nPPVs is roughly 40%, suggesting minimal or absent serological cross-reactivity.

At present, no published data have quantified the effect of nPPVs on PRF or their impact on RPPs. The current study provided several notable insights. First, PCV3 showed a statistically significant correlation with FR ($p < 0.05$), while PPV4 and PPV5 tended to exhibit similar associations with this reproductive indicator. Furthermore, the relationship between PCV3 and FR shifted by more than 10% once PPV4 status was considered, confirming that PPV4 acts as a confounding factor in this association. This implies that PPV4 may be implicated in PRF, as previously suggested for PPV1 and PPV7 [67]. These findings represent the first field-based evidence of a confounding relationship between nPPVs and PRF. Additional work is still required to clarify whether PPVs are direct contributors to reproductive failure in single or mixed infections and to determine how herd-level parameters (PPRs) may be influenced by concurrent pathogens or management-related variables [73].

Another major outcome of this study concerns herd management factors. Although no risk variables directly linked to nPPV presence were identified, the MCA analysis highlighted two relevant trends: (i) herds containing PPV4 or PPV7 tended to exhibit a higher number of circulating viruses (up to six), and (ii) this increased viral diversity occurred predominantly in larger herds (≥1000 breeding females) with replacement rates ≥50%. These patterns underline how population size and replacement intensity can shape the epidemiology of porcine viruses,

as documented by prior research [74, 75]. Such circumstances may promote the establishment of nPPVs, supported by their robust structural and physicochemical resilience [71]. Within the nPPV group, some unique genomic traits are worth noting—PPV4, for instance, carries a circular DNA genome, unlike the linear configuration seen in most PPVs. This could enhance its persistence in swine by allowing the virus to maintain an episomal state within the nucleus of infected cells, protecting it from nuclease degradation [21]. Likewise, PPV7 is classified within a distinct subfamily of Parvoviridae, giving it specific biological and molecular properties that set it apart from other known parvoviruses [76].

Conclusion

Finally, these results contribute to two prevailing hypotheses concerning nPPVs. The first posits that most of these viruses may cause subclinical infections, functioning as commensal members of the porcine virome (the so-called "iceberg model" of viral infections) [25, 77]. The second hypothesis proposes that they can establish persistent infections [78]. In the present study, detection of nPPVs in serum from clinically healthy gilts might support the first hypothesis, implying that infection does not necessarily equate to disease. However, the simultaneous detection of PRF-related viruses in these same animals suggests a more complex interaction. It is therefore plausible that both primary PRF viruses and nPPVs require a certain threshold—possibly a critical viral load—to act as either direct pathogens (causing lesions) or indirect ones (through immunosuppression or modulation). The second hypothesis, which considers some nPPVs as potential agents contributing to PRF [15] or PRDC [14, 79], is further supported by our data showing an association between PPV4 and reduced RR, along with correlations between PPV4 and various RPPs, as well as coinfections involving other PRF viruses. These findings reinforce the possibility of PPV4's active role in PRF pathogenesis [68, 80].

To substantiate these assumptions, future studies should aim to isolate these viruses and characterize their pathogenic and epidemiological properties to clarify their actual influence on porcine health. Based on our findings, gilts examined in this work were already infected with several viral agents, including nPPVs, at insemination. Hence, from a veterinary perspective, it is recommended to routinely screen both gilts and multiparous sows for primary PRF viruses (PPV1, PCV2, and PRRSV) throughout the reproductive cycle to better understand infection dynamics. From a production standpoint, continuous viral monitoring and periodic review of vaccination and feedback programs against key PRF pathogens are strongly advised.

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