

# Detection of *Parvoviridae* and *Circoviridae* family species from blood of gilts in central Mexico backyard farms

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## ABSTRACT

**Objective:** To determine the DNA prevalence of Parvoviridae family species (PPV1, PPV2, PPV5, and PPV6) and Circoviridae family species (PCV2 and PCV3) in PCV2-unvaccinated backyard farms in central Mexico through gilt blood samples.

**Design/Methods/Approach:** Blood samples were collected from 60 gilts raised in backyard farms located in the State of Mexico, Hidalgo, and Querétaro. Genomic DNA was extracted using a commercial kit. Nested PCR was performed on each sample using previously reported primers and amplification conditions for PPV1, PPV2, PPV5, and PPV6, PCV2, and PCV3.

**Results:** Positive cases were detected for each viral species tested: 5.2% (3/58) for PPV1, 89.6% (52/58) for PPV2, 67.2% (39/58) for PPV5, 25.9% (15/58) for PPV6, 50% (29/58) for PCV2, and 69% (40/58) for PCV3. The most prevalent species was PPV2, followed by PCV3 and PPV5, while PPV1 showed the lowest prevalence. Overall, 96.5% of the samples exhibited co-infection with at least one other species, with triple assortments being the most frequent, particularly in backyard farms from the State of Mexico. However, double assortments were predominant in Querétaro. The most common viral combinations were PPV2/PCV3 and PPV2/PPV5. The former was dominant in Querétaro, while the latter was widespread in Hidalgo and the State of Mexico. Additionally, PCV2 was significantly associated with co-infections in Querétaro, whereas both PCV2 and PCV3 were predominant in the State of Mexico and Hidalgo.

**Study Limitations/Implications:** Backyard farms have a low number of sows and limited accessibility for sampling. Increasing the sample size would provide a more comprehensive understanding of the interactions between these viral species in non-industrial farms.

**Findings/Conclusions:** All viral species analyzed were detected in the blood of backyard sows. Prevalence and co-infection patterns varied by state and farm. Notably, PPV5 was the predominant virus in Hidalgo and the State of Mexico, often co-occurring with PPV2, PCV2, and PCV3, in that order. In contrast, PPV2 and PPV5 were the most frequent double co-infections in Querétaro. This is the first report documenting the DNA prevalence of PPV2, PPV5, PPV6, and PCV3 in backyard farms. Given the high co-infection rates observed in reproductive sows, further studies are necessary to assess their impact on swine health and productivity.

**Key words:** *Parvoviridae*, *Circoviridae*, backyard farms, sows, PCR, co-infection.

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## INTRODUCTION

In Mexico, porcine production is a highly sustainable economic sector that appeals to large corporations while also playing a crucial role in household economies. Nationwide, backyard farms account for approximately 30% of the swine trade (SENASICA, 2020). These farms are considered high-risk areas due to the lack of rigorous husbandry, biosecurity, and immunization protocols (Carrero, 2005), creating an environment where controlled diseases can spread within the porcine population. Reproductive issues in the swine industry lead to significant economic losses, ultimately affecting consumer prices. Viral agents are among the primary causes of reproductive failure (RF) in sows, as they can induce pregnancy miscarriages, with maternal viremia being essential for transplacental transmission (Zimmerman *et al.*, 2019). RF in sows encompasses a broad spectrum of clinical manifestations, with *Protoparvovirus unguulate* 1 commonly known as porcine parvovirus 1 (PPV1) being one of the main pathogens involved (Chen *et al.*, 2023). However, due to widespread vaccination over the past 40 years, the global incidence of PPV1 has remained low. PPV1 outbreaks are primarily linked to vaccination deficiencies, suggesting that infections may be more frequent in backyard farms where immunization practices are infrequent. Similarly, Porcine circovirus 2 (PCV2) is a well-documented pathogen associated with sow RF (Chen *et al.*, 2023). Although PCV2 vaccination has been available since 2008, its implementation has been largely restricted to commercial farms, with backyard farms exhibiting a low immunization rate. Meanwhile, Porcine circovirus 3 (PCV3), a recently identified agent, has been reported in Mexico. Although global studies have suggested its involvement in piglet diseases and RF outbreaks, its precise role in disease progression remains unclear. Furthermore, data on PCV3 prevalence in Mexico are scarce, with no available reports on backyard farms. Recently, seven emerging porcine parvovirus species (PPV2-8) have been identified, all belonging to the same family as PPV1 (Walker *et al.*, 2022). Numerous studies have investigated their potential role in disease, either as primary pathogens or in co-infections with other agents (Li *et al.*, 2021). In Mexico, a retrospective study analyzing PCV2-unvaccinated commercial farms from 2001 to 2015 reported a high prevalence of PPV2 to PPV6, markedly differing from global prevalence trends (García-Camacho *et al.*, 2020). However, no studies have assessed the prevalence of these viral agents in backyard farms, particularly those associated with sow RF. Therefore, the aim of this study is to determine the DNA prevalence of PPV1-6, PCV2, and PCV3 in backyard swine farms in central Mexico using blood samples from gilts.

## MATERIAL AND METHODS

**Sampling:** Fifty-eight blood samples were collected from gilts in 2023 from PCV2-unvaccinated backyard farms located in three Mexican states: Hidalgo (Tepatepec, 16 samples), State of Mexico (Teotihuacán de Arista, 10 samples; Melchor Ocampo, 4 samples; Teoloyucan, 7 samples), and Querétaro (San Juan del Río, 21 samples). Venipuncture was performed via the jugular vein using EDTA-coated tubes (Vacutainer™).

**DNA extraction:** DNA extraction from each sample was performed using a commercial kit (QuickGene DNA Whole Blood, Fujifilm, United Kingdom) following the manufacturer's

instructions. The total DNA concentration was measured using a spectrophotometer (NanoDrop Lite, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA).

**Nested PCR:** Each genomic DNA sample was used as a template in 25  $\mu$ L reactions containing 1 $\times$  master mix (RealQ Plus 2 $\times$  Master Mix Green, Ampliqon, Denmark). Nested PCR was carried out in a Master Cycler Gradient (Eppendorf, Hamburg, Germany) using previously reported primers and amplification conditions: García-Camacho *et al.* (2020) for PPV1, PPV2, PPV5, and PPV6; Kim *et al.* (2001) for PCV2; and Agatón-Flores (2024) for PCV3. Amplicons were visualized by electrophoresis on 1.5% agarose gels.

### RESULTS

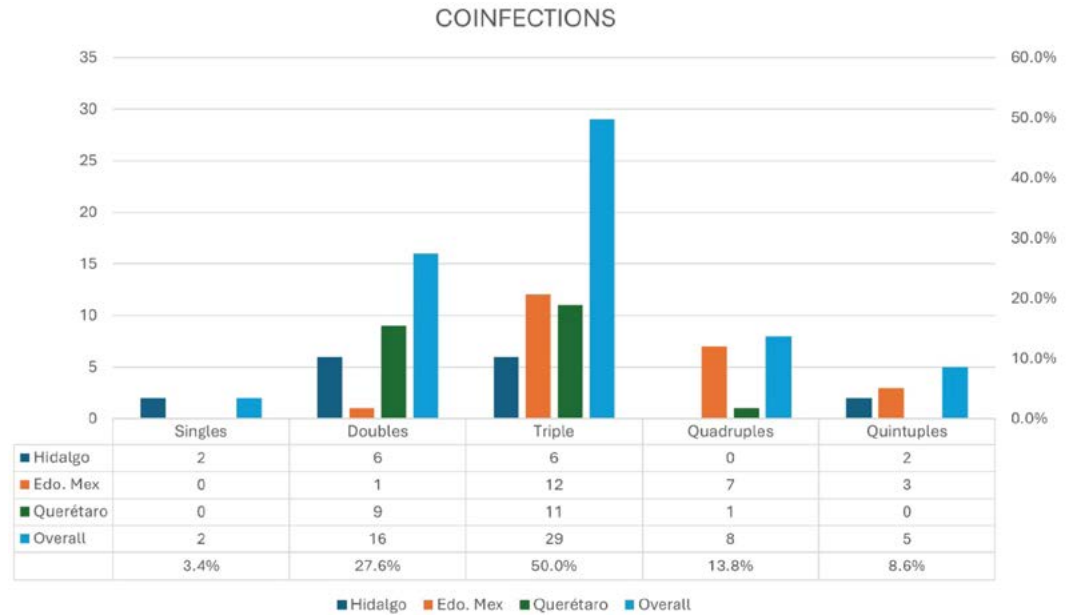
Positive cases were detected for each viral agent tested in this study, as summarized in Table 1. The overall prevalence for PPV1, PPV2, PPV5, PPV6, PCV2, and PCV3 was 5.2%, 89.6%, 67.2%, 25.9%, 50%, and 69%, respectively. In general, viral frequencies were similar across states, though some variations were observed. PPV2 was the most prevalent, with a lower proportion in Hidalgo (56.2%), while PPV1 was the least prevalent, ranging from 0% to 10%. Notably, PPV5 was less frequent in Querétaro (9.5%) than in Hidalgo and the State of Mexico. Similarly, PPV6 prevalence was lower in Querétaro (9.5%) compared to Hidalgo (37.5%) and the State of Mexico (33.3%, on average). Fluctuations were also observed in PCV2 prevalence, with the highest frequency in the State of Mexico (71.4%, on average), followed by Querétaro and Hidalgo. Conversely, PCV3 showed the highest prevalence in Querétaro (100%), surpassing levels found in the State of Mexico and Hidalgo.

Regarding co-infections, 96.5% of the samples exhibited at least one type of co-infection (Figure 1). The most frequent were triple co-infections (50%), followed by double co-infections (27.6%) and quadruple co-infections (13.8%).

Quintuple co-infections accounted for 8.6% of cases, while no sextuple co-infections were identified. In the State of Mexico, co-infections involving three or more viruses were predominant, whereas double and triple co-infections were more common in Querétaro

**Table 1.** Frequency of viral species detected by nested PCR in the study population.

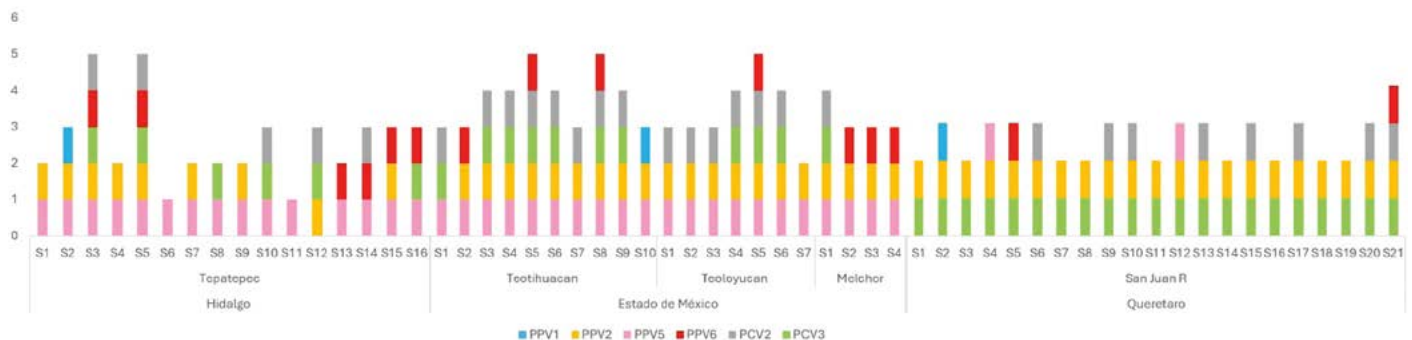
Farm	Samples	PPV1		PPV2		PPV5		PPV6		PCV2		PCV3	
		#	%	#	%	#	%	#	%	#	%	#	%
<b>Hidalgo</b>													
Tepatepec	16	1/16	6.2	9/16	56.2	15/16	93.7	6/16	37.5	5/16	31.2	6/16	37.5
<b>State of Mexico</b>													
Teotihuacan	10	1/10	10	9/10	90.0	10/10	100	3/10	30.0	8/10	80.0	7/10	70.0
Melchor Ocampo	4	0/4	0	4/4	100	4/4	100	3/4	75.0	1/4	25.0	1/4	25.0
Teoloyucan	7	0/7	0	7/7	100	7/7	100	1/7	14.2	6/7	85.7	3/7	42.8
Average	21	1/21	4.8	20/21	95.2	21/21	100	7/21	33.3	15/21	71.4	11/21	52.4
<b>Querétaro</b>													
San Juan del Río	21	1/21	4.8	21/21	100	2/21	9.5	2/21	9.5	8/21	38.0	21/21	100
Overall	58	3/58	5.2	52/58	89.6	39/58	67.2	15/58	25.9	29/58	50.0	40/58	69.0



**Figure 1.** Frequency of co-infections by state.

and Hidalgo. Notably, Hidalgo was the only location where single infections were observed (two cases). The distribution of viral assortments in co-infections is illustrated in Figure 2. Among triple co-infections, the most frequently identified combinations were PPV2/PCV2/PCV3 (28.57%), PPV2/PPV5/PPV6 (17.2%), PPV2/PPV5/PCV2 (13.8%), and PPV2/PPV5/PCV3 (6.9%). In double co-infections, the most prevalent combination was PPV2/PCV3 (56.2%), primarily observed in Querétaro, followed by PPV2/PPV5 (37.5%). The overall frequency of PPV5 co-occurring with either PPV6 or PCV3 was 6.25%. Regarding quadruple co-infections, two viral assortments were identified: PPV2/PPV5/PCV2/PCV3 (87.5%) and PPV2/PPV6/PCV2/PCV3 (12.5%). A single combination was detected in quintuple co-infections, consisting of PPV2/PPV5/PPV6/PCV2/PCV3.

Regarding the distribution of viral combinations by state, in Hidalgo, PPV5 was detected in all cases except one. Two single infections were observed, while PPV5 showed a strong association with PPV2. The next most frequently linked viruses in co-infections were PCV3 and PCV2, in that order. A similar pattern was observed in backyard farms in the State of Mexico, where PPV5 and PPV2 were closely associated. However, a greater



**Figure 2.** Assortments of viruses in co-infections by state. S=sample.

number of viral species were involved in this region, with a high prevalence of PCV2 and PCV3. In contrast, the most frequent viral combination in Querétaro was PPV2/PCV3, with PCV2 playing a significant role in triple co-infections.

## DISCUSSION

In porcine backyard farms, basic diagnostic practices are rarely implemented. As a result, the prevalence of infectious agents and diseases remains largely unknown, potentially creating conditions favorable for disease outbreaks (Alonso & Maqueda, 2020). In this study, PPV2, PCV3, and PPV5 were the most prevalent viruses detected, exhibiting distinct distributions and assortments across different states and farms. Globally, PPV2 has consistently been reported as the most prevalent Parvoviridae species (Streck *et al.*, 2013; Saekhow & Ikeda, 2015; Saekhow *et al.*, 2014; García-Camacho *et al.*, 2020), a finding that aligns with the results of this study. The potential association of PPV2 with disease has not yet been fully elucidated. Regarding PPV5, its reported seroprevalence in China, South Korea, and Poland ranges from 20.0% (Li *et al.*, 2021; Kim *et al.*, 2022; Milek *et al.*, 2020) to 41.3% in oral fluid samples (Milek *et al.*, 2019), which is lower than the overall PPV5 prevalence (67.2%) observed in this study. In Mexico, a retrospective study using PCV2-affected and PCV2-unaffected paraffin-embedded tissues reported an overall PPV5 prevalence of 32.4% and a prevalence of 28.0% in reproductive failure (RF) cases. That study found a significant association between PPV5 and Porcine circovirus-associated disease (PCVAD), as well as with RF cases in PCV2-affected farms. Although PPV2 was the most prevalent Parvoviridae species in that case series, no direct association with PCVAD or RF was established (García-Camacho *et al.*, 2020). In this study, PCV3 exhibited a high overall prevalence (69.0%), followed by PCV2 (50.0%). While PCV2 is widely recognized as a pathogenic virus causing PCVAD, PCV3 has been detected in pigs with clinical conditions such as Porcine Dermatitis and Nephropathy Syndrome (PDNS), RF, and multisystemic inflammation (Palinski *et al.*, 2017; Phan *et al.*, 2016), suggesting its potential involvement in disease. However, because the gilts sampled in this study were clinically healthy, no direct correlation with disease can be made. Nonetheless, co-infections among different viral species are increasingly common in animal populations, and these viral interactions may contribute to disease severity. The impact of specific viral co-infections on animal health remains unclear (Vargas-Bermúdez *et al.*, 2023). The distinct viral distribution patterns identified in this study could play a role in disease outbreaks, as the detected viruses have either been directly associated with disease or reported in clinical cases. Hidalgo and the State of Mexico displayed particularly high PPV5 prevalence (93.7% and 100.0%, respectively). In Hidalgo, PPV5 appeared to be a key viral agent, detected in single infections and primarily co-infecting with PPV2, followed by PCV3. Notably, the only sample negative for PPV5 exhibited a viral assortment of PPV2/PCV3/PCV2, emphasizing the relevance of these four viruses in the local farm population. Similarly, in the State of Mexico, the PPV5/PPV2 combination was dominant (20 out of 21 samples). Triple and quadruple co-infections involving three or more viruses were more frequent in this region than in the other states, including quintuple co-infections. In this setting,

PCV2 and PCV3 were detected in 71.4% (15/21) and 52.4% (11/21) of the samples, respectively. In contrast, PPV5 prevalence was significantly lower in Querétaro (9.5%). Interestingly, co-infection of PPV2 with PCV3 was observed in all samples from this location, primarily as double co-infections, with a considerable presence of PCV2 in triple co-infections. This finding contradicts previous studies, where PCV3 was detected at lower rates (30.2%-40.9%) in RF cases (Saporiti *et al.*, 2021; Wang *et al.*, 2019; Chang *et al.*, 2020; Xu *et al.*, 2021). It is well established that co-infections involving multiple viruses can exacerbate disease severity. For example, PCV2 co-infection with other pathogens, such as PPV1 and Porcine Reproductive and Respiratory Syndrome Virus (PRRSv), has been linked to increased pathogenicity (Cui *et al.*, 2023). Additionally, viral co-infections may enhance the likelihood of recombination events, potentially leading to increased virulence or the emergence of new genotypes (Kwon *et al.*, 2017). These findings suggest that the observed viral assortments may contribute to disease outbreaks. PPV5 has already been associated with PCVAD in Mexico (García-Camacho *et al.*, 2020), a possible link between PPV2 and PCV2 has been proposed (Novosel *et al.*, 2018), and ongoing research continues to investigate PCV3's role in disease (Phan *et al.*, 2016). Additionally, PCV3 prevalence has shown an increasing trend in both the U.S. and Taiwan (Wang *et al.*, 2019; Chang *et al.*, 2020). Conversely, PPV6 and PPV1 were the least prevalent species in backyard farms, consistent with previously reported data indicating prevalence rates of 19.4%-25.8% for PPV6 (Milek *et al.*, 2020; Kim *et al.*, 2022; Faustini *et al.*, 2024) and 0.0%-3.5% for PPV1 (Cui *et al.*, 2017; Milek *et al.*, 2019; Kim *et al.*, 2022). A potential role of PPV6 in RF has been suggested, as its prevalence in fetal heart tissue from aborted fetuses was high (57.0%), particularly in PCV2-affected farms (García-Camacho *et al.*, 2020). However, in this study, the PPV6/PCV2 co-infection rate was relatively low (12.5%). Since the gilts were clinically healthy with no reproductive history, no assumption can be made about PPV6's role in RF in backyard farms, though its involvement seems unlikely based on these findings. As for PPV1, its low prevalence was expected, as it has been successfully controlled through vaccination. In fact, in the backyard farms sampled in this study, the pig population is vaccinated against PPV1, similar to high-density commercial farms. Further follow-up studies in these farms could provide valuable insights into the role of each viral agent and the potential impact of viral co-infections on disease development.

## CONCLUSIONS

In this study, all assessed viral species (PPV1, PPV2, PPV5, PPV6, PCV2, and PCV3) were detected in gilts from backyard farms. This represents the first report on the prevalence of PPV2, PPV5, PPV6, and PCV3 in such settings. Among the experimental population, PPV2 was the most prevalent species, followed by PCV3, a recently described virus in Mexico. The co-existence of multiple viral species within gilts exhibited distinctive patterns that warrant further investigation to determine their potential contribution to disease.

## ACKNOWLEDGEMENT

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