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Porcine Circovirus Type 2 Genotypes and PCV3 in Swine Clinical Samples From Brazil

Genótipos de Circovírus Suíno Tipo 2 e PCV3 em Amostras Clínicas de Suínos no Brasil

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Abstract: Porcine circovirus type 2 (PCV2), an important pathogen in swine, causes PCV disease (PCVD). Although PCVD is effectively controlled using commercial vaccines, its clinical presentation is changing. Moreover, PCV2 is genetically evolving, with new genotypes emerging in vaccinated or unvaccinated pigs. In this study, we aimed to verify the presence of the PCV2a, PCV2b, and PCV2d genotypes in PCV-positive porcine samples. Furthermore, to identify coinfections between the PCV2 genotypes and/or PCV3, which can also induce disease in pigs, we employed a quick, effective, and low-cost PCR diagnostic test. In this study, 333 PCV2 PCR and clinically positive samples from various production stages and herds across Brazil were analyzed. Among these, 266 samples were genotyped, with PCV2b emerging as the most predominant genotype (56.77% of the positive samples), mainly observed in nursery pigs. PCV2d was also identified in 33.10% of the samples, primarily from finishing pigs and breeding sows. The employed PCR test was compared with a commercial kit, proving effective in PCV2 genotyping. This study demonstrates the significance of PCV2 genotyping, showing PCV2b as the most predominant genotype responsible for disease in pig farms in Brazil. PCV2a, the prevalent genotype used in commercial vaccines, was not detected in any of the analyzed samples. While pigs infected with other PCV2 genotypes may receive some heterologous protection from PCV2a vaccines, adequate diagnosis and vaccine monitoring for updates must be considered.

Keywords: Genotyping; PCV2a; PCV2b; PCV2d

Resumo: O Circovírus Suíno tipo 2 (PCV2) é um importante patógeno para suínos e causador da circovirose suína. Embora a circovirose seja bem controlada pelas vacinas comerciais, a sua apresentação está mudando. Além disso, o PCV2 está evoluindo genomicamente, com novos genótipos emergindo em suínos vacinados ou não vacinados. O objetivo deste estudo foi verificar a presença de os genótipos PCV2a, PCV2b e PCV2d em amostras de suínos positivas para PCV. Além disso, identificar coinfecções entre genótipos de PCV2 e/ou com PCV3, que também podem causar doenças em suínos, utilizando um teste de diagnóstico PCR rápido, eficaz e de baixo custo. Foram analisadas 333 amostras clínicas para PCV2 positivas por PCR e provenientes de diferentes fases de produção e rebanhos do Brasil. Destas, 266 foram genotipadas, sendo o PCV2b o genótipo mais predominantemente encontrado em 56,77% das amostras positivas, principalmente provenientes de crechários. O PCV2d também

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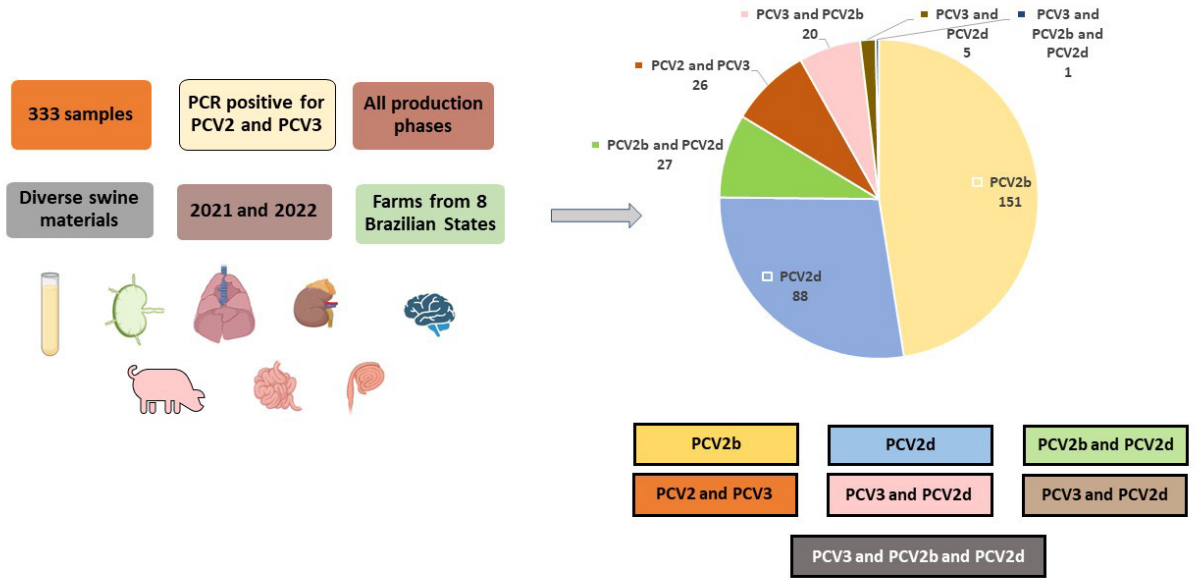
foi detectado em 33,10% das amostras, principalmente em suínos de terminação e porcas reprodutoras. O teste PCR utilizado neste estudo foi comparado a um kit comercial e foi eficaz na genotipagem do PCV2. Este estudo demonstrou a importância da genotipagem do PCV2 e que o PCV2b continua sendo o genótipo mais predominante nas granjas de suínos no Brasil, causando a doença. O PCV2a, o genótipo mais comum utilizado nas vacinas comerciais, não foi detectado em nenhuma amostra analisada. Os suínos infectados com outros genótipos de PCV2 podem obter alguma protecção heteróloga das vacinas contra PCV2a, mas o diagnóstico adequado e monitoria da vacina para atualizações devem ser considerados.

Palavras-chave: Genotipagem; PCV2a; PCV2b; PCV2d

Resumo gráfico



1. Introduction

Porcine circovirus type 2 (PCV2) is the causative agent of porcine circovirus–associated disease (PCVD). PCV2 was initially identified in 1998 in the tissues of pigs suffering from postweaning multisystemic wasting syndrome (PMWS)[1, 2], a syndrome first observed in pigs within high-health Canadian herds in 1995. Since then, PCV2 has been linked to various clinical manifestations. These include systemic, enteric, respiratory, and reproductive diseases, as well as porcine dermatitis and nephropathy syndrome, now collectively recognized as PCV2-associated disease, PCVAD, or PCVD[3, 4]. PMWS, the most prevalent and severe clinical manifestation of PCV2 infection, is characterized by loss and severe lesions in lymphoid tissue and is classified as systemic or subclinical. Although PCV2 infection was first identified in Brazil in 2000[5], retrospective studies have revealed its presence as early as 1978[6]. Since its initial detection, PCV2 infection has been widely distributed across Brazilian pig herds, leading to sanitary and production losses[7]. While PCVD can be managed by correcting risk factors and using vaccines[4], it remains a challenge in Brazil, primarily due to its evolving manifestations.

PCV2 belongs to the Circovirus genus within the Circoviridae family[4]. It is the smallest single-stranded DNA virus with autonomous replication, characterized by nonenveloped, circular symmetry[1]. The evolution of this genotype in recent years has been characterized

by severe outbreaks of PCVD, notably reported in North America from 2004 to 2006. During this period, the genomic composition of PCV2 isolates found in affected herds was compared to that of previously found isolates[8-10]. Subsequently, a new PCV2 variant was detected in PCV2-vaccinated herds across North America, China, Brazil, and numerous swine-producing countries[11-13]. This evolution was linked to PCV2 vaccine failures. In Brazil, a PCV2 mutant associated with vaccine failures was initially identified in 2012, with the viral strain closely resembling that observed in China between 2004 and 2008 [13]. The virus was isolated from pigs vaccinated against PCV2 that exhibited typical clinical symptoms of PCVD and a positive laboratory diagnosis of PCV2. Genomic analyses revealed additions or substitutions of nucleotides, resulting in modifications within the genome of the virus, called mPCV2. These alterations, in turn, induced changes in amino acids located within epitopes responsible for immune system activation. Notably, at the end of the open reading frame 2 (ORF2) sequence for the viral capsid protein, the variant exhibited additional nucleotides coding for three extra amino acids[13].

Due to the high genomic variability observed in PCV2, isolates have been categorized into nine genotypes, ranging from PCV2a to PCV2i, based on ORF2 or complete genome sequences[14-16]. Presently, numerous commercial vaccines are available, most of which are based on genotype "a" (PCV2a) of PCV[4, 17]. However, given the rise in circulating genotypic diversity, more effective vaccines that incorporate updates and/or include a broader range of genotypes are being considered[17]. In addition to PCV2, numerous PCDV-related illnesses involve other agents (e.g., porcine circovirus type 3, PCV3), aggravating the severity of clinical cases[8]. Thus, investigating PCV3 and PCV2 coinfections, including genotyping, is imperative in clinical PCVD scenarios[18, 19].

Brazil is the fourth-largest pork producer and exporter globally, making it a significant player in the international market[20, 21]. Despite the prominence of swine production in Brazil, with over 2 million sows housed and 5 million tons of pork produced in 2022 [21], only a few laboratories conduct molecular analyses, particularly specialized analyses such as viral genotyping [22]. This makes it difficult for veterinarians to interpret clinical cases, especially when vaccines are used to manage diseases such as PCVD. Considering the losses inflicted by PCV2 infection on global pork production and the resulting economic impact, coupled with the ongoing detection of PCV2 in vaccinated herds, we aimed to characterize PCV2 genotypes to assess the prevalence of PCV2a, PCV2b, and PCV2d in the field, delineated by region, production phase, and collected material. Additionally, we aimed to determine the occurrence of coinfections among genotypes and identify which genotype predominates in coinfections with PCV3 through a quick, low-cost, and effective diagnostic method.

2. Materials and Methods

2.1 Analyzed samples.

A total of 333 samples from suspected PCVD clinical cases submitted to the Animal Health Diagnostic Center (CEDISA) between 2021 and 2022 were used in this study. These samples

originated from eight Brazilian states: Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina, and São Paulo. The samples were collected from various organs and materials based on clinical conditions and compatibility with PCVD lesions. The sample types comprised 105 serum samples, 10 sow uterus samples, four rectal swab samples, three kidney samples, 50 lung samples, six lymph node samples, 31 intestine samples, 61 fetus samples (including stillbirths and mummified fetuses), two brain samples, and 58 organ pool samples (comprising liver, kidney, lung, spleen, lymph node, and heart tissue). The samples were obtained through a technical-scientific agreement between the Embrapa Swine and Poultry Research Center and CEDISA (Agreement 21000.18/0009-7)[22].

All samples underwent viral DNA detection for PCV2 or PCV3 using quantitative real-time PCR (qPCR). The samples were pretreated overnight with tissue lysis buffer for nucleic acid purification (ATL, Qiagen, Venlo, Netherlands) and proteinase K (Qiagen) at 56°C under agitation. Viral DNA extraction was conducted using an IndiMag Pathogen Kit (Indical Bioscience, Leipzig, Germany) with an automated extraction system, IndiMag 48 s (Indical Bioscience). The qPCR for PCV2 diagnosis targeted the amplification of a Cap gene (ORF2) region using the specific primers PCV2_F (5'-CCAGGAGGGGCGTTGTGACT-3') and PCV2_R (5'-CGCTACCGTTGGAGAAGGAA-3') and the probe PCV2_S (5'-AATGGCATCTTCAACACCCGCCTCT-3'), as outlined in a previous study[23]. For the qPCR, 2.5 µL of extracted DNA was added to a GoTaq® Probe qPCR master mix (Promega Corporation, Madison, WI, USA), along with 1.25 µM and 0.5 µM concentrations of the specific primer and probe, respectively. Fluorescence signal detection was conducted at the end of each extension cycle phase using the QuantStudioTM Flex 6 thermocycler (Applied Biosystems, Foster City, CA, USA). All samples with Ct < 38.00 (Cycle threshold) were considered positive. For PCV3 detection, the conserved replication gene was targeted using the primers PCV3_535_F (5'-TGA CGG AGA CGT CGG GAA AT-3') and PCV3_465_R (5'-CGG TTT ACC CAA CCC CAT CA-3'), along with the probe PCV3_535_F (5'-TGA CGG AGA CGT CGG GAA AT-3'), as described in a previous study.[14] For the qPCR procedure, 2 µL of extracted DNA was added to a GoTaq Probe qPCR master mix (Promega), along with 0.8 µM and 0.4 µM of the specific primer and probe, respectively. The cycling parameters consisted of an initial denaturation step at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min using a QuantStudioTM Flex 6 thermocycler (Applied Biosystems). The fluorescence signal was acquired at the end of each cycle extension phase.

2.2 PCV2 DNA–positive controls

To obtain positive controls for the reaction, 20 samples were randomly selected and subjected to sequencing. The sequencing reaction utilized previously described primers[24] to amplify the ORF2 region of PCV2. Following genomic sequencing analysis, positive control samples were selected for the PCV2b and PCV2d genotypes. For PCV2a, a previously sequenced reference sample from the "Collection of microorganisms of interest to swine and poultry farming" at Embrapa Swine and Poultry was used[13]. Subsequently, the sequenced samples were tested to validate the PCR assays. The results of the positive control test are illustrated in Figure 1.

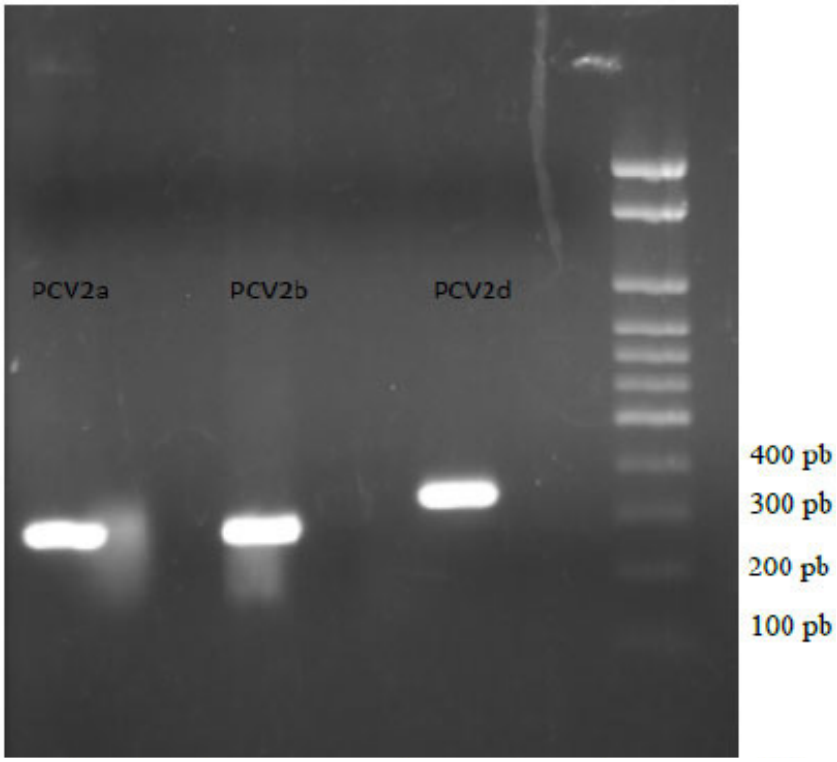


Figure 1 Agarose gel electrophoresis showing the separation of amplified DNA fragments of PCV2 genotypes by molecular weight, with PCV2a at 277 bp, PCV2b at 277 bp, and PCV2d at 343 bp

2.3 Conventional PCR (cPCR) used for PCV2 genotyping.

Each sample was individually tested for the three PCV2 genotypes of interest. The PCR targeted the ORF2 region of PCV2, known for its increased diversity rates. The primer sequences used[25-27] are shown in Table 1. The primers were diluted to 10 pmol, and the PCR mixture comprised 2.5 µl of buffer, 1 µl with 25 mmol of MgCl2, 1 µl of dNTPs, 0.6 µl of each primer, and 2 µl of DNA from each sample. The following PCR conditions were employed: initial denaturation at 94°C for 1 min, followed by 35 cycles with denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min[25-27]. The PCR products underwent 1% agarose gel electrophoresis and were visualized under ultraviolet light after staining with 2% ethidium bromide. Due to the identical amplicon size of the PCV2a and PCV2b genotypes, each sample was submitted separately to PCR to distinguish between the genotypes.

Table 1 Primers used in the PCV2 genotyping analyses

Primer Name	Nucleotide sequence (5'-3')	Product size
PCV2ab - F	GGT TGG AAG TAA TCA ATA GTG GA	277 bp
PCV2a - R	GGG GAA CCA ACA AAA TCT C	
PCV2ab - F	GGT TGG AAG TAA TCA ATA GTG GA	277 bp
PCV2b - R	GGG GCT CAA ACC CCC GCT C	

PCV2d - F	GGT TGG AAG TAA TCG ATT GTC CT	343 bp
PCV2d - R	TCA GAA CGC CCT CCT GGA AT	

Table 1 Forward (F) and reverse (R) primer nucleotide sequences, amplified DNA product size in base pairs (bp), and PCR conditions adapted from a previous study[25-27]

2.4 PCV2 genotyping using a commercial qPCR kit.

Eighteen samples underwent genotype detection using a commercial qPCR kit (Kylt® PCV-2 Typing kit, SAN Group Biotech, Höltinghausen, Germany). The results were then compared with those obtained from cPCR to assess sensitivity and specificity. Kylt® PCV2 Typing kits utilize a multiplex real-time PCR approach and are designed to differentiate the viral DNA of PCV2a, PCV2b, and PCV2d. The positive or negative results of each sample, including coinfections, were subjected to Cohen's Kappa test to evaluate the agreement between the two tests.

2.5 Ethics approval

All samples used in this study were obtained from pigs presented for diagnostic testing at the CEDISA laboratory in Concordia, Brazil. Since the study did not involve human samples, it was not presented to any ethics committee.

3. Results

PCV2b or PCV2d were detected in 266 of the 333 analyzed samples subjected to genotyping. However, genotype detection was not feasible in 67 samples, as outlined in Table 2. Among the 266 genotyped samples, 151 were positive for PCV2b (51.7%), 88 for PCV2d (30.1%), and 27 for both PCV2b and PCV2d (9.2%) (Table 2). However, no sample tested positive for PCV2a.

Table 2 Percentage and type of PCV2-genotyped samples.

Sample or tissue	Number of analyzed samples	Positive for PCV2b	Positive for PCV2d	Positive for PCV2b and PCV2d (coinfection)
Organs (pool)	52	41 (78.85%)	9 (17.30%)	2 (3.85%)
Brain	2	2 (100%)	0 (0%)	0 (0%)
Fetuses	40	29 (72.5%)	10 (25%)	1 (2.5%)
Feces	3	3 (100%)	0 (0%)	0 (0%)
Intestine	29	13 (44.82%)	13 (44.82%)	3 (10.34%)
Lymph nodes	6	6 (100%)	0 (0%)	0 (0%)
Lungs	44	28 (63.63%)	13 (29.54%)	3 (6.81%)
Kidney	3	2 (66.67%)	0 (0%)	1 (33.33%)

Serum	81	23 (28.4%)	43 (53.1%)	15 (18.51%)
Rectal swab	3	1 (33.33%)	0 0%)	2 (66.67%)
Uterus	3	3 (100%)	0 (0%)	0 (0%)

Table 2 PCV2 PCR Genotype results per analyzed sample (organs and fluids). The table presents the number of samples and the percentage of positive results for each PCV2 genotype or coinfection.

PCV2b was detected in brain samples (2/2), lymph nodes (6/6), feces (3/3), and uterus tissue (3/3) among the tested samples. Conversely, serum samples exhibited the highest frequency of positivity for PCV2d, with 43 positive samples out of the 81 total positive samples (43/81). Hence, it is evident that PCV2b was present in all types of tested samples. As depicted in Table 2, rectal swab samples exhibited the highest percentage of coinfection between the PCV2b and PCV2d genotypes.

The eight Brazilian states whose samples were analyzed produce over 90% of all pork in Brazil[20]. Most of the samples originated from the three southern states (Paraná, Rio Grande do Sul, and Santa Catarina), which represent the largest swine production region in the country. Concerning genotype distribution by state, PCV2b was predominantly detected in samples from Paraná (61.99%), whereas PCV2d was more prevalent in samples originating from São Paulo. Mato Grosso do Sul exhibited the highest coinfection rates between the two genotypes (PCV2b and PCV2d), and genotyping was feasible in 100% of the tested samples (Figure 2).

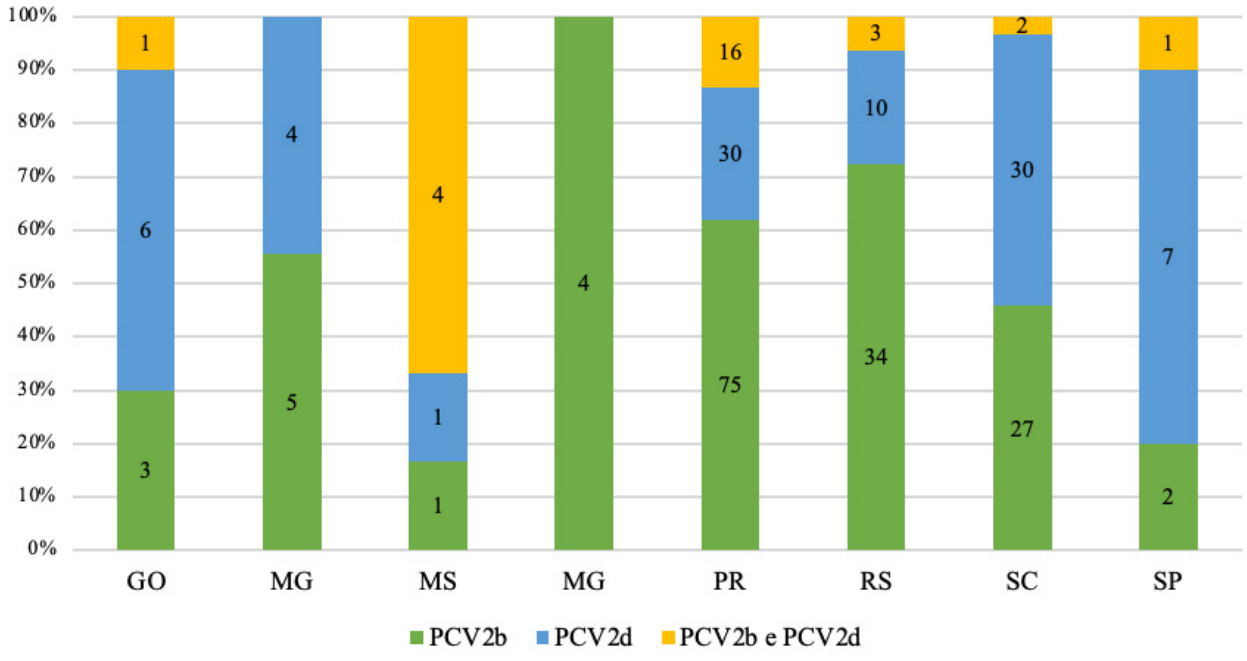


Figure 2 PCV2 genotype detection per sample by each Brazilian state, showing the most prevalent genotype in each sample.

Regarding the production phase, the finishing phase yielded the highest number of genotyped samples. Among these, PCV2d was the most frequently detected genotype in 62 samples, while PCV2b was detected in 41 samples. Additionally, PCV2d exhibited a higher prevalence in the sow samples. In the nursery phase, the most prevalent genotype was PCV2b,

present in 51 samples, while PCV2d was detected in 11 samples. Notably, the highest rates of coinfection of PCV2b and PCV2d were observed in the finishing phase samples, totaling 20. Furthermore, the highest rates of PCV2b were identified in samples for which the production phase was not specified, as outlined in Table 3.

Table 3 PCV2 genotype detection per production phase, showing the most positive genotypes and the total number of positive samples.

Production Phases	Number of analyzed samples	Positive for PCV2b	Positive for PCV2d	Positive for PCV2b and PCV2d (coinfection)
Replacement pigs	12	8 (66.67%)	1 (8.33%)	3 (25%)
Sows	9	3 (33.33%)	5 (55.56%)	1 (11.11%)
Gestation sows	4	2 (50%)	2 (50%)	0 (0%)
Farrowing sows	24	17 (70.83%)	6 (25%)	1 (4.16%)
Nursery pigs	64	51 (79.68%)	11 (17.18%)	2 (3.12%)
Finishing pigs	119	42 (35.3%)	57 (47.9%)	20 (16.8%)
Uninformed	34	28 (82.35%)	6 (17.65%)	0 (0%)

Table 3 PCV2 PCR genotype results per analyzed sample (pig production phase). The table displays the number of analyzed samples, accounting for the origin of the sample and the percentage of positive results for each PCV2 genotype or coinfection.

3.1 Occurrence of PCV2 genotypes and/or PCV3 coinfections

Twenty-seven samples exhibited coinfection between the PCV2b and PCV2d genotypes, accounting for 9.2% of the cases. However, no other coinfection between the PCV2 genotypes was identified, with the majority of coinfections detected in samples obtained from swabs constituting 66.67% of positive samples. Moreover, coinfections with PCV3 were evident in 26 samples involving both PCV2 and PCV3, accounting for 8.9% of cases. Samples obtained from fetuses exhibited the highest positivity for coinfection between PCV2 and PCV3. Furthermore, a higher prevalence of "b" genotype samples was observed in these coinfections, with 20 samples testing positive for PCV2b and five testing positive for PCV2d. Only one sample exhibited positivity for coinfection involving PCV2b, PCV2d, and PCV3 (data not shown).

3.2 Assessment of the agreement between the two tests for PCV2 genotyping

Eighteen samples were selected based on PCR results and subsequently tested with the commercial kit (Kylt® PCV-2 Typing kit) for comparison with the cPCR. The analysis revealed that most samples exhibited coinfection between the genotypes (Table 4). The results underwent the Kappa test to assess the agreement between the tests, and the findings are presented in Table 5.

Table 4 Comparison between the two PCV2 genotyping tests

Sample	Conventional PCR	Commercial Kit
1	PCV2b	PCV2b
2	PCV2b	PCV2b and PCV2d

3	Negative	Negative
4	PCV2b and PCV2d	Negative
5	Negative	Negative
6	PCV2b and PCV2d	PCV2b and PCV2d
7	Negative	Negative
8	PCV2b and PCV2d	PCV2b and PCV2d
9	PCV2b and PCV2d	Negative
10	PCV2b and PCV2d	PCV2b and PCV2d
11	PCV2b and PCV2d	PCV2b and PCV2d
12	PCV2b	Negative
13	PCV2b	Negative
14	PCV2b and PCV2d	PCV2b and PCV2d
15	PCV2b and PCV2d	PCV2b and PCV2d
16	PCV2b and PCV2d	PCV2b and PCV2d
17	PCV2b and PCV2d	PCV2b and PCV2d
18	PCV2b and PCV2d	PCV2b and PCV2d

Table 5 Cohen-Kappa statistical test to assess the agreement between the two tests.

Genotype	KAPPA	Accuracy
PCV2b	0.478 (0.094 – 0.863)	77.8
PCV2d	0.658 (0.308 – 1.000)	83.33
PCV2	0.478 (0.094 – 0.863)	77.8

Table 5 Comparison of PCV2 genotype results obtained using cPCR and a commercial test kit (Kylt® PCV-2 Typing kit) for each sample, along with Cohen's kappa coefficient. Cohen's kappa statistical measure evaluates the possibility of agreement occurring by chance. The table shows the accuracy of both tests, with coefficients ranging from −1 to 1. Here, 1 indicates perfect agreement, and 0 indicates agreement that is no better than chance.

The cPCR was more sensitive in particular samples, resulting in the successful genotyping of four samples. Conversely, the commercial kit failed to characterize the genotypes in the analyzed samples. Only one sample (Sample 2) exhibited a discrepancy between the commercial kit and cPCR, wherein the commercial kit identified a coinfection (PCV2b and PCV2d), while the cPCR test detected only PCV2b. The data analysis between the genotypes and the two tests revealed consistent results. The PCV2b test and the general PCV2 (cPCR and commercial kit) exhibited the same accuracy of 77.8%. In the PCV2d test, the accuracy was higher, reaching 83.33%. The kappa test revealed a confidence interval greater than 0, indicating that both tests could identify and demonstrate agreement between the genotypes. However, the highest agreement was observed in the identification of PCV2d, as it exhibited accuracy and a larger confidence interval.

4. Discussion

In this study, we examined clinical samples diagnosed with PCVD that had previously tested positive for PCV2 at the CEDISA laboratory, a diagnostic laboratory for swine diseases in Brazil[22]. As established for diagnosing PCV2 infections[4], the CEDISA laboratory includes

tests within its scope for detecting antigens and nucleic acids, as well as the examination of micro and macroscopic lesions to correlate with the clinical signs of the disease. However, at CEDISA, PCV2 genotyping was not yet available as part of the diagnostic service. To supplement the diagnosis of PCV2-vaccinated herds exhibiting signs of PCVD, our study involved genotyping 79.88% (266/333) of the samples for PCV2. The results revealed that PCV2b was the predominant genotype detected in 151 of the 266 analyzed samples. This observation aligns with numerous studies worldwide, indicating that PCV2b and PCV2d are presently the most prevalent genotypes of PCV2 infection in pigs[11, 28]. Furthermore, our study revealed that the PCV2b and PCV2d genotypes were present in samples from all Brazilian states examined, confirming their widespread occurrence in the country. Interestingly, despite genotyping PCV2-positive samples from clinical cases, PCV2a was not detected. Previous studies have indicated a shift in the predominant genotypes in pig production over time, transitioning from the PCV2a genotype to PCV2b in 2002[29]. Moreover, in 2012, there was another shift from PCV2b to PCV2d[4, 13, 17]. Additionally, it is worth noting that PCV2a-based vaccines are predominantly used in Brazilian swine herds (M. Donin, personal communication).

Coinfections between genotypes (PCV2b and PCV2d) are becoming more prevalent[15, 17, 28, 30], and the same was observed in 27 samples in our study. Additionally, we observed coinfection with PCV3 in 26 samples. Again, this finding is consistent with worldwide research, where coinfections between PCV2 and PCV3 genotypes have been identified[16, 17, 31]. However, none of this research specifies which PCV2 genotype is predominantly involved in coinfections with PCV3.

Lymph node samples are a reliable indicator of the extent of PCV2 infection in pig farms[32]. In our study, genotyping was feasible for all lymph node samples received. Alongside meticulous diagnosis during animal assessment, ensuring high-quality samples is essential. PCV2b was identified in all samples, indicating its role in systemic animal infection. Conversely, PCV2d was detected in lung, fetus, intestine, and blood serum samples. An important observation in this study was the detection of PCV2b in brain samples from pigs in the finishing phase. Brain lesions due to PCV2 can be occasional[4]. However, in our study, correlating the clinical symptoms presented by the animals with the PCV2 genotype detected was not feasible.

PCV2d emerged as the most predominant genotype in the finishing phase. This finding aligns with a study conducted in South Korea, which reported a higher prevalence of PCV2d in pig lymph nodes at slaughter[32]. Conversely, in the nursery phase, PCV2b was the most prevalent genotype. To our knowledge, this is the first study to uncover such data and explore the correlation between genotype and the production phase. The finishing phase yielded the most positive samples for PCV2, which could be genotyped. This is likely attributed to the decline in vaccine and maternal immunity, rendering pigs more susceptible to PCV2 infections during this phase[32].

5. Conclusion

PCV2 genotyping is crucial to discerning the predominant genotype within each farm, aiding in disease control and vaccine efficacy monitoring. Therefore, in our analyses, cPCR demonstrated greater effectiveness than commercial kits, making it a cost-effective alternative for quick diagnosis. PCV2b continues to be prevalent in farms, alongside an observed rise in the detection of PCV2d in the samples analyzed. Notably, coinfections between genotypes, including those with PCV3, were observed. These findings emphasize the significance of monitoring vaccine efficacy and the emergence of clinical circovirus disease. Hence, studying the evolution of PCV2 into various genotypes and assessing the updating of existing vaccines on the market is crucial, given the high mutation rate of the virus. Controlling circovirus disease should entail a blend of effective vaccine usage with biosafety and sanitary management practices to enhance pig health.

Declarations Conflict of interest/Competing interests:

The authors declare that they have no conflicts of interest. competing interests.

Data availability

The data generated from this research are published here and can also be requested from the authors.

Author contributions:

Rovian Miotto: Methodology, Data curation, Writing- Original draft preparation, Validation. Formal Analysis. Caroline Pissetti: Methodology, Data curation, Validation, Resources. Luiz Carlos Bordin: Investigation, Resources. Janice Reis Ciacci Zanella: Supervision. Conceptualization, Methodology, Resources.Writing- Reviewing and Editing, Project administration, Funding acquisition.

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