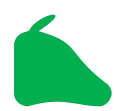
https://doi.org/10.22319/rmcp.v16i2.6787

Article



Determination of seroconversion induced by two treatments against porcine parvovirus and identification of porcine parvovirus 4 in sows with reproductive signs

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Abstract:

Porcine parvovirus causes mummified fetuses and stillbirth. Immunization programs are based on commercial vaccines and feedback (oral administration of a macerate of parvovirus-mummified fetuses). The present study assessed the seroconversion of these two methods on a farm in Sonora, Mexico, with reproductive problems. Replacement gilts are divided into: T1, feedback at 24 wk of age and T2, commercial vaccine (porcine parvovirus) at 24 and 27 wk of age. Multiparous sows are split into: T3, feedback at weaning and T4, commercial vaccine 2 wk before farrowing. Serum samples were taken the week before administrating the treatments (T0) and three weeks later to evaluate the immune response. T1, T2, T3, and T4 seroconverted 90, 83, 78, and 50 %, respectively. The presence of the porcine parvovirus genome was analyzed by endpoint PCR, using specific indicators (genotypes 1 to 6) with the presence of porcine parvovirus 4, in samples of T1, T2, and T4, which are analyzed by next-generation sequencing and porcine parvovirus 4 is confirmed, in addition to the identification of other viruses, bacteria, fungi, and parasites. The presence of porcine parvovirus 4 emphasizes the importance of evaluating preventive medicine programs, under the specific conditions of each farm, to optimize the prevention of reproductive conditions since commercial vaccines may not be effective and feedback treatment may favor the dissemination of other pathogens that are present on the farm.

Keywords: Vaccine, Feedback, Porcine Parvovirus, Parvovirus 4, Control Strategies.

Received: 11/10/2024

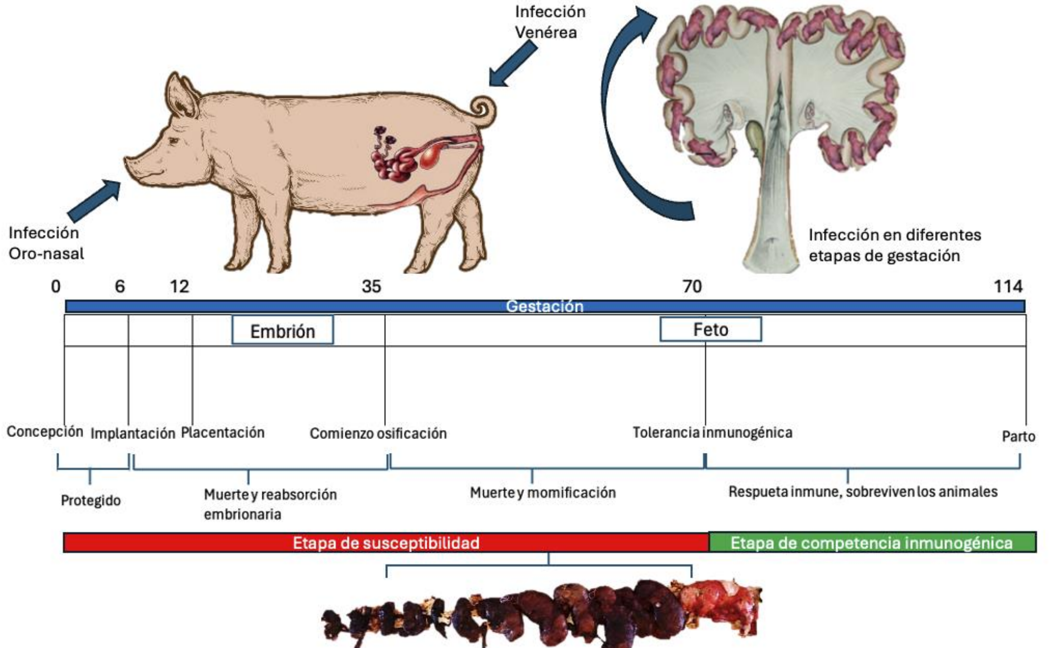
Accepted: 22/04/2025

Introduction

Porcine parvovirus (PPV), which is important in the breeding herd, belongs to the SMEDI group[1,2] due to the symptoms it presents: stillborn piglets, mummified piglets, and infertility in sows[3,4]. PPV is stable in the environment and is ubiquitous in pigs[5,6], so it is important to develop a correct immunization program against it[7,8,9].

The development of the clinical disease in animals is limited to sows that are in the reproductive stage; if the infection takes place during the first half of gestation, it can cause the death of the embryo or fetus, followed by embryonic resorption, or fetal mummification. If transmission occurs after the first half of gestation, fetuses can survive in utero without clinical effects (Figure 1)[10].

Figure 1: Timeline of PPV infection during gestation. Modified from Mészáros[10]



The immunization programs used in pig farms are based on two main methods, the first consists of using commercial vaccines (in Mexico, only inactivated vaccines are employed), which can be alone or accompanied by bacterial agents, such as *Erysipelothrix rhusiopathiae* and *Leptospira* spp. The second method is the oral administration of crushed fecal matter, mummies, and placenta (feedback) to replacement gilts[4,7,11,12,]; the latter presents the risk of disseminating other pathogens existing on the farm[4,11]; furthermore, because the concentration or viral load of the inoculum is not determined, it is possible that there is no induction of an adequate immune response[13].

Porcine parvovirus has several genotypes that can affect the epidemiology of the disease and its control in pig farms. Surveillance and proper management are crucial to prevent outbreaks and minimize the impact on pig production. In the European and Asian continents, there are reports of the circulation of PPV genotypes 1 to 7[8,14,15,16], and in North America, PPV genotypes 1, 2, 5 and 6 have been reported[17,18]. It has also been shown that there may be co-infections with different PPV genotypes[19,20]. Studies regarding vaccination against PPV show that there is seroconversion, but it is not homogeneous since there may be susceptible subpopulations[2]. In Mexico, epidemiological studies are limited to the detection of specific antibodies against PPV. Recently, García-Camacho *et al*[21] reported the presence of different PPV genotypes in different states of the Mexican Republic, but there is no information associating their presence with reproductive signs.

This study aimed to determine the seroconversion induced by two treatments (feedback and a commercial vaccine) against parvovirus and to identify the circulating genotype in gilts in the adaptation area and in multiparous sows.

Material and methods

History of the farm

The evaluated pig farm is located in northwestern Mexico (Ciudad Obregón, Sonora) and has an inventory of 3,000 breeding sows of Pig Improvement Company (PIC) genetics, with a sanitary status negative for PRRSV (Porcine Reproductive and Respiratory Syndrome Virus) and *Mycoplasma hyopneumoniae* and positive for swine influenza. The farm's health program includes the application of a bacterin against *Salmonella* spp at two weeks of age (Enterisol SC Boehringer Ingelheim®), two applications against porcine circovirus type 2 (PCV-2) (Circumvent PCV2-MSD®) at three and six weeks of age, two administrations of a vaccine against ileitis (*Lawsonia intracellularis*) (Porcilis Ileitis MSD®) at 8 and 20 wk of age. For porcine parvovirus, the health program is carried out through two treatments (a controlled exposure, feedback, and the administration of a commercial vaccine) to replacement and multiparous sows. In 2020, reproductive behavior was mainly affected by the increase in piglets born mummified (11.12 %) and stillbirths (4.46 %), the decrease in the conception rate by 13 %, from 93 to 80 %, and in fertility at birth, from 88 % to 65 %, in the most critical stage. The management protocol and treatments carried out in this work were approved by the Subcommittee on the Care and Use of Experimental Animals (SICUE, for its acronym in Spanish) of the Faculty of Veterinary Medicine and Zootechnics of UNAM (MC-2019/3-10).

Treatments

The present study was conducted in two different scenarios: in the first, replacement gilts were evaluated, and in the second, multiparous sows. They were not evaluated together as replacement gilts are in the genetic development unit (GDU) until before they are incorporated into the production line.

Two immunization protocols against porcine parvovirus were used, feedback and a vaccine (Farrowsure B â Zoetis) that contains porcine parvovirus-1 apathogenic strain type NADL-7 (PPV). In the preparation of the feedback, liquefied mummies no larger than 16 cm in length (from young sows 1-2 parities) were used, which were macerated with purified water in the presence of antibiotics (streptomycin 1.25 mg) and frozen in 50 ml tubes until use, the macerate was analyzed to rule out the presence of PRRSV by PCR.

Treatment 1 (T1) consisted of a single administration of 20 ml of feedback at 24 wk of age to 30 replacement gilts. Treatment 2 (T2) consisted of the administration of 2 ml of the vaccine (Farrowsure B â Zoetis) at 24 wk of age to 30 replacement gilts, with a second application at 27 wk of age. Treatment 3 (T3) consisted of the administration of 20 ml of feedback to 80 multiparous sows at the time of weaning. Treatment 4 (T4) consisted of the administration of 2 ml of the vaccine (Farrowsure B ® Zoetis) to 80 multiparous sows, 2 wk before farrowing.

Antibody determination

Serum samples were collected for the determination of antibodies by the hemagglutination inhibition (HI) test using the Beta method, using 0.5 % guinea pig erythrocytes and vaccine Parvovirus (Synparv, strain NADL-8 Syva®) adjusted to 4 hemagglutinate units (HAU), for T1, T2, T3 and T4. The positivity criterion was established at a value of 1:480 (2.68 log10), as reported by Cobos *et al*[22]. For treatments T1 and T2, serum samples were taken at intervals of 3 wk each (24, 27, and 30 wk of age). In the case of treatments T3 and T4, samples were taken at weaning.

Statistical analysis

Seroconversion in replacement gilts was analyzed using descriptive statistics. The effect of treatments T1 and T2 was compared using the Kaplan-Meier survival test and the Mantel-Cox log-rank test with a significance of $P<0.05$[23,24]. The analysis of treatments T3 and T4, of multiparous sows, was performed using a mixed-effects model, and the results were expressed in Log_{10}. The fixed effect was treatment, and farrowing was evaluated as a random effect since this was a condition of the animal that was not considered for the selection of the experimental units[25,26].

Mixed-effects model used for the assessment of treatments:

Y_ijk=μ+δ_(i(j))+τ_j+ 〖(τP)〗_jk+ε_ijk

Where:
Yijk is the response in log^{10} in the i-th individual in the j-th farrowing in the k-th treatment;
μ is the general mean;
δi(j) is the random effect associated with the i-th individual in the j-th farrowing;
τi is the effect of the k-th treatment;
(δτ)jk is the interaction of the j-th farrowing in the k-th treatment.

All analyses were performed with SAS OnDemand for Academics (SAS Institute, Cary, North Carolina, USA) with the (PROC) MIXED procedure.

PCR test

The HI-positive sera were grouped into pools of five sera (31 pools were analyzed), DNA extraction was performed using the Taco™ DNA/RNA automatic extraction system (GeneReach Biotechnology, Taiwan), the extraction methodology was carried out following the manufacturer's instructions. To detect the presence of PPV genotypes 1-6, previously reported primers were used for each genotype (Table 1)[21].

Table 1: Primers used for the amplification of porcine parvovirus

Primers	Sequence 5´3´	T° of annealing	Size (bp)	Genbank position/accession number
PPV1-F PPV1-R	CACACCAGCAGCACCTAGAA TCCTACCTGAGCTGGCCTAA	60°C	454	3364-3817/U44978a
PPV2-F PPV2-R	AACCACGCCAAATCAAAGTC TAACAGACCCGTCCATTTCC	60°C	413	4015-4427/EU200677[a]
PPV3-F PPV3-R	ATTGTGCGCATTAATATACCA TGGTCCCAAGCAATAGGAT	57°C	578	3590-4167/GU938300[a]
PPV4-F PPV4-R	TAGCACTATGGCGAGCAAA AAGCAAGCAGTGTAGCAGAGTT	59°C	675	3800-4474/NC014665[a]
PPV5-F PPV5-R	TTTGGGCGCCATATATTGAT ATGCGGAAAAGAGCTAAGCA	58°C	521	3363-3883/JX896318
PPV6-F PPV6-R	ATTCCCAACAGACAGACAGAA TTAGGTCGGAAGGCATCAT	58°C	550	3791-4340/KR709268

PPVs= parvoviruses. [a]ICTV reference sequence.

For amplification, the PCR test was performed with a reaction adjusted to 25 µL using the primers at a concentration of 25 µM, the reaction used a Qiagen® Taq PCR Master mix [containing 5 U Taq DNA polymerase, the buffer 1X magnesium chloride 25µM, 10 µM of each dinucleotide triphosphate (dNTP), 0.5 µM of each primer, and 25 ng of template DNA] and they were amplified in a C1000 Touch Thermal Cycler, BIO-RAD™, under the following reaction conditions: 94 °C for 3 min, 35 cycles at 94 °C for 1 min, annealing temperature of 60 °C for PPV1 and PPV2, 57 °C for PPV3, 59 °C for PPV4, and 58 °C for PPV5 and PPV6, 72 °C for elongation for 1 min, and final extension of 72 °C for 10 min. The amplified PCR product was separated by horizontal capillary electrophoresis with 1.5 % agarose gel for the visualization of the fragments.

Metagenomic sequencing

The samples positive for parvovirus by endpoint PCR were analyzed by next-generation sequencing (Shotgun), which was carried out at the Sequencing Unit of the Institute of Biotechnology, UNAM. For the construction of the genomic libraries, the concentration of the genetic material extracted from the samples was quantified using the 737,501 Genova Nano microvolume spectrophotometer, Jenway, Staffordshire, United Kingdom. Subsequently, the DNA Library Preparation Kit (Nextera XT.) was used with 5 µL of DNA. Sequencing was performed on the Illumina NextSeq 2 x 75 platform, with a depth of 1 million readings. The quality of the raw sequences was evaluated with the FASTQC program (Babraham Bioinformatics, United Kingdom), the elimination of adapters was carried out with Cutadapt v1.11. Likewise, the clean reads were initially filtered using the Bowtie2

alignment software against the reference genome of Sus scrofa (GCF_000003025.6) to remove the genome from the host.

The filtered sequences were assembled using the SPADES program, and the analysis of the assemblies was performed with Kraken2. In addition, alignments were made for the specific search for readings compatible with porcine parvovirus (Porcine parvovirus 4 isolate PPV4/COL/Valle575/2021, complete genome) using the Bowtie2 program (bowtie2 --no-unal --sensitive-local).

Results

The seroconversion results for T1 (feedback) at the 24th, 27th, and 30th wk of age seroconverted 23.2, 63.3, and 90 %, respectively, while in group T2 (vaccine), seroconversion was 33.3, 53.3, and 83.3 %, respectively (Table 2). The comparative analysis performed between the two treatments, T1 and T2, using the Kaplan-Meier survival test showed no significant difference (P>0.05).

The seroconversion results obtained for the treatments performed on the multiparous females, T3 and T4, showed a seroconversion of 78.7 % of the sows (P<0.05) with T3, whereas for T4, only 50 % of the sows showed seroconversion (P<0.05).

Table 2: Percentage of sows with seroconversion of T1, T2, T3, T4 by the hemagglutination inhibition test

Treatment	**T1 (Feedback) n=30/age**			**T2 (vaccine) n=30/age**			**T3 n=80**	**T4 n=80**
Age in weeks	24	27	30	24	27	30	Multiparous	Multiparous
Positive	7	19	27	10	16	25	63	40
Negative	23	11	3	20	14	5	17	40
% Seroconversion	23.3	63.3	90	33.3	53.3	83.3	78.7	50

The results obtained showed a significant effect on T3 by farrowing number (P<0.0292). The analysis of seroconversion by farrowing number and not by treatment was not statistically significant (P>0.05) (Table 3).

Table 3: Sows seropositive by hemagglutination inhibition (HI) test, by treatment (T3 and T4) and farrowing number in multiparous sows

	Feedback T3						**Vaccine T4**					
	Farrowing number						**Farrowing number**					
HI titer	1	2	3	4	5	6	1	2	3	4	5	6
1:60	1	-	-	-	-	-	6	3	3	1	-	-
1:120	1	-	2	1	-	-	4	3	2	4	1	2
1:240	3	5	3	1	-	-	2	3	-	2	4	-
1:480	4	5	2	2	3	2	-	1	-	1	1	-
1:960	3	5	2	2	2	2	-	2	3	1	2	-
1:3840	4	2	-	3	3	1	3	6	2	-	1	-
1:7680	4	3	3	2	2	2	5	1	3	2	4	2

Positive titer starting at 1:480; -= no sample.

Figure 2 shows the PCR result of the analyzed sera that were positive for porcine parvovirus by HI, of the four treatments. Of these, four positive samples of porcine parvovirus type 4 are corroborated by PCR in treatments T1, T2, and T4 (two positive), which represents 9.67 %.

Figure 2: Parvovirus 4 amplification products

Lane 1 M= 1000kb; Lane 2: positive sample (fragment of 675 bp) obtained from treatment 1; Lane 3: positive samples from treatment two; and Lanes 4 and 5: positive samples from treatment 4.

Metagenomics results

From the sequenced samples, a total of 2,199 million readings were obtained for the analyzed sample of treatment T1, of which 0.04 % corresponded to viral genomes, and 0.04 % to bacterial genomes; 2,145 million for treatment T2, of which 0.4 % corresponded to viral genomes, and 0.5 % to bacterial genomes; 21,938 million for treatment T3, of which 0.03 % corresponded to viral genomes, and 0.01 % to bacterial genomes, and 12,379 million for treatment T4, of which 0.1 % corresponded to viral genomes, and 0.008 % to bacterial genomes. The presence of reported bacteria includes environmental microorganisms that may be associated with different stages of production in pigs, as well as revealing the initial development of the microbiome; this stage is crucial as the early microbiome is linked to immune system development, organ maturation, and overall health of animals, and the presence of pathogens associated with reproductive problems in sows.

Table 4 summarizes the species of viruses and bacteria identified by sequencing and their importance on farms; as mentioned, some of them are related to environmental bacteria, such as *Diaphorobacter* sp.

Table 4: Microorganisms found by metagenomics

Microorganism	Importance	References
Clostridium botulinum	It is rare in pigs; the infection causes incoordination, motor paralysis, anorexia, and death. It is associated with contaminated food or feeding with unconventional diets (waste), and it is of public health importance.	(27)
Staphylococcus aureus	It is involved in skin lesions, as well as causing septicemia, mastitis, vaginitis, metritis, osteomyelitis, and endocarditis, and it is of public health importance.	(28)
Cupriavidus neocaledonicus	Microorganism capable of growing in soils rich in heavy metals; no associations with diseases in humans or animals are reported.	(29)
Comamonas testosteroni	It is considered a commensal bacterium; however, it has been reported in humans with septicemic processes.	(30)
Haloterrigena sp.	Bacteria found in desert environments; there are no reports or associations with disease in animals and humans.	(31)
Diaphorobacter sp.	Bacteria that can be isolated from mud; they are part of the intestinal microbiota and have been frequently found in humans with cancer; there are no reports in domestic animals.	(32,33)
Malassezia restricta	Associated with skin diseases, otitis in animals and humans; it has not been documented in pigs.	(30,34)
Leishmania mexicana	It causes skin diseases and is of public health importance. Leishmaniasis can be classified as an occupational	(35)

	disease where veterinarians and workers in the agricultural and animal sector are mostly affected.	
Porcine gammaretrovirus C	Porcine type-C oncovirus is an endogenous virus; it has great relevance, especially in animals that are destined for xenotransplantation, since it can infect human cells and represents a risk of infection to humans with retroviruses.	(36,37)
Porcine parvovirus 4	Virus that affects pigs; association with diseases has not been reported in studies; nevertheless, it is mentioned that it may be involved in co-infections with other viral agents.	(38,39)

Porcine parvovirus 4 identification

Based on the results of the PCR analysis of the samples, the sequences obtained were also analyzed to identify the genome of porcine parvovirus type 4. The reference genome of PVP4 OR359295.1 Porcine parvovirus 4 isolate PPV4/COL/Valle575/2021 was used and sequences were aligned to map the low reference genome. The recovered contigs that aligned with the reference were analyzed using the NCBI's Blastn tool to verify their identity with PVP4 sequences.

Of the four samples analyzed, sequences corresponding to PVP4 were identified in two of them. In sample T1 (feedback treatment at 24 wk of age in replacement gilts), 9 contigs were obtained that aligned along the reference genome, of which six were concordant. These identified sequences have 96 % coverage and 100 % identity with samples reported in China in 2017 (Table 5). Likewise, sample T4 (Table 6) obtained 8 readings that aligned along the reference genome, all concordant. These identified sequences have between 40 and 80 % coverage and 100 % identity with samples reported in China between 2017 and 2022 and the KY586146 strain reported in Brazil in 2008.

Table 5: Recovered contigs corresponding to PVP4, from the sample of treatment T1

Reading	Genome position	Sequence
R1	480	GTGTATAAGAGACAGGAGCAGAACTCCGTCGTTTTCG GCCTGTATTTGAAGATGGA
R2	460	CGGCCTGTATTTGAAGATGGAAACCTACTGGACAGGT ATTTCTGTCTCTTATACAC
R2	2456	CGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTATT ATACCGATACTAACATGGG
R1	2940	GATTCTGATGACACCAGTAAAGAAAAATTCTATGCAT TTATGGAAAAAATTCAGC
R2	2838	GAAGCTCCTGTTACAGCAGTTCCGGTTGAGACATCACC AACTGAAAAACAGGTAC
R1	4636	AAGATTTAAAGACGGTGATTATTCAATAACAGGTCCA GGAGATAGAGATAAAACA
R2	4726	CACCAGAGATACCTATCTCTTCTCCAGCGATTTAGCTA AAACTGAAAGAGAACAGC
R1	5280	GCGCCTGCAGCGGATCCATTCCAAGTAATACATATTTG AACCAATATTGCCAATTT
R2	5259	CAAGTAATACATATTTGAACCAATATTGCCAATTTTTG CTTACATATGAAATGGAA

Table 6: Recovered contigs corresponding to PVP4, from the sample of treatment T4

Reading	Genome position	Sequence
R1	339	GCATGATTTATGCAAAGAGGAAGTTAACCTGATTGGTCAGTT TTTTTGGCGGGA
R2	279	TTGATTGGACGGGAACTCAAGTCCTAATTTGAATTGACGTGG ACCAATCAGAATC
R2	1062	TAAGCTTCTTTGTGCCCCATAAGAATAAACACGGGGCCTGGA AGAGCACGGACG
R1	946	CACCATCGGTGGAGCCATCGGTGATGCTTGCCTAAATACTGA TAAGAGAAAGGAGT
R2	1245	AGGAGTTGCTGGATAATAGACAGGACCCAGCGGTGATCGAA GAGCTCTCAGCTCCC
R1	1112	CAGATGGGAAAATAAGATTGCTCTAAGTCTATACTCGTTTCT GGCCACCCAGGCAG
R2	4189	TTTAATACACCATGGTACTATTATGATCTAAACATTATGTCCT GCCATTTCTCTCC
R1	3911	ACCGGGACAACCATATCAACTACCAAAATACTCATACAGAA CTGTTGGAAAA

The results of the Blastn analysis of the contigs obtained from samples T1 and T4 are shown in Table 7, including the percentage of identity and coverage.

Discussion

Porcine parvovirus (PPV) is a crucial pathogen in pig production; its ubiquity in pigs highlights the need for effective preventive strategies, such as vaccination and the use of exposure methods such as feedback[4,13]. Recently, seven PPV genotypes have been identified in different regions, with reproductive symptoms[21,40-43].

Several studies provide a comprehensive view of seroconversion and the immune response to porcine parvovirus type 1 (PPV1) in replacement gilts and their reproductive implications[6,44]; nevertheless, little has been described about the other genotypes of porcine parvovirus. Luevano *et al*[45] observed that antibodies against PPV1 in gilts can persist up to 20 wk of age; this finding suggests that the antibodies detected in the present work in the first sampling at 24 wk could be of maternal origin and a critical factor to consider when interpreting the immune response in gilts. The prolonged presence of maternal antibodies can interfere with the efficacy of vaccination, and it should be borne in mind when planning vaccination and sampling schedules[45,46]; it may also explain the heterogeneous response in vaccinated sows, both in reported positivity and in antibody titers.

As mentioned, there are commercial vaccines against porcine parvovirus 1, and they have been evaluated by various groups; Noguera *et al*[47] evaluated three commercial vaccines against PPV1, the percentages of seroconversion varied between 50 and 70 %, similar to what was obtained in the present study. In the present study, the results obtained with the conventional treatment of feedback (T1) and with a commercial vaccine (T2) showed 90 and 83.3 seropositivity in gilts, respectively; this result is consistent with what was reported by other researchers[48], who reported seroconversion of 70 % after vaccination with a commercial PPV1 vaccine. The results of the present research indicate a good initial immune response, but also reflect that the percentage of seroconversion may not be absolute, similar to what has been observed in other reports[46,47]; furthermore, in these studies, it was observed that, despite vaccination, mummified piglets were present, which emphasizes the importance of appropriate vaccine selection and its potential impact on the reproductive health of sows. In the present study, it was found that, in the groups with vaccination (T2 and T4), mummified piglets continued to be present, which may be associated with the fact that the component of the vaccine is parvovirus genotype 1 and does not protect against other genotypes of porcine parvovirus.

The effect of farrowing number in sows on vaccination is an important issue in pig production since it influences the effectiveness of immunization and the reproductive health of the herd. Normally, gilts may have a more robust immune response due to their lack of prior exposure to pathogens. Nonetheless, multiparous sows, which have been exposed to multiple reproductive cycles and potentially more pathogens, may have a less predictable immune response due to variability in their exposure history and overall health status. The results of the present study for groups T3 and T4, with seroconversions of 78.7 % and 50 %, respectively, reveal that, despite vaccination, seropositivity can vary significantly. It is particularly noteworthy that the result of group T3, with a high seroconversion without prior vaccination, suggests previous exposure to the virus, whereas in group T4, despite continuous vaccination, half of the animals did not show antibodies against PPV1; it should be noted that, in the cases where antibodies were found by the hemagglutination inhibition technique, some of the titers were high (1:7680), which could indicate variability in the individual immune response, the farrowing number, or problems in vaccine coverage and administration. The feedback results are consistent with those reported by Streck *et al*[48], who report a seropositivity of 84.7 % in sows of 4 or more parities, while it decreases with more parities.

Failure to use the appropriate vaccine for the specific porcine parvovirus genotype can cause several problems in pig production; if the vaccine used does not cover the genotype of the virus prevalent on the farm, the induced immune response may be insufficient to protect sows against the virus. This can result in partial or no protection against infection, compromising the effectiveness of vaccination. Molecular studies have identified several PPV genotypes, classified as PPV1, PPV2, PPV3, up to PPV7, these genotypes may have variations in their ability to cause disease and in their geographical distribution. In order to determine the genotype of the parvovirus present on the farm analyzed in this study, endpoint PCR analyses were performed for genotypes 1-6, and the results showed positive samples for genotype 4. In Mexico, the presence of parvovirus 8 was reported in co-infections with porcine circovirus 2[21]. Brown mentions that it has not been possible to directly associate it with any reproductive disease in pig farms[25]. However, it has been mentioned that PPV4 may have reproductive implications[49].

In this work, the presence of porcine parvovirus 4 is reported for the first time in farms with reproductive problems. In addition, samples positive by PCR for porcine parvovirus genotype 4 were analyzed using next-generation sequencing, which allows the simultaneous sequencing of millions of DNA fragments in a single run, generating large volumes of data and confirming the presence of porcine parvovirus 4 and bacteria, fungi and parasites. Next-generation sequencing has been used to characterize microbial communities in animals and humans, as well as environmental environments. This is a valuable tool for non-specific detection of diverse microorganisms in samples, offering unique advantages for the detection of emerging pathogens, fastidious or non-culturable pathogens, and mixed infections.

Compared to bacterial culture and PCR testing, metagenomic sequencing demonstrates advantages in detecting difficult-to-culture bacteria and viruses that are less common or found in low numbers in samples. The composition of the microbial community is dynamic and can develop from complex interactions between the host and external environmental factors, where viral infections can modify these interactions. The results showed the presence of *Clostridium botulinum*, *Cupriavidus neocaledonicus*, *Staphylococcus aureus*, *Diaphorobacter* sp, *Comamonas testosteroni*, *Haloterrigena* sp, *Malassezia restricta*, *Leishmania mexicana*, as well as species of plant origin, such as *Beta vulgaris*, *Solanum pennellii*, *Vitis vinifera*, *Telopea speciosissima*, *Solanum stenotomum*, *Zingiber officinale*, *Manihot esculenta*, *Vigna angularis*, *Physcomitrium patens*, and *Gossypium raimondii*. The presence of viruses of the porcine type-C gammaretrovirus family is also reported; porcine endogenous retroviruses (PERVs) are not well studied; they are released by normal pig cells and are infectious. PERV-A, PERV-B, and PERV-C viruses have been reported, A and B viruses are polytropic viruses that infect cells of several species, including humans, making them a risk for xenotransplantation. PERV-C is an ecotropic virus that only infects pig cells. The infectivity of these viruses has been demonstrated in *in vitro* co-culture experiments. In recent years, evidence has increased that PERVs, unlike human endogenous retroviruses (HERVs), are still active *in vivo* in pigs. While HERV express themselves as messenger RNAs, proteins, and non-infectious particles, PERVs are still active in pigs. The number of copies of PRV in different breeds of pigs is variable. The biological significance of the presence of these agents in the samples analyzed must be studied in the context of interactions between the microbiota and pathogens (viruses or bacteria).

Porcine parvovirus type 4 is an emerging pathogen; its presence in pigs could result in the presence of other viral or bacterial infections, which can complicate diagnosis and disease management, making surveillance and study of this virus even more important. The economic consequences due to the reduction in production and the additional costs for infection control and treatment can generate a serious problem for pig production in Mexico.

Conclusions and implications

The methods used for the prevention of PPV, such commercial vaccines or feedback, are widely employed in pig production units and depend on many factors, such as the health status of the farm, immunization programs and therefore maternal antibodies, which makes their effectiveness more difficult to evaluate; on the other hand, the use of vaccines with the appropriate plan and immunogen help prevent or control the virus; it is important to mention that, at the moment, no vaccine against porcine parvovirus has PPV4 as a vaccine component. The variability in seroconversion and incidence of reproductive problems highlights the need

for careful assessment of vaccination programs and adaptation of strategies to ensure optimal protection and minimize reproductive losses. The identification of PPV4, in farms with reproductive signs, stresses the need to improve strategies for surveillance, diagnosis and control of viral diseases in pigs. PPV4 is relatively new compared to other better-known swine pathogens, so its study can provide valuable insights into the virology of porcine parvoviruses, their transmission mechanisms, how it affects pigs and how it can be controlled, as well as its potential interaction with other viruses or bacteria present in pig farms.

Acknowledgements

We are grateful to the DGAPA-UNAM for financing the PAPIIT project Detection and genetic diversity of porcine parvovirus IN216921, to the zootechnical veterinarians who helped obtain the farms and data for their analysis, as well as to the Technological Institute of Sonora, and to the Laboratory of Comprehensive Diagnostics in Animal Pathology for establishing contact with the production units. To the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico (FMVZ-UNAM, for its acronym in Spanish). To the National Council of Humanities, Sciences and Technologies (CONAHCYT, for its acronym in Spanish) and to the master's and doctoral programs in Animal Production Sciences for the financial support during this project. To the MTI Juan Manuel Hurtado and Dr. Gamaliel López Real for facilitating the use of the server and to Dr. Luis Lozano Aguirre Beltrán of the Bioinformatics Analysis Unit (UAB, for its acronym in Spanish) and the IT Administration Unit (UATi, for its acronym in Spanish) of the Center for Genomic Sciences, UNAM.

Conflicts of interest

The authors declare that there is no conflict of interest in the publication of this document.

Table 7: Alignment of the obtained sequences with the PPV4 reference sequences

Accession no	Country/year	Description	Scientific name	Max score	Total score	Query cover	E value	% Ident	Acc. Len
MK092421.1	China, 2015	Porcine parvovirus 4 isolate PPV4-DJH18, partial genome	Porcine parvovirus 4	104	104	96%	2.00E-18	100.00	5036
MK378290.1	China, 2017	Porcine parvovirus 4 isolate PPV4_VIRES_TJ01_C2 capsid protein gene, partial cds	Porcine parvovirus 4	104	104	96%	2.00E-18	100.00	1482
MK378288.1	China, 2017	Porcine parvovirus 4 isolate PPV4_VIRES_SX02_C2 capsid protein gene, partial cds	Porcine parvovirus 4	104	104	96%	2.00E-18	100.00	1355
MK378286.1	China, 2017	Porcine parvovirus 4 isolate PPV4_VIRES_SX01_C1 replicase gene, partial cds; and ORF3 and capsid protein genes, complete cds	Porcine parvovirus 4	104	104	96%	2.00E-18	100.00	4835
MK378278.1	China, 2017	Porcine parvovirus 4 isolate PPV4_VIRES_NX02_C1 replicase, ORF3, and capsid protein genes, complete cds	Porcine parvovirus 4	104	104	96%	2.00E-18	100.00	5174
MK378276.1	China, 2017	Porcine parvovirus 4 isolate PPV4_VIRES_NM02_C1 replicase gene, partial cds;	Porcine parvovirus 4	104	104	96%	2.00E-18	100.00	4835

		and ORF3 and capsid protein genes, complete cds							
MK378270.1	China, 2017	Porcine parvovirus 4 isolate PPV4_VIRES_JL01_C1 replicase gene, partial cds; ORF3 gene, complete cds; and capsid protein gene, partial cds	Porcine parvovirus 4	104	104	96%	2.00E-18	100.00	4786
MK378269.1	China, 2017	Porcine parvovirus 4 isolate PPV4_VIRES_HuN02_C1 replicase gene, partial cds; ORF3 gene, complete cds; and capsid protein gene, partial cds	Porcine parvovirus 4	104	104	96%	2.00E-18	100.00	4721
MK378268.1	China, 2017	Porcine parvovirus 4 isolate PPV4_VIRES_HuN01_C1 replicase, ORF3, and capsid protein genes, complete cds	Porcine parvovirus 4	104	104	96%	2.00E-18	100.00	5500
MK378264.1	China, 2017	Porcine parvovirus 4 isolate PPV4_VIRES_HeN01_C1 replicase gene, partial cds; ORF3 gene, complete cds; and capsid protein gene, partial cds	Porcine parvovirus 4	104	104	96%	2.00E-18	100.00	4778

Acc. Len= Accession length.

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