Infection dynamics of porcine circovirus type 3 in longitudinally sampled pigs from four Spanish farms

Francini Klaumann,1,2 Florencia Correa-Fiz,2 Marina Sibila,2 José Ignacio Núñez,2 Joaquim Segalés3,4

Abstract
Porcine circovirus type 3 (PCV-3) is a recently discovered virus in domestic pigs and wild boar. The virus has been described in pigs with different clinical/pathological presentations and healthy animals, but the dynamics of infection is currently unknown. The aim of this study was to longitudinally monitor PCV-3 infection in 152 pigs from four different healthy farms (A, B, C and D) by means of PCR in serum. The selected animals were sampled five (farm A) or six (farms B–D) times from weaning until the end of the fattening period. PCV-3 genome was found in pigs from all tested ages and farms; few animals had an apparent long-term infection (4–23 weeks). PCV-3 frequency of detection remained fairly uniform along tested ages within farms A and C, but was more variable among sampling times in farms B and D. Eight partial genome sequences were obtained from six different animals. Phylogenetic tree and pairwise distance analysis showed high similarity among sequences and with available genomes from different countries. This is the first study on PCV-3 infection dynamics in longitudinally sampled pigs. Most pigs got infection during their life, although PCV-3 did not appear to be linked with any specific age.

Introduction
Recently, an emerging circovirus species was discovered and named porcine circovirus type 3 (PCV-3).1,2 The newly described virus belongs to the family Circoviridae, genus Circovirus.3 Circovirus virions have a non-enveloped, icosahedral structure containing a circular single-stranded DNA (ssDNA) molecule. Viral DNA includes two major ORFs, which encode for capsid and replicase proteins.4,5

PCV-3 is the third member of this genus able to infect pigs. PCV-1 was the first described member of this genus and is considered non-pathogenic for pigs.6–8 In contrast, PCV-2 is associated with several clinical/pathological conditions and considered one of the most important pathogens of the pig industry causing important economic losses.9

Since the first description in North America,1,2 many reports have identified PCV-3 in Europe,10–12 Asia,13–16 and South America,17–18 suggesting a worldwide circulation. Moreover, retrospective studies have shown PCV-3 circulation at least since the 1990s19–21 and, according to phylogenetic analyses, the common ancestor was dated around 50 years ago.18,22 The virus has also been detected recently in wild boar with fairly high prevalence, suggesting a potential role as reservoir for the domestic pigs.23,24

The first metagenomics analyses revealed PCV-3 genome in sows with porcine dermatitis and nephropathy syndrome and chronic reproductive failure.1 Subsequently, PCV-3 was found in tissue homogenates in pigs with a causally unexplained myocarditis.2 Thereafter, reports identified PCV-3 genome in nursery and fattening pigs with different clinical/pathological presentations as respiratory disorders,20,25 and in neonatal piglets with congenital tremors.26 In addition, the genome was detected in apparently healthy sows and fattening pigs as well as in stillborns.11,25,27 Based on current published data, it is not demonstrated whether PCV-3 infection is linked to a particular pathological condition or any specific age.19

E-mail for correspondence: joaquim.segales@uab.cat
Provenance and peer review Not commissioned; externally peer reviewed.
Received October 8, 2018
Revised February 25, 2019
Accepted March 10, 2019
Available literature indicates that PCV-3 is present in almost all pig ages (from fetuses to adults). However, a comprehensive study of the infection dynamics of this virus in a healthy pig population has not been described so far. Therefore, the aim of the present study was to longitudinally assess the dynamics of PCV-3 infection in a set of pigs from four clinically healthy conventional farms from Spain.

**Materials and methods**

**Study design**

Serum samples corresponding to 152 pigs from four selected clinically healthy conventional farms from Spain were chosen for this study (table 1). Samples were collected longitudinally (sampling the same individual repeatedly) during years 2012 and 2016 for different study purposes. In the first farm (farm A), 34 piglets were sampled at 2, 8, 13, 18 and 24 weeks of age. In farm B, 44 piglets were sampled at 2, 7, 12, 18, 22 and 25 weeks of age. From farm C, 28 animals were followed up at 2, 6, 10, 14, 18 and 25 weeks. Finally, 46 piglets were sampled at 4, 8, 12, 16, 21 and 25 weeks of age from farm D. The weeks were grouped according to the production phase (lactation, from one to four weeks of age; nursery, from five to nine weeks of age; and growing/fattening, >10 weeks of age) (figure 1).

**DNA extraction and specific PCR for PCV-3 detection and sequencing**

DNA was extracted from 200 µl of serum using MagMAX Pathogen RNA/DNA Kit (Applied Biosystems) according to the manufacturer’s protocol. Double-distilled water and a plasmid containing the full-length PCV-3 genome included into a PCV-3 negative serum were used as negative and positive controls, respectively.

To detect the presence of PCV-3 DNA in tested samples, a conventional PCR assay was performed based on a previous protocol described by Franzo and colleagues, with slight modifications. Three microlitres of extracted DNA was added to a PCR mix and amplified using the below described thermal protocol. The PCR thermic protocol was carried out in a final volume of 50 µl mixture containing 1 x PCR buffer, 100 µM of dNTPs, 0.2 µM of forward primer located in genomic positions 233–255 (5′-AAAGCCCGAAACACGGTGGTGT-3′), 0.2 µM of reverse primer placed between nucleotide positions 742 and 718 (5′-TTTTCCCGACATCCTGGAGA4CAAT-3′), one unit of DNA polymerase Platinum SuperFi (Invitrogen) and double-distilled water. The PCR thermic protocol was 98°C for
were aligned using Clustal Omega with 74 complete sequences as 0.05. An exact test was performed. The significance level was set at 0.05.

Statistical analyses were performed using XLSTAT. To test for significant differences, statistical analyses were performed using XLSTAT. The obtained sequences were deposited at the GenBank (references MH780665-MH780672).

Sequence analyses
PCV-3 positive samples were selected and submitted to Sanger sequencing, which was performed with BigDye Terminator v3.1 Cycle Sequencing Kit, following the manufacturer’s protocol at the Genomic and Bioinformatics Service of the Universitat Autònoma de Barcelona (Barcelona, Spain). The sequencing reactions were analysed using an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems).

Sequences and chromatograms were manually explored to trim bad-quality bases with BioEdit V.7.2.3. The assembly of the consensus sequences extracted from different fragments was attempted using DNASTAR Lasergene software. The partial genomes obtained were aligned using Clustal Omega with 74 complete genome sequences available at the GenBank (online supplementary table 1) and trimmed accordingly for comparison purposes. A phylogenetic tree was constructed with the maximum likelihood method based on the best predicted substitution model (lowest Bayesian information criterion score) by means of the Tamura-Nei plus Gamma substitution model using MEGA software V.7. The robustness of the clade was evaluated with 1000 bootstrap replicates. The obtained sequences were deposited at the GenBank (references MH780665-MH780672).

Statistical analyses
Statistical analyses were performed using XLSTAT 365 Microsoft Excel 2016. To test for significant differences between weeks of age in each tested farm, the Fisher’s exact test was performed. The significance level was set at 0.05.

Results
PCV-3 detection by PCR
PCV-3 genome was detected in all tested farms and sampling points during the study period. Overall, PCV-3 PCR positivity was found in 28 out of 34 (82.35 per cent), 32 out of 44 (72.72 per cent), 22 out of 28 (78.57 per cent) and 34 out of 46 (71.74 per cent) pigs in farms A, B, C and D, respectively. Results of the PCV-3 frequency of detection obtained by PCR in each age group are summarised in figure 1. Individual PCR results for each pig from each farm are displayed in the online supplementary table 2.

Globally, the PCV-3 positive percentage was fairly uniform within each tested farm (figure 1). In farm A, PCV-3 DNA detection frequency ranged from 23.53 per cent (8 out of 34 pigs) at the second sampling to 32.35 per cent (11 out of 34 animals) at the last one. In farm B, PCV-3 genome presence varied from 9.09 per cent (4 out of 44, first sampling) to 36.37 per cent (15 out of 44, fifth sampling). Such frequency ranged from 10.71 per cent (3 out of 28, fifth sampling) to 34.71 per cent (10 out of 28, fourth sampling) in farm C, and from 6.52 per cent (3 out of 46, third sampling) to 34.78 per cent (16 out of 46, second sampling) in farm D. No statistically significant differences were found across the tested weeks of age (P>0.05) in farms A and C; however, differences in PCV-3 frequency were detected among tested ages in farms B and D (figure 1).

In most of the cases, the detection of PCV-3 was either intermittent or found once in life (online supplementary table 1). In farm A, 3 out of 28 (10.7 per cent) animals showed infection intermittently and 10 animals (35.71 per cent) had a continuous PCR-positive result during a period ranging from 5 to 22 weeks; only one pig was positive at all sampling times. In farm B, intermittent detection of PCV-3 was observed in 10 out of 44 animals (22.7 per cent); eight more pigs (18.18 per cent) showed continuous PCR positivity during a period of 4–23 weeks; again, one of them was PCV-3 PCR positive at all sampling points. In farm C, 8 out of 28 (28.6 per cent) animals had PCV-3 DNA in serum intermittently and only two more animals (7.14 per cent) were positive during two consecutive samplings. Finally, in farm D, most pigs were PCV-3 PCR positive once during the study period (26 out of 46; 56.52 per cent), 5 out of 46 (10.87 per cent) had an intermittent detection of PCV-3 during a period from 5 to 17 weeks and, finally, three more had continuous PCR positive PCR detection ranging from four to nine weeks. The numbers of animals that are PCV-3 PCR positive in more than one sampling are depicted in table 2.

Sequence alignment and phylogenetic analysis
In total, eight PCV-3 partial sequences were finally obtained across two tested farms (farms B and C) corresponding to six different animals; from two of them, sequences at two sampling points were obtained. Sequences were retrieved from four farm B pigs at 12, 18, 22 and 18 plus 22 weeks of age, respectively, and one animal at 10 and 18 weeks and another at 25 weeks of...
age from farm C. The obtained sequences comprised part of the rep protein gene (954 nucleotides). The phylogenetic tree and pairwise distance demonstrated high similarity among obtained PCV-3 partial sequences and also with the corresponding sequence fragment of the complete Spanish genome from a domestic pig available at GenBank (>99 per cent) (figure 2). In fact, most sequences obtained from farm B (four out of five) were identical to the one obtained from a 25-week-old pig from farm C, and clustered close to US and Chinese sequences. The two sequences from the same pig (10 and 18 weeks of age) of farm C were identical, and very close (99.9 per cent) to the existing Spanish complete genome sequence from the GenBank from a domestic pig. One sequence from farm B clustered together with a German sequence, although nucleotide identity was greater than 99 per cent as well.

**Discussion**

Several epidemiological reports have detected PCV-3 genome in pigs from all production phases, associated or not with pathological disorders.\(^1\)\(^2\)\(^25\)\(^27\) However, the lack of an existing comprehensive approach on the dynamics of infection justified to carry out specific research on longitudinally sampled animals and assess how the virus is circulating in conventional healthy farms. Moreover, already published studies testing PCV-3 frequency in different age groups are fragmented and comparisons are not possible since information came from different sources, farms and countries. Therefore, the present study represents the first approach to investigate the PCV-3 infection dynamics in the same subset of animals.

Obtained results confirmed that this virus is apparently widespread (at least in the four selected farms), able to infect pigs at all tested ages and to cause long-term infection in few animals. In fact, there was not a particular PCV-3 infection dynamics pattern that could be inferred from the frequency of detection in the four studied farms. The higher frequency of PCV-3 genome detection occurred at different time points in the studied herds, which might be linked with the potential existence of maternally derived immunity or its duration.

However, while this might be the case for farms B, C and D (lower frequency of PCV-3 infection at early ages), a different situation was found in farm A, where a moderate percentage of infected piglets was already detected at two weeks of age (around 26 per cent). It is possible that such amount of PCV-3 PCR positive pigs at early ages is related with intrauterine infections, but the fact that a low-moderate percentage of pigs were found PCV-3 infected at all tested ages poses certain discussion elements on how the pig immune system reacts against this virus. Definitively, further studies are needed to assess the circulation patterns of PCV-3 as well as to develop techniques to monitor the immune response against the virus, still lacking at present.

The most obvious comparison of PCV-3 infection dynamics is with that of PCV-2, another member of the Circoviridae family. In the specific case of this latter infectious agent, the virus is considered of ubiquitous nature\(^18\) and can be found in different age groups. However, a distinct pattern of dynamics of infection is seen for PCV-2 in non-vaccinated farms, with usual low or very low prevalence during the lactating period, loss of maternally derived immunity between 6 and 10 weeks of age and subsequent peak of infection during the late nursery or early fattening period.\(^39\)\(^-\)\(^41\) In general, the prevalence at the peak of infection can be rather high, being close to 90–100 per cent of infected pigs in some cases,\(^60\)\(^41\) which is fairly different from current observations for PCV-3. An interesting point would have been the study of the infection status of sows, since at least for PCV-2 is known that infection at early ages is correlated with the percentage of infection in sows.\(^61\) Sow sera were not available for the present study, but PCV-3 has already been detected in 29 per cent of the tested serum from sows in farms located in Poland and 47.37 per cent in Thailand.\(^13\)\(^42\)

In the present study a quantitative PCR described by Franzo and colleagues\(^31\) was attempted in some of the PCV-3 positive samples (data not shown). High Ct values were obtained in most of the cases, and the viral load was below the quantification limit of the PCR (10 copies of DNA/µl). These results are in agreement with studies that detected low amount of PCV-3 DNA in serum samples,\(^12\)\(^25\)\(^32\) which would suggest a subclinical infection. Moreover, this was probably the main reason why only a few number of PCV-3 sequences were obtained.

Phylogenetic analyses and pairwise distance estimation with the eight PCV-3 partial sequences obtained throughout this study demonstrated high similarity with the corresponding sequences available

---

**Table 2** Number and percentage of porcine circovirus type 3 (PCV-3) PCR positive and negative pigs during all the study period and number of PCV-3 PCR positive pigs during one, two, three and four or more sampling times

| Farm ID | PCV-3 PCR positive pigs along the study period (%) | PCV-3 PCR positive pigs at one sampling (%) | PCV-3 PCR positive pigs at two samplings (%) | PCV-3 PCR positive pigs at three samplings (%) | PCV-3 PCR positive pigs in at least four samplings (%) | PCV-3 PCR negative pigs at all samplings (%) |
|---------|---------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|------------------------------------------|-------------------------------------------|
| Farm A  | 28/34 (82.35)                                    | 15/34 (44.12)                            | 10/34 (29.41)                             | 2/34 (5.88)                              | 1/34 (2.94)                             | 6/34 (17.65)                              |
| Farm B  | 32/44 (72.73)                                    | 14/44 (31.82)                            | 7/44 (15.91)                              | 3/44 (6.82)                              | 8/44 (18.18)                             | 12/44 (27.27)                             |
| Farm C  | 22/28 (78.57)                                    | 12/28 (42.86)                            | 6/28 (21.43)                              | 3/28 (10.71)                             | 1/28 (3.57)                              | 6/28 (21.43)                              |
| Farm D  | 34/46 (73.91)                                    | 26/46 (56.52)                            | 5/46 (10.87)                              | 3/46 (6.52)                              | 0/46 (0)                                 | 12/46 (26.09)                             |
at GenBank. Moreover, the sequences from the same animal (farm C) at 10 and 18 weeks of age were identical, as well as the sequences from the animal (farm B) analysed at 18 and 22 weeks. These results would suggest possible long-lasting or persistent infections of PCV-3 in some animals with the same viral variant. Taking into account the low number of sequences obtained, it was not possible to assess if
more than one PCV-3 strain was circulating in the same animal over time. However, at least two different strains were detected in both farms B and C taking into account the phylogenetic distribution of obtained sequences, indicating that the potential circulation of more than one strain in the same farm and, eventually in the same animal, is feasible. In any case, all sequences obtained were very closely phylogenetically related, indicating the low variability found so far with PCV-3 in comparison with other circoviruses. Importantly, the potential long-lasting or persistent infections seem to be relatively frequent based on obtained results; a variable percentage ranging from 6.5 per cent (farm D) to 25 per cent (farm B) of analysed pigs were PCR positive during three or more samplings. Long duration of infection is rather typical of ssDNA viruses infecting pigs such as PCV-2 and Torque tenus viruses. Obtained partial sequences were very close to each other although a broad mixing among sequences from Spain and different countries was found. However, in all cases the nucleotide identity among them was very high (>99 per cent), suggesting that minimal variation does currently exist among PCV-3 strains. Of course, the complete genome would have been more accurate in order to distinguish potential different variants infecting the studied farms.

In summary, this is the first longitudinal study to assess the infection dynamics of PCV-3 in commercial healthy farms. Although a particular general infection dynamics pattern was not able to be ascertained, the obtained data confirmed that PCV-3 circulated in the chosen clinically healthy farms at all tested ages and most pigs got infection during their lifetime.

References
1. Palmquist R, Pinhey P, Shank P, et al. A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. J Virol 2017;91:e01879–16.
2. Phan TG, Gannotti F, Rossov S, et al. Detection of a novel circovirus PCV3 in pigs with cardiac and multi-systemic inflammation. Vet Res 2016;47:84.
3. ICTV. International Committee for the Taxonomy of Viruses, ICTV 2017 www.ncbi.nlm.nih.gov/ICTVdb/ (Accessed 5th Aug 2018).
4. Ritchie BW, Niagro FD, Latimer KS, et al. Ultrastructural, protein composition, and antigenic comparison of psittacine beak and feather disease virus purified from four genera of psittacine birds. J Wildl Dis 1990;26:196–203.
5. Rosario K, Breitbart M, Harrach B, et al. Revisiting the taxonomy of the circoviridae: establishment of the genus cytocivirus and removal of the genus gyrovirus. Arch Virol 2016;161:1447–63.
6. Stocker C, Tggermehlin W, Verberkwe W, et al. A very small porcine virus with circular single-stranded DNA. Nature 1982;295:64–6.
7. Stocker C, Rasch K, Taggermehlin G. Characterization of papovavirus-and picornavirus-like particles in permanent pig kidney cell lines. Zentralbl Bakteriol 1974;226:153–67.

8. Allan GM, McNeill F, Cassidy JP, et al. Pathogenesis of colostrum deprived piglets and examination of pig foetal material. Vet Microbiol 1995;44:49–64.
9. Segales J. Porcine circovirus type 2 (PCV2) infections: clinical signs, pathology and laboratory diagnosis. Virus Res 2012;160:10–19.
10. Faccini S, Babeti E, Gilioli A, et al. Detection and genetic characterization of porcine circovirus type 3 in Italy. Transbound Emerg Dis 2017;64:1661–4.
11. Franzo G, Legnard M, Hjulsager CK, et al. Full-genome sequencing of porcine circovirus 3 field strains from Denmark, Italy and Spain demonstrates a high within-Europe genetic heterogeneity. Transbound Emerg Dis 2018;65:602–6.
12. Sadejek T, Wozniak A, Milek D, et al. First detection of porcine circovirus type 3 on commercial pig farms in Poland. Transbound Emerg Dis 2017;64:1530–5.
13. Xu K, Den F, Liu P, et al. Identification and characterization of porcine circovirus type 3 in China. Transbound Emerg Dis 2017;64:703–8.
14. Kwon T, Yoo SJ, Park CT, et al. Prevalence of novel porcine circovirus 3 in Korean pig populations. Vet Microbiol 2017;207:178–80.
15. Shen H, Lu X, Zhang F, et al. Genotypic characterization of a porcine circovirus type 3 in Shandong China. Transbound Emerg Dis 2018;65:264–6.
16. Hayashi S, Ohshima Y, Funuya Y, et al. First detection of porcine circovirus type 3 in Japan. J Vet Med Sci 2018;80:1468–72.
17. Exs C, Lima DA, Varela APM, et al. Full-Genome Sequence of Porcine circovirus 3 recovered from serum of sow with stillbirths in Brazil. Transbound Emerg Dis 2018;65:5–9.
18. Sanao GL, Vidigal PMP, Fetto JL, et al. Evolutionary analysis of Porcine circovirus 3 (PCV3) indicates an ancient origin for its current strains and a worldwide dispersion. Virus Genes 2018;54:376–84.
19. Klauhammer F, Franzo G, Sohrmann M, et al. Retrospective detection of Porcine circovirus 3 (PCV-3) in pig serum samples from Spain. Transbound Emerg Dis 2018;65:1290–6.
20. Sun J, Zou L, Lu Z, et al. Retrospective study on porcine circovirus 3 infection in China. Transbound Emerg Dis 2018;65:607–13.
21. Ye X, Berg M, Fossom C, et al. Detection and genetic characterisation of porcine circovirus 3 from pigs in Sweden. Virus Genes 2018;54:466–9.
22. Fu X, Fang B, Ma J, et al. Insights into the epidemic characteristics and evolutionary history of the novel porcine circovirus type 3 in southern China. Transbound Emerg Dis 2018;65:e296–e303.
23. Franzo G, Tucciaroni CM, Drigo M, et al. First report of wild boar susceptibility to Porcine circovirus type 3: High prevalence in the Colli Euganei Regional Park (Italy) in the absence of clinical signs. Transbound Emerg Dis 2018;65:957–62.
24. Klauhammer F, Dias-Alves A, Cabezon O, et al. Porcine circovirus 3 is highly prevalent in serum and tissues and may persistently infect wild boar (Sus scrofa scrofa). Transbound Emerg Dis 2019;66:91–101.
25. Zhai SL, Zhou X, Zhang H, et al. Comparative epidemiology of porcine circovirus type 3 in pigs with different clinical presentations. Virol 2017;14:222.
26. Chen GH, Mai KJ, Zhou L, et al. Detection and genome sequencing of porcine circovirus 3 in neonatal pigs with congenital tremors in South China. Transbound Emerg Dis 2017;64:1650–4.
27. Zheng S, Wu X, Zhang L, et al. The occurrence of porcine circovirus 3 without clinical infection signs in Shandong Province. Transbound Emerg Dis 2017;64:1337–41.
28. Fraile L, Sibila M, Novalias M, et al. Effect of sow and piglet porcine circovirus type 2 (PCV2) vaccination on piglet mortality, viramia, antibody titre and production parameters. Vet Microbiol 2012;161:229–34.
29. Oliver-Ferrando S, Segales J, López-Soria S, et al. Evaluation of natural porcine circovirus type 2 (PCV2) subclinical infection and seroconversion dynamics in pigs vaccinated at different ages. Vet Res 2016;47:121.
30. Feng H, Segales J, Fraile L, et al. Effect of high and low levels of maternally derived antibodies on porcine circovirus type 2 (PCV2) infection dynamics and production parameters in PCV2 vaccinated pigs under field conditions. Vaccine 2016;34:3044–50.
31. Franzo G, Legnardi M, Centelleghie C, et al. Development and validation of direct PCR and quantitative PCR assays for the rapid, sensitive, and economical detection of porcine circovirus 3. J Vet Diagn Invest 2018;30:538–44.
32. Fux K, Söckler C, Link EK, et al. Full-genome characterization of porcine circovirus type 3 isolates reveals the existence of two distinct groups of virus strains. Viral 2018;15:25.
33. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser 1999;41:95–8.
34. Butand TD, In: Misener S, Kowert DA, eds. DNASPUR’s Lasergene Sequence Analysis Software BT - Bioinformatics Methods and Protocols. 91. Totowa, NJ: Humana Press, 1999. doi.
35. Thompson DJ, Gibson TJ, Plewniak F, et al. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 1997;25:4876–82.
36. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 1992;9:615–21.
37. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 2016;33:1780–7.
38. Segales J, Allan GM, Domingo M. Porcine circovirus diseases. Anim Health Res Rev 2011;12:119–42.
39. Lanoechelle R, Magar R, D’Alieira S. Comparative serologic and virologic study of commercial swine herds with and without postweaning multisystemic wasting syndrome. Can J Vet Res 2003;67:114–20.
40. Sibila M, Calsamiglia M, Segales J, et al. Use of a polymerase chain reaction assay and an ELISA to monitor porcine circovirus type 2 infection in pigs from farms with and without postweaning multisystemic wasting syndrome. Am J Vet Res 2004;65:88–93.
41. Grau-Roma L, Hjulsager CK, Sibila M, et al. Infection, excretion and seroconversion dynamics of porcine circovirus type 2 (PCV2) in pigs from post-weaning multisystemic
wasting syndrome (PMWS) affected farms in Spain and Denmark. Vet Microbiol 2009;135:272–82.

42 Kedkovid R, Woonwong Y, Arunorat J, et al. Porcine circovirus type 3 (PCV3) shedding in sow colostrum. Vet Microbiol 2018;220:12–17.

43 Sibila M, Martínez-Guinó L, Huerta E, et al. Torque teno virus (TTV) infection in sows and suckling piglets. Vet Microbiol 2009;137:354–8.

44 Nieto D, Aramouni M, Sibila M, et al. Lack of effect of piglet vaccination against Porcine circovirus type 2 (PCV2) on serum viral loads of Torque teno sus virus 2 (TTSuV2). Vet Microbiol 2012;157:8–12.