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Porcine circovirus 2-genotypes in fetuses from litters suspicious for stillbirth, mummification, and embryonic death syndrome (SMEDI) in Germany

Vorkommen von Genotypen des porzinen Circovirus 2 in Feten Stillbirth, mummification, and embryonic death syndrome (SMEDI) verdächtiger Würfe

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Keywords

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ABSTRACT

Objective The present study examined the occurrence of the 3 major genotypes of porcine circovirus 2 (PCV2a, PCV2b, PCV2d) in tissue samples from fetuses origination from litters suspicious for stillbirth, mummification, embryonic death, and infertility syndrome (SMEDI) affected litters from Germany.

Material and methods A total of 53 litters suspicious for SMEDI of 27 farms with a total of 469 fetuses sent in for diagnostic purposes between 2021–2023 from veterinarians in the field were available for the present examination. Veterinarians were asked to submit all dead piglets of SMEDI-litters to allow random sampling. Four fetuses per litter were randomly selected for the study. In total, 209 fetuses were examined by qPCR for PCV2 DNA and in case of a positive result, further genotyped by PCV2 genotype-specific qPCR or sequencing of the open reading frame 2. Farm specific data was collected on a voluntary base and included in the analyses.

Results In total 40.7 % (11/27) of farms, 37.7 % of litters (20/53) and 21.1 % (44/209) of fetuses were positive for PCV2 DNA. Genotyping by qPCR was successful in 37 PCV2 positive tissue samples. For 4 additional samples, sequencing of PCV2 DNA was performed to support the genotype assignment. After all, 29.6 % (8/27) of farms, 30.2 % (16/53) of litters and 19.1 % (40/209) of fetuses were positive for PCV2d. One farm (3.7 %) had a PCV2a + PCV2d positive fetus (0.5 %) in one litter (1.9 %).

Conclusions PCV2d could be identified as the main PCV2 genotype in SMEDI-associated fetuses. This finding is in line with the overall observed genotype shift toward PCV2d as the predominating PCV2 genotype in the domestic pig population not only in Germany but also in most all relevant pig producing countries worldwide. Moreover, these findings also indicate that diaplacental transmission may play a major role in the spread of PCV2 to downstream pig populations and thus, also for the observed genotype shift. The high Cq-values in tissue samples indicated that PCV2 was not the etiological pathogen in most cases.

Clinical relevance The occurrence of SMEDI in a piglet producing herd needs diagnostic attention and may also include PCV2 diagnostics including genotyping in PCV2 associated cases. This approach could enable adjustment of the vaccination protocol on farm level and early detection of newly introduced PCV2 genotype in a pig herd.

ZUSAMMENFASSUNG

Ziel In der vorliegenden Studie wurde das Vorkommen der in Europa vorherrschenden PCV2-Genotypen (PCV2a, PCV2b, PCV2d) in Gewebeproben von SMEDI-assoziierten Feten untersucht.

Material und Methoden Für die vorliegende Untersuchung standen insgesamt 53 SMEDI-verdächtige Würfe aus 27 Betrieben mit insgesamt 469 Föten zur Verfügung, die zwischen 2021 und 2023 von Tierärzten zu diagnostischen Zwecken eingeschickt wurden. Für die Studie wurden nur Einsendungen ganzer Würfe (nur tot geborene Föten) berücksichtigt. Es wurden pro Wurf 4 Föten nach dem Zufallsprinzip für die Studie ausgewählt und somit insgesamt 209 Föten mittels qPCR auf PCV2-DNA untersucht. Im Falle eines positiven Ergebnisses wurde eine PCV2-Genotyp-spezifische qPCR oder die Sequenzierung des Open Reading Frame 2 zur Genotypisierung durchgeführt. Zusätzlich wurden bestandsspezifische Daten auf freiwilliger Basis erhoben und in die Analyse einbezogen.

Ergebnisse Insgesamt waren 40,7 % (11/27) der Betriebe, 37,7 % der Würfe (20/53) und 21,1 % (44/209) der Föten positiv für PCV2-DNA. Die Genotypisierung mittels PCR war bei 37 PCV2-positiven Gewebeproben möglich. Bei 4 weiteren Proben wurde eine Sequenzierung der PCV2-DNA durchgeführt, um die korrekte Genotypen-Zuordnung abzusichern. Insgesamt waren 29,6 % (8/27) der Betriebe, 30,2 % (16/53) der Würfe und 19,1 % (40/209) der Föten positiv für PCV2d. In einem Betrieb (3,7 %) konnte in einem positiven Fötus (0,5 %) eines Wurfes (1,9 %) gleichzeitig PCV2a und PCV2d nachgewiesen werden.

Schlussfolgerung PCV2d konnte als der vorherrschende PCV2-Genotyp bei SMEDI-assoziierten Föten identifiziert werden. Dieses Ergebnis steht im Einklang mit dem global beobachteten Genotypen-Shift mit PCV2d als derzeit vorherrschendem PCV2-Genotyp in der Hausschweinepopulation. Darüber hinaus deuten diese Ergebnisse auch darauf hin, dass die diaplazentare Übertragung von PCV2 eine wichtige Rolle bei der Ausbreitung des Virus auf nachgelagerte Produktionsstufen spielen könnte und so auch für die Veränderung des Vorkommens von PCV2 Genotypen in Hausschweinepopulationen von Bedeutung ist. Die hohen Cq-Werte in fetalen Geweproben deuten darauf hin, dass PCV2 in den meisten Fällen nicht das ätiologische Agens für das klinische Bild darstellte.

Klinische Relevanz Das Auftreten von SMEDI in Ferkelerzeugerbetrieben erfordert diagnostische Aufmerksamkeit und sollte auch eine PCV2-Diagnostik einschließlich Genotypisierung umfassen. Dieser Ansatz könnte die Anpassung des Impfschemas auf Bestandsebene und die frühzeitige Erkennung eines neu eingeschleppten PCV2-Genotyps in einem Schweinebestand ermöglichen.

Introduction

The porcine circovirus 2 is known as the etiological agent of the so-called porcine circovirus diseases (PCVD) that comprises PCV2 systemic disease (PCV2-SD), porcine dermatitis and nephropathy syndrome (PDNS), reproductive disease (PCV2-RD), or subclinical infections (PCV2-SI), extensively reviewed elsewhere [1]. Currently, 8 different PCV2 genotypes (PCV2a-PCV2h) can be distinguished [2], additionally a ninth genotype (PCV2i) was proposed from the USA [3]. Within the known PCV2 genotypes, PCV2a, PCV2b and PCV2d are of particular importance and are colloquially referred to as "major genotypes". However, all 3 genotypes were associated with PCVD in different eras of swine production. Whereas PCV2a was the predominant genotype when PCVD was first recognized in the 1990s [4, 5], PCV2b became the most prevalent genotype in 2006/2007 [6–9], marking the first so-called PCV2 genotype shift. PCV2d was first reported in 2008 in China [10] and subsequently emerged in countries all over the world [11–14], depicting the second PCV2 genotype shift. Meanwhile, based on clinical samples, PCV2d is known as the most prevalent genotype in the domestic pig population [2, 15–17]. In several field studies, tissue samples of fetuses or newborn piglets [18, 19], or pre-suckle blood samples [20, 21] were examined to elucidate the relevance of diaplacental infections for the spread of PCV2 to the pig population. Shortly, vertical in utero infection of fetuses can result in clinical inapparent piglets at time of birth and subsequent horizontal spread of the virus, or lead to death and result in stillborn or mummified fetuses. Within the frame of the PCV2-RD, the stillbirth, mummification, embryonic death, and infertility (SMEDI) syndrome of swine is also described in association with PCV2 [22, 23]. The clinical appearance of this syndrome depends on the time of infection of the embryos or fetuses, respectively. Embryonic death and subsequently a reduced litter size appears in cases of early infections before mineralization of the bones, which is around 35 days of gestation. Later infections until day 70 of gestation result in fetal death characterized by mummification or stillbirth [24, 25]. As it is assumed that the infection spreads horizontally within the uterus, affected fetuses are of variable size. Fetuses that become infected after the 70th day of gestation are assumed to be immunocompetent and can appear as clinically healthy at time of birth [25]. Less is known about the occurrence of PCV2 genotypes in cases of reproductive disorders at the moment. However, a case of PCV2d associated SMEDI-like disorder was recently published from Austria [26]. Within the present cross-sectional study, the proportion of PCV2 genotypes in SMEDI cases was evaluated. Therefore, 209 fetuses from 53 SMEDI affected litters of 27 farms were examined for PCV2 DNA by qPCR. Positive samples were further examined for PCV2 genotypes by genotype specific PCR or sequencing of PCV2 DNA.

Material and Methods

The present study was evaluated and approved by the internal ethical commission of the veterinary faculty of the Ludwig-Maximilians-Universität München (AZ 382–06–12–2023).

Sample collection

For the present examination all dead born piglets and mummies out of 53 SMEDI affected litters (in total 469 fetuses) were sent in for diagnostic purposes between 2021–2023 from veterinarians in the field. Only transmittals of whole litters (all dead born fetuses) were considered for the study. All litters were frozen when they reached the LMU Munich. Each litter was photographed (piglets were sorted by size), and the crown-rump length (CRL), bodyweight and phenotypical appearance (mummified, stillborn, either fresh or macerated) was documented. All fetuses received an individual numeration (farm.sow.litter.fetus) (▶**Fig. 1**). Based on recommendations for the diagnosis of abortion and reproductive diseases, we sampled 4 fetuses per litter [23] by systematic random sampling with respect to the litter size. To calculate the sample interval for each litter, we divided the number of available fetuses per litter by 4. The calculated number was rounded up and depicted the sample interval (e. g. number of available fetuses is 9: 9/4 = 2.25, every second fetus will be examined). To further consider the different phenotypes, we shifted the starting point for each litter, starting at one (smallest fetus) up to 4 and then starting at one again. If only 4 or less fetuses were available, all the fetuses from this litter were chosen The sample population (all, ▶**Fig. 2a**) and randomly selected (▶**Fig. 2b**) sorted by size and bodyweight is available from ▶**Fig. 2**.

To estimate the approximate age of the fetuses, a regressions equation was calculated based on the examinations published by Evans et al. [27]: $y_{(age)} = 23.27 + 0.28 * x_{(crl)}$. Based on the calculated age, the fetuses were sub-grouped as follows: embryonic phase (EP, day 0 to day 35), fetal phase before immunocompetence (PBI, day 35 to day 69), phase of immunocompetence (POI, day ≥ 70).

▶**Fig. 1** Exemplary photo documentation of farm 6, litter 2, fetuses 1–15. Source: Clinic for Swine, LMU Munich.

▶**Abb. 1** Beispielhafte Fotodokumentation von Bestand 6, Wurf 2, Feten 1–15. Quelle: Klinik für Schweine, LMU München.

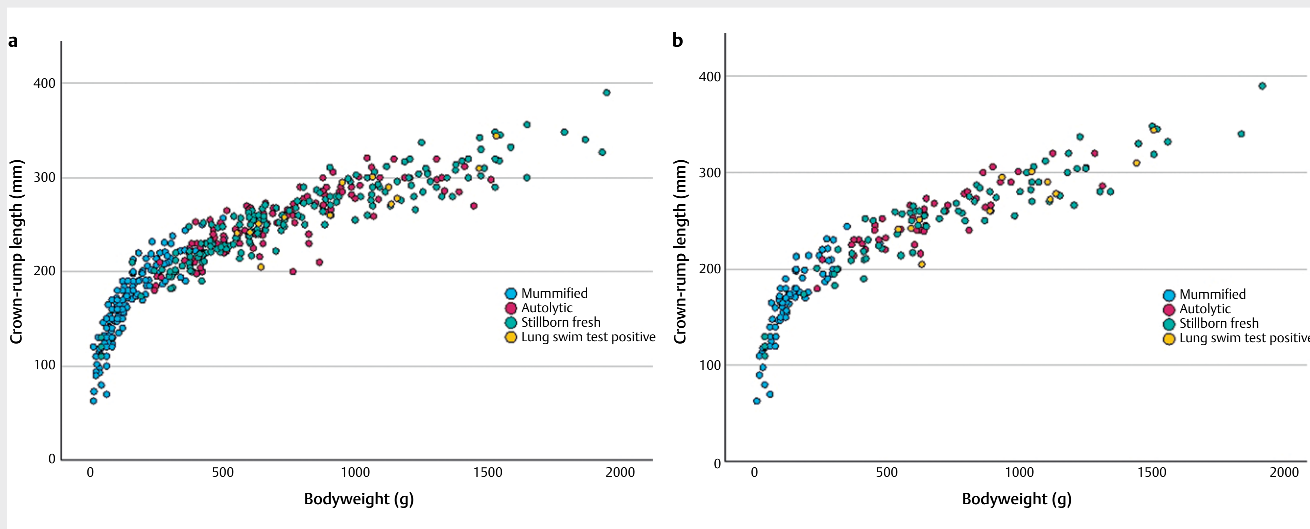
Sample collection

The fetuses were thawed at room temperature and subsequently opened in a supine position. To evaluate whether the fetus was stillborn or weak born, a lung flotation test was done. From each fetus a tissue pool consisted of myocardium, lung, thymus, and spleen was generated. Tissue pools were numbered corresponding to the fetus (▶**Fig. 1**). To avoid cross contamination of tissues, the set of instruments for the sample collection was cleaned, dipped in alcohol (96 %), and flame-treated after each sampling [28].

Molecular biological examinations

DNA from homogenized organ pools was isolated manually using the QIAamp DNA Mini Kit according to the manufacturer's instructions. For detection of PCV2 specific DNA a TaqMan PCR described recently was used [29]. Cq-value ≤ 35 was rated as positive.

Genotype specific qPCR was conducted on all tissue pools with a Cq-value of ≤ 35. Therefore, we used a recently described set of singleplex TaqMan PCRs [29], which specifically detect the genotypes PCV2a, PCV2b and PCV2d. Briefly, we used for PCV2a specific PCR: primers PCV2a_V1-F: ATC AAT AGT GGA RTC RAG AAC AG (400nM), PCV2a_V2-F: ATC AAT RGT GGA ATC AAG GAC (400nM), PCV2a-R: CGG TGG ACA TGM TGA GAT (800nM) and probe PCV2a: FAM-GGT ATA GAG ATT TTG TTG GTC C-BHQ1 (200nM); for PCV2b specific PCR: primers PCV2b-F: TCA ATA GTG GAA TCT AGG ACA GG (800nM), PCV2b-R: CGG TKG ACA TGM TGA GAT T (800nM), and probe PCV2b: FAM-ACA GAG CGG GGG TTT GA-BHQ1 (200nM); for PCV2d specific PCR: primers PCV2d-F: AAT CGA TTG TCC YAT CAA G (800nM), PCV2d-R: AAC GCC CTC CTG GAA T (800nM), and probe PCV2d: FAM-ACA GTG AGG GGG TTT GA-BHQ1 (200nM). Underlined nucleotides are modified (locked nucleic acid, LNA) to increase the specific binding of oligonucleotides. All primers and TaqMan probes (including LNA modified oligonucleotides) were obtained from Merck (Sigma-Aldrich Chemie GmbH, Germany). For real-time PCR we used the Luna Universal Probe qPCR kit (New



▶ **Fig. 2** Crown-rump length and phenotytical appearance of all available (**a**) and randomly selected (**b**) SMEDI fetuses. Source: M. Eddicks, Clinic for Swine, LMU Munich.

▶ **Abb. 2** Scheitel-Steiß-Länge und phänotypisches Erscheinungsbild aller verfügbaren (**a**) und zufällig ausgewählten (**b**) SMEDI-Feten. M. Eddicks, Klinik für Schweine, LMU München.

England BioLabs). The thermal profile of the PCR was: 95 °C for 1 min, and 40 cycles of 95 °C for 15 s, 59 °C for 20 s and 68 °C for 20 s. We used the AriaMx real-time PCR system (Agilent) and the corresponding Aria 1.7 software to perform and analyze the qPCRs.

Sequencing of PCV2 DNA was performed according to the method described previously published by Gagnon and colleagues [8]. In cases of low viral load in single samples, we performed a multiply primed rolling circle amplification (RCA) prior to amplification of PCR products for sequencing. For RCA we used the TempliPhi 100 amplification kit (GE Healthcare) according to the manufacturer's protocol. Overlapping PCR fragments were purified and sequenced using the sequencing service of Eurofins Genomics (Ebersberg, Germany). DNASTAR Lasergene and MEGA7 software was used for assembly, alignment and analysis of the sequences.

Statistical analysis

The qPCR results (qualitative and quantitative [Cq-values]) were tested for associations with independent variables *PCV2 sow vaccination* (yes/no), *sow parity* (gilt, old sow), *gilt quarantine* (yes/no), *phenotype of fetus* (mummified, stillborn) and *CRL of fetus* (cm). For binary data, Chi2 Test and in case of more than one significantly associated variable, binary logistic regression was calculated. Metric data was checked for associations by either Mann-Whitney-U Test or Kruskal-Wallis Test. In cases of more significantly associated variables, a generalized Estimating Equations model (GEE) with farm as subject effect and fetus as inner-subject effect was calculated. The significance level of this study was 0.5 % with a confidence interval of 95 %.

Results

A summary of the composition of the study population under consideration of the PCV2 vaccination scheme, gilt quarantine, and parity of the sows on farm, litter, and fetus level is presented in ▶ **Table 1**.

Based on the age of the fetuses estimated by the CRL, 100 % of the fetuses (n = 209) were assigned to fetal phase (FP). Within this group 18.7 % (39/209) of the fetuses were below 70 days of age at the day they presumably died and assigned to phase before immunocompetence (PBI). Moreover, 81.3 % (170/209) of all fetuses were 70 days or older and accordingly assigned to phase of immunocompetence (POI).

Chi2-test revealed significantly more mummified fetuses in the group of PBI compared to POI ($p < 0.001$) and more stillborn (including macerated and fresh) fetuses in the group of POI compared to PBI ($p < 0.001$). In ▶ **Table 2** the percentage of fetuses categorized by phenotype and age group are available.

Qualitative PCR results

In total, 40.7 % (11/27) of farms, 37.7 % of litters (20/53), and 21.1 % (44/209) of fetuses were positive for PCV2 DNA. Data concerning the parity of the corresponding sows was available for 50 sows and 197 fetuses. In total 33.3 % (3/9) of litters from gilts and 41.5 % (17/41) of litters from old sows were positive for PCV2. Fetuses from gilts were PCV2 positive in 19.4 % (7/36) of the cases whereas 23.0 % (37/161) of fetuses from old sows revealed a PCV2 positive PCR result.

Under consideration of the vaccination scheme against PCV2, 8 out of 13 (61.5 %) farms with solely gilt vaccination against PCV2, and 3 out of 8 (37.5 %) farms with no PCV2 sow vaccination had PCV2 positive SMEDI litters. Farms with gilt and sow (n = 5), or only sow vaccination (n = 1), had no PCV2 positive SMEDI litters. On litter level, 37.7 % (20/53) litters were PCV2 positive, all from farms with either only gilt (60.0 %, 15/25) or no (31.3 %, 5/16) PCV2 vaccinating farms. Within these litters 27.3 % (12/44) and 72.7 % (32/44) of the PCV2 positive fetuses were from farms with no or

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▶ **Table 1** Percentage and numbers of farms, litters and fetuses assigned to PCV2 vaccination scheme, quarantine for gilts, and parity of the sows.

▶ **Tab. 1** Prozentsatz und Anzahl der Betriebe, Würfe und Feten, die dem PCV2-Impfschema, der Quarantäne für Jungsauen und der Parität der Sauen zugeordnet wurden.

Factor		Farms	Litters	Fetuses
PCV2 vaccination	Gilts	48.1 % (13/27)	47.2 % (25/53)	49.3 % (103/209)
	Gilts + old sows	18.5 % (5/27)	20.8 % (11/53)	19.1 % (40/209)
	Old sows	3.7 % (1/27)	1.9 % (1/53)	1.9 % (4/209)
	None	29.6 % (8/27)	30.2 % (16/53)	29.7 % (62/209)
Quarantine for gilts		70.4 % (19/27)	64.2 % (34/53)	65.6 % (137/209)
Parity	Gilts	20.0 % (5/25)	18.0 % (9/50)	18.3 % (36/197)
	Old sows	63.0 % (17/25)	82.0 % (41/50)	81.7 % (161/197)
	Gilts + old sows	12.0 % (3/25)	–	–

▶ **Table 2** Summary of the study population. Percentage (%) and numbers (n) of all study animals assigned to phenotype and fetal development status (phase before immunocompetence [PBI] and phase of immunocompetence [POI]).

▶ **Tab. 2** Zusammenfassung der Studienpopulation. Prozentsatz (%) und Anzahl (n) aller Studientiere, die dem Phänotyp und dem fetalen Entwicklungsstatus (Phase vor der Immunkompetenz [PBI)] und Phase der Immunkompetenz [POI]) zugeordnet sind.

Phenotype	PBI (n = 39)	POI (n = 170)	All (n = 209)
Mummified	92.3 % (36/39)	25.3 % (43/170)	37.8 % (79/209)
Stillborn total	7.7 % (3/39)	67.6 % (115/170)	56.5 % (118/209)
Stillborn macerated	0.0 % (0/39)	24.1 % (41/170)	19.6 % (41/209)
Stillborn fresh	7.7 % (3/39)	42.9 % (73/170)	36.4 % (76/209)
Weak born	0.0 % (0/39)	7.1 % (12/170)	5.7 % (12/209)

▶ **Table 3** Percentage of PCV2 DNA positive fetuses of all study animals assigned to phenotype and fetal development status (phase before immunocompetence [PBI] and phase of immunocompetence [POI]).

▶ **Tab. 3** Prozentsatz der PCV2-DNA-positiven Feten aller Studientiere, die dem Phänotyp und dem fetalen Entwicklungsstatus (Phase vor der Immunkompetenz [PBI] und Phase der Immunkompetenz [POI]) zugeordnet wurden.

Phenotype	PBI (n = 39) PCV2 positive	POI (n = 170) PCV2 positive	All (n = 209) PCV2 positive
Mummified	12.8 % (5/39)	7.6 % (13/170)	8.6 % (18/209)
Stillborn total	0.0 % (0/39)	14.7 % (25/170)	11.9 % (25/209)
Stillborn macerated	0.0 % (0/39)	6.4 % (11/170)	5.2 % (11/209)
Stillborn fresh	0.0 % (0/39)	8.2 % (14/170)	6.7 % (14/209)
Weak born	0.0 % (0/39)	0.6 % (1/170)	0.5 % (1/209)
Total	12.8 % (5/39)	22.9 % (39/170)	21.1 % (44/209)

only gilt vaccination against PCV2, respectively. There was no significant difference in the number of PCV2 positive fetuses between stillborn (total, macerated and fresh) and mummified phenotype.

More detailed results referring to the subgroups and the PCV2-qPCR results are depicted in ▶ **Table 3**.

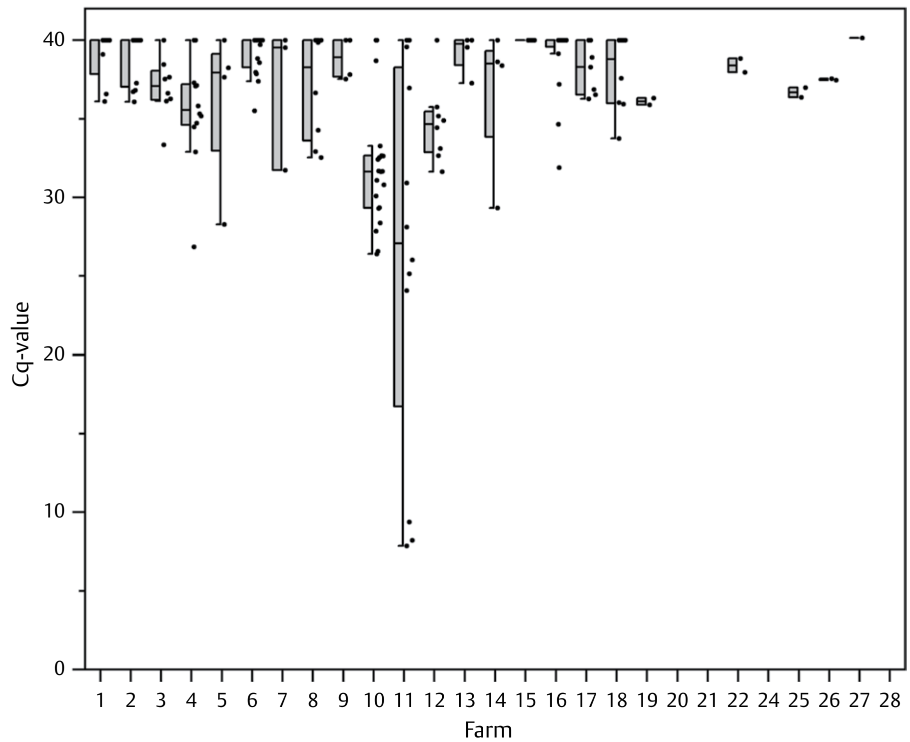
There was no significant difference in the number of PCV2 positive fetuses between stillborn (total, macerated and fresh) and mummified phenotype.

Quantitative PCR results

Mean Cq-value of all PCV2 positive samples (n = 44) was 29.46 (Min.: 7.86, Max.: 34.89, SD: 6.37, median: 31.64). The PCV2-qPCR Cq-values of fetal tissue pools for each single farm are shown in ▶ **Fig. 3**. The Cq-values by phenotype did not differ significantly. Details are presented in ▶ **Table 4**.

Related to Cq-values under consideration of the vaccine scheme, only fetuses from farms with solely gilt vaccination or without any sow vaccination were PCV2 DNA positive. Fetuses from farms with no PCV2 vaccination revealed significant lower Cq-values in the PCR (Mean: 24.43, SD: 10.19, Min.: 7.86, Max.: 34.28, Median: 27.07) compared to fetuses from farms with gilt vaccination (Mean: 31.35, SD: 2.46, Min.: 26.41, Max.: 34.89, Median: 31.70) ($p = 0.035$). Subsequently, a GEE was calculated with farm as subject effect and fetus as inner-subject effect. This test confirmed the observations reported above ($p = 0.008$).

For single fetuses, the solely PCV2 vaccination of old gilts (OR: 3.531, 95 % CI: 1.699–7.337, $p < 0.001$), PCV2 vaccination of gilts and old sows (0.785, 95 % CI: 0.695–0.826) and gilt quarantine (OR: 5.33, 95 % CI: 1.998–14.230, $p < 0.001$) were associated with the detection of PCV2 DNA in the univariate analysis and where further considered for binary logistic regression. This analysis revealed a significant association between the presence of a gilt quarantine on farm and the detection of PCV2 DNA positive fetuses (OR: 3.71. 95 % CI: 1.357–10.145, $p = 0.011$).



▶ **Fig. 3** Halboxes of PCV2 qPCR Cq-vales of fetal tissue pools for each farm. Source: M. Eddicks, Clinic for Swine, LMU Munich.

▶ **Abb. 3** Boxplot von PCV2 qPCR Cq-Werten von fetalen Gewebepools für jeden Bestand. M. Eddicks, Klinik für Schweine, LMU München.

▶ **Table 4** Cq-values of the positive PCV2 qPCR results (n = 44) including Mean, N, SD, Min., Max. and Median in association with the phenotype of the SMEDI-associated fetuses.

▶ **Tab. 4** Cq-Werte der positiven PCV2 qPCR-Ergebnisse (n = 44) einschließlich Mittelwert, N, SD, Min., Max. und Median in Verbindung mit dem Phänotyp der SMEDI-assoziierten Feten.

	Cq-value					
Phenotype	**Mean**	**N**	**Standard deviation**	**Minimum**	**Maximum**	**Median**
Mummified	30.65	18	6.03	7.86	34.89	32.22
Stillborn total	28.48	25	6.68	8.23	34.73	30.93
Stillborn macerated	29.23	11	7.33	8.23	34.28	31.69
Stillborn fresh	27.90	14	6.34	9.38	34.73	28.79
Weak born	32.54	1	–	32.54	32.54	32.54
Total	29.46	44	6.37	7.86	34.89	31.64

On litter level the vaccination of gilts and old sows (OR: 0.667, 95 % CI: 0.524–0.849, $p = 0.003$) and vaccination of only gilts (OR: 6.900, 95 % CI: 1.967–24.209) were significantly and gilt quarantine tendentially (OR: 3.529, 95 % CI: 0.965–12.913, $p = 0.076$) associated with detection of PCV2 DNA in the univariate analysis. The subsequently conducted binary logistic regression including these 3 factors revealed no significant associated factors with the Odds to detect a PCV2 positive litter.

Results of PCV2 genotyping

In total, 42 tissue samples from 11 farms and 19 litters with Cq-values ≤ 35 were available and considered for subsequent PCV2-genotyping by PCR. Genotyping by PCR was successful and clear in 37/42 tissue samples. For 4 additional samples sequencing of PCV2 DNA was performed to support the genotype assignment. Based on this, for 9 farms and 17 litters a genotype could be assigned. After all, 29.6 % (8/27) of farms, 30.2 % (16/53) of litters and 19.1 % (40/209) of fetuses were positive for PCV2d. In one farm (3.7 %) one fetus (0.5 %) of one litter (1.9 %) was infected simultaneously with PCV2a and PCV2d. Thus, within the positive findings, 88.8 % (8/9) of farms, 94.1 % (16/17) litters and 97.6 % (40/41) of the positive fetuses were PCV2d positive, whereas 11.1 % (1/9) of farms, 5.9 % (1/17) of litters and 2.4 % (1/41) of fetuses were positive for PCV2a and PCV2d. Metric analysis was waived due to no relevant subgroups.

Discussion

Within the present study, we evaluated the occurrence of the PCV2 major genotypes PCV2a, PCV2b and PCV2d in fetuses from SMEDI affected litters. Moreover, epidemiological data from the corresponding farms were collected and set in association with the laboratory diagnostic findings.

The relevance of young sows and gilts for the maintenance of PCV2 circulation in a sow herd without PCV2 vaccination was shown earlier [31]. Subsequently, PCV2 associated reproductive disorders are assumed to be associated with gilts or herds with a high proportion of young sows [1, 22], whereas broad level of endemic infection may reduce the potential of PCV2 as a fetal pathogen due to herd immunity in sows in later stages of production [32] and thus, in the domestic pig population [30]. Interestingly, within our study population, only 18 % of SMEDI affected litters were from gilts. Referring to PCV2, 33.3 % of the litters from gilts and 41.5 % of the litters from old sows were positive and no significant association between parity and PCV2 detection was obvious. On the other hand, the presence of a quarantine for gilts, which might be an indicator for purchasing gilts from gilt breeding herds, significantly increased the probability to detect a PCV2 DNA positive fetus. Thus, the introduction of new animals into a herd seems to be associated with PCV2 positive SMEDI fetuses rather than the parity of the dams. A possible explanation might be the high proportion of farms with gilt vaccination (48.1 %) within our study. The vaccination of gilts might be beneficial to reduce the number of PCV2 shedding sows in the main herd but also changes the epidemiology of PCV2 infections on these farms. Subpopulations with more susceptible old sows due to a reduced PCV2 infectious pressure on old sows and less susceptible gilts due to their vaccination might be the consequence. A comparable assumption was made by Segales et al. [33] who postulated different epidemiological scenarios as a consequence of different vaccination schemes against PCV2. However, under current vaccination schedules, especially when only gilts are vaccinated against PCV2, PCV2-RD might no longer be restricted to gilts, whereas in farms where old sows were vaccinated against PCV2 the probability to detect PCV2 in SMEDI cases tended to be reduced. This observation corresponds to other study results when PCV2 sow vaccination was identified as a protective factor concerning the PCV2 detection in suckling piglets [19, 31, 32] or SMEDI affected fetuses [23].

With 88.9 %, PCV2d was the most often detected genotype within SMEDI affected PCV2 positive farms. Concerning reproductive disorders, large scale studies on PCV2 genotypes in terms of reproductive disorders are scarce up to date, but a recently published case report also describes PCV2d associated reproductive failure on a piglet producing farm in Austria [26]. However, our results go in line with a globally observed PCV2 genotype shift to PCV2d as the most prevalent one in the domestic pig population [2, 15, 17]. Interestingly, PCV2a but not PCV2b was detected in the study population. This is remarkable, as PCV2b was suspected to be the most prevalent genotype in clinical cases from 2006 [6–8] until the emerging of PCV2d in the pig population around 2010 [10–12]. Thus, our results support the theory of a genotype shift particularly from PCV2b to PCV2d as also observed by others [33].

Based on our results, the high percentage of the “new” PCV2 genotype PCV2d in cases of SMEDI is rather of epidemiological nature e. g. as the result of the introduction of new animals in the farm, than an indicator for an increased virulence. As a low herd immunity seems to be associated with PCV2-RD, the introduction of a new strain might end up in clinical signs as long as this strain becomes endemic. However, the Cq-values in our study animals were mostly high which indicates an endemic PCV2 situation in the farms and disagrees with the diagnostic criteria for PCV2-reproductive disease [33, 37]. This implicates that PCV2 was not the causing agent in most of the present cases. This might additionally be supported by the missing differences concerning the viral loads between the defined fetal phenotypes in the present study. Moreover, only a few studies report on differences in virulence concerning different PCV2 genotypes [34, 35] or strains [36] whereas the majority of studies reveal no indications concerning differences in virulence between PCV2a, PCV2b and PCV2d [35, 37, 38].

CONCLUSION FOR PRACTICE

PCV2d was the most prevalent genotype in fetal tissue samples of litters suspicious for SMEDI. PCV2 detection in these litters was restricted to farms without PCV2 vaccination in sows and gilts or farms with solely gilt vaccination, but not generally restricted to gilts or young sows. Thus, solely gilt vaccination might change the epidemiology of PCV2 infections in sow herds to susceptible subpopulations in these farms. None of the litters from farms with sow or sow and gilt vaccination were PCV2 positive.

Conflict of interest

The authors declare that they have no conflict of interest.

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