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Short Communication

Detection of a new cluster of porcine circovirus type 2b strains in domestic pigs in Germany

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ABSTRACT

PCV2 can be divided into three different genotypes: PCV2a, PCV2b and PCV2c. Since 2004/2005 PCV2b has become the predominant genotype in the domestic pig population worldwide. In the years 2010 and 2012 PCV2b mutant strains (mPCV2), classified as PCV2b-1C strains, were detected in porcine circovirus diseases (PCVD) affected pigs in China and the United States, respectively. Within one year (April 2013–April 2014) newly emerging mPCV2 strains were isolated in seven German pig farms routinely vaccinating against PCV2. Histopathological, clinical and molecular biological findings including in-situ hybridization (ISH) and real-time PCR indicate PCVD in the affected animals. Characterized isolates from five farms were closely related to the PCV2b-1C reference strain BDH (GenBank no. HM038017), whereas strains from two other farms were only 99.1% and 99.0% identical (based on the nucleotide sequence of the complete genome) to mPCV2 strain BDH, respectively.

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1. Introduction

Porcine circovirus type 2 (PCV2) is associated with several disease syndromes like the postweaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), reproductive disorders and others, collectively named porcine circovirus diseases (PCVD) (Segales et al., 2005). Based on pairwise sequence comparisons (PASC) of the open reading frame 2 (ORF2) PCV2 can be divided into three genotypes (PCV2a, PCV2b, and PCV2c) (Segales et al., 2008). Olvera et al. (2007) described different clonal lineages of PCV2 isolates below the genotype level. PCV2a was divided into 5 clusters (group 2A-E) and PCV2b into 3 clusters (group 1A–C). While PCV2c could only be identified in Danish samples from the 1980s (Dupont et al., 2008), PCV2a and PCV2b are present all over the world. Since 2004/2005 PCV2b has become the predominant genotype in the domestic pig population worldwide (Cheung et al., 2007; Gagnon et al., 2007). In 2010, Guo et al. (2010) reported about newly emerging PCV2 strains in China. One of them ("BDH", GenBank no. HM038017) was characterized by a mutation in the stop codon of the ORF2 that leads to a capsid protein with 234 amino acids (aa). Following infection experiments with strain "BDH" seem to confirm a higher virulence of this new emerging PCV2 mutant in vivo (Guo et al., 2012). Two years later a PCV2 strain with 99.9% identity on nucleotide level and 100% identity on amino acid level with strain "BDH" was isolated in the

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Table 1
Assignment of strain notations to case numbers and their corresponding farms.

Farm	Case (strain notation)
1 Finishing farm	1 (DE 125-13)
2 Nursery	2 (DE 136-13); 3 (DE 137-13); 4 (DE 138-13)
3 Finishing farm	5 (DE 222-13)
4 Nursery	6 (DE 263-13); 7 (DE 264-13)
5 Finishing farm	8 (DE 03-14)
6 Finishing farm	9 (DE 06-14); 10 (DE 07-14)
7 Finishing farm	11 (DE 143-14); 12 (DE 144-14)

Table 2
PCV2 specific PCR2 (Zhao et al., 2010) cannot detect PCV2b-1C. 30 PCV2b-1C strains from GenBank[a] were aligned. In all PCV2b-1C sequences were nine mismatches to the binding site of the reverse primer sequence.

Reverse primer	CCT GTC CTA GAT TCC CCT ATT GAT T
Binding site PCV2-1C (reverse complement)	CCT GTC CCT GAT AGG ACA ATC GAT T

[a] AY181946, AY181947, AY291317, AY484410, AY510375, AY556473, AY682991, AY682994, AY682996, AY686763, AY686765, AY713470, AY943819, EF524517, EF524539, EF675241, FJ158607, GQ404800, HM038017, HM038030, HM038031, HM161711, HQ378158, HQ395061, JQ413808, JQ809463, JQ809464, KC473168, KC753771 and KF742543.

Midwestern USA from growing pigs that were suffering from PCVD despite routine vaccination against PCV2 (Opriessnig et al., 2013). This investigation we report about newly emerging mPCV2 strains in German domestic pigs with clinical signs, microscopic lesions and laboratory findings that indicate PCVD in the affected animals.

2. Material and methods

2.1. Case history and animal selection

In April 2013 a pig (case 1) from a finishing farm (farm 1) with clinical signs of PCVD was sent in for routine PCV2 diagnostics. In-situ hybridization (ISH) and real-time PCR (PCR1, Olvera et al. (2004)) of the superficial inguinal lymph nodes gave positive results. However, a second real-time assay (PCR2, Zhao et al. (2010)) was not able to detect PCV2 in these samples. Sequencing of the complete genome of this PCV2 isolate revealed a PCV2b-1C strain. From then on, cases from routine diagnostics in which the superficial inguinal lymph node was PCV2 positive in ISH and PCR1, but negative when tested by PCR2 were included in our investigation. Within one year (April 2013–April 2014) 12 cases from 7 farms fulfilled these requirements. For this purpose PCV2 in serum of the pigs was quantified and sequenced. Histopathological investigations including ISH were performed in addition. Table 1 displays the assignment of strain notations to case numbers and their corresponding farms.

2.2. Real-time PCR and screening for novel PCV2b variant

PCV2 DNA was isolated from serum samples using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. For all real-time PCRs the QuantiTect probe PCR kit (Qiagen, Hilden, Germany) was used. Primers (PCV2F 5′-CCA GGA GGG CGT TGT GAC T, PCV2R 5′-CGC TAC CGT TGG AGA AGG AA) and probe (PCV2S FAM-AAT GGC ATC TTC AAC ACC CGC CTC T-BHQ1) as previously described by Olvera et al. (2004) were used for PCV2 detection (PCR1). Screening for the new variant of PCV2b was performed with a real-time assay (PCR2) published by Zhao et al. (2010) (Primer F 5′-CGG ATA TTG TAK TCC TGG TCG TA, primer R 5′-CCT GTC CTA GAT TCC CCT ATT GAT T, probe Fam-CTA GGC CTA CGT GGT CTA CAT TTC-BHQ1). This assay was designed, based on a PCV2b-1B strain (BJ0804, EU921257). Because of nine mismatches at the binding site of the reverse primer, this assay cannot detect PCV2b-1C strains (Table 2). Samples positive in PCR1 but negative in PCR2 were suspicious to contain novel PCV2b strain. Viral DNA from these samples was amplified and sequenced.

2.3. Sequencing and phylogenetic analyses

Two previously published sets of primers (Gagnon et al., 2007) (PCV_Seq1F 5′-GGA CCC CAA CCC CAT AAA A and PCV_Seq1R 5′-CCC TCA CCT ATG ACC CCT ATG T; PCV_Seq2F

Table 3
Results of the PCV2 in-situ hybridization of different tissues and real-time PCR from serum samples.

Case	In-situ hybridization[a]									Real-time PCR[b]
	Inguinal lymph node	Kidney	Spleen	Lung	Liver	Ileum	Peyer's patch	Heart	Brain	Serum
1	+	++	+	+	–	+	+	–	–	1.3×10^{5}
2	++	–	+	+	–	–	+	–	–	1.2×10^{6}
3	++	+	++	+	–	–	++	–	–	3.8×10^{6}
4	+	–	–	–	–	–	–	–	–	3.2×10^{6}
5	+++	+	++	+++	+	++	+++	+	+	4.0×10^{6}
6	+++	–	–	–	–	–	+	–	–	1.6×10^{6}
7	+	+	n.a.	+	–	–	–	–	–	1.0×10^{7}
8	++	–	–	+	–	n.a.	n.a.	–	n.a.	3.8×10^{6}
9	+++	+++	+++	+++	+++	n.a.	n.a.	n.a.	++	3.0×10^{9}
10	+++	+++	n.a.	++	n.a.	n.a.	n.a.	n.a.	+	1.7×10^{8}
11	+++	+++	+++	+++	+++	+++	+++	–	n.a.	2.0×10^{10}
12	+	–	–	–	–	–	–	–	–	2.5×10^{7}

[a] Amount of PCV2 genome in tissue: –, negative; +, low; ++, moderate; +++, high; n.a., tissue sample not available.
[b] PCV2 DNA copies/ml serum.

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