



Comparative genetic characterization of *Porcine Circovirus* type 2 samples from German wild boar populations

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Received 22 September 2004; received in revised form 12 May 2005; accepted 2 June 2005

Abstract

PCV-2 is involved in “postweaning multisystemic wasting syndrome” (PMWS), “porcine dermatitis and nephropathy syndrome” (PDNS), respiratory and reproductive disorders, and thereby plays a crucial role in today’s swine production worldwide. The virus is apparently ubiquitous in domestic pigs and has also been demonstrated in wild pigs. Up to now, a characterization of PCV-2 samples from wild pigs, which might help to estimate the possible role of wild pigs as sources of domestic pig infection, has not been carried out. Spleen samples from 16 PCV-2-positive wild pigs from hunting grounds of four regions in Germany were used for the analysis of the viral genome. In one sample, the complete sequence of the genome was determined. In the other, a 742 nucleotide fragment from the highly variable capsid sequence of the ORF2 was sequenced. Analysis of the sequences led to the identification of three PCV-2 strains. One strain, representing 14 of the 16 samples, was closely related with Chinese, but not with German strains. The genome of this strain was shortened by one nucleotide by a deletion close to the end of ORF2. The deletion led to a shift of the stop-codon and to the insertion of a further codon. Two further strains differed in up to 4.7% of nucleotides and up to 10.5% of amino acids (aa). These strains were aligned with clusters of PCV-2 samples from mainly French and German domestic origin.

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Keywords: *Porcine Circovirus*; PCV-2; Epidemiology; Genetic analysis

1. Introduction

Porcine Circovirus (PCV) is a small non-enveloped virus of the family Circoviridae, genus *Circovirus*, that contains a single-stranded circular DNA of about 1.76 kb. Two species of PCV have been identified in

swine. PCV-1 was first detected as a contaminant of the porcine kidney PK15 cell line (Tischer et al., 1974). PCV-2 was initially isolated from a Canadian swine herd in 1991 (Harding, 1996). Serological prevalences estimated by different studies indicate that both PCV-types are widespread in swine (Allan and Ellis, 2000; Segales and Domingo, 2002). While PCV-1 is classified as non-pathogenic, PCV-2 has been associated with the postweaning multisystemic wasting syndrome (PMWS; Harding, 1996; Ellis et al., 1999), porcine dermatitis and nephropathy syndrome

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(PDNS; Segales et al., 1998; Wellenberg et al., 2004) and respiratory (Pesch et al., 2000; Segales et al., 2004), reproductive disease (Pensaert et al., 2001; Cariolet et al., 2001). Genomic sequences of both PCV-1 and PCV-2 have been determined and aligned (Meehan et al., 1997, 1998; Hamel et al., 1998; Morozov et al., 1998; Niagro et al., 1998; Fenaux et al., 2000) leading to homologies within species-isolates of over 90% and of 68–76% between species.

Besides domestic pigs, circoviruses have also been demonstrated in wild pigs either by serological (Tischer et al., 1986; Sanchez et al., 2001; Segales et al., 2002; Ellis et al., 2003; Schulze et al., 2003; Vicente et al., 2004) or direct methods (Segales et al., 2003; Schulze et al., 2003; Exel et al., 2004; Knell et al., in press). However, the virus has not yet been characterized in wild pigs. A characterization might help to understand epidemiological features in wild pig populations and to estimate the possible role of wild pigs as a source of domestic pig circovirus infection.

2. Materials and methods

2.1. Material studied

Spleen samples from 238 wild pigs from 10 hunting areas, situated in four regions of Rhineland–Palatinate and Hesse, were collected from November 2003 to March 2004. Distances between hunting areas were 63.5 ± 51.8 km, ranging between 7.4 and 140.4 km. West–East distances were between 2 and 106 km, North–South distances between 2 and 93.5 km.

Pigs were collected after the hunt at central places. They were numbered, rated according to age (dentation) and body scores, and samples were taken half an hour to 2 h after death. Tissues were stored at -20 °C for not more than 1 week before use.

3. Methods

3.1. Isolation of DNA from spleen tissues

DNA was extracted from spleen samples with the Puregene[®] Genomic DNA Purification Kit (Gentra Systems, Minnesota, USA) according to the protocol

supplied by the manufacturer. For each extraction, 20 mg of spleen sample was used. The resulting DNA was eluted in 50 μ l DNA hydration solution (Gentra Systems) and stored at 4 °C.

3.2. Identification of PCV-2 infection

PCV-2-specific DNA was amplified by PCR with AmpliTaq Gold Hot Start polymerase (Applied Biosystems, Foster City, USA) in a T-gradient cycler (Whatmann, Biometra, Goettingen, Germany). The PCR consisted of 35 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 30 s, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. Primers PCV-2IS and PCV-2IAS (Table 1) were described by Larochelle et al. (2000).

3.3. PCR amplification of the complete genome of PCV-2

Extracted DNA of one of the PCV-2-positive wild pigs was used for the complete amplification and sequencing of the PCV-2 genome. One fragment of 1085 bp (bases 501–1585 of PCV-2) was produced by PCR with oligonucleotides Porcirc1 (Table 1) and PCV-2IAS. PCR conditions were as described above, with the exception of an annealing temperature of 62 °C. The fragment was cloned into a p-Drive Cloning Vector (QIAGEN PCR Cloning^{plus} Kit, Qiagen, Hilden, Germany). Plasmids were raised in transformed *Escherichia coli*, identified by PCR, and prepared for forward and reverse sequencing with M13 primers (Table 1). Three clones were sequenced. There were no differences between them. Further, smaller fragments were amplified by PCR and sequenced directly (Table 1). A 632 bp fragment (bases 1–632) was amplified with oligonucleotides F1 and Porcirc3, a 446 bp fragment (bases 1322–1767) with oligonucleotides PCV-2IS and 1767r, and a 879 bp fragment (bases 1322–433) with oligonucleotides PCV-2IS and 433r. Remaining gaps and joining sequences were sequenced in an overlapping manner with oligonucleotides Porcirc1 and Porcirc2 (241 bp, bases 501–741), PCV-2IS and PCV-2IAS (264 bp, bases 1322–1585), PCV-2IAS and nPCRfwd (742 bp, bases 844–1585), Porcirc1 and PCV-2IAS (1085 bp, bases 501–1585). Oligonucleotide 1696f was used as a direct sequencing primer.

Table 1
Oligonucleotide primers used in this study

Primer	Primer sequence	Application ^a	Position and orientation ^b
F1 ^c	5'-ACC AGC GCA CTT CGG CAG-3'	PCR, sequencing	1–18, f
433R ^c	5'-TCC AGC AAG GTA CTC A-3'	PCR, sequencing	418–433, r
Porcirc1	5'-GCT GAA CTT TTG AAA GTG AGC-3'	PCR, sequencing	501–521, f
Porcirc3	5'-GTA TGT GGT TTC CGG GTC-3'	PCR, sequencing	615–632, r
Porcirc2	5'-CACACAGTCTCAGTAGATCATCC-3'	PCR, sequencing	719–741, r
nPCRfwd	5'-CAA CTG CTG TCC CAG CTG TAG-3'	PCR, sequencing, comparative sequencing	844–864, f
PCV-2IS ^d	5'-TAG GTT AGG GCT GTG GCC TT-3'	PCR, sequencing	1322–1341, f
PCV-2IAS ^d	5'-CCG CAC CTT CGG ATA TAC TG-3'	PCR, cloning, sequencing, comparative sequencing	1566–1585, r
1696F ^c	5'-GGT GTC TTC GTC TTC GGT AAC G-3'	Sequencing	1695–1716, f
1768R ^c	5'-AAT ACT TAC AGC GCA CTT CTT TCG-3'	PCR, sequencing	1744–1767, r
M13 fwd	5'-ACGACGTTGTAAAACGACGGCCAG-3'	Sequencing	f
M13 rvs	5'-TTCACACAGGAAACAGCTATGACC-3'	Sequencing	r

^a Oligonucleotides used for PCR, sequencing, comparative sequencing and/or cloning.

^b Relative positions of the oligonucleotides; f: forward; r: reverse.

^c Kim and Lyoo (2002).

^d Larochelle et al. (2000).

3.4. PCR amplification of the highly variable region within ORF2 (capsid protein) for comparative sequence analysis

From DNA samples of 16 pigs, a highly variable fragment of 742 bp (bases 844–1585) was amplified with oligonucleotides nPCRfwd and PCV-2IAS (Table 1) and directly sequenced.

3.5. Nucleotide sequencing, sequence and phylogenetic analyses

PCR products for direct sequencing were separated in 1.5% agarose gels and then extracted with a QIAquick[®] Gel Purification Kit (QIAGEN). Both strands were sequenced with the CycleReader[™] Auto DNA Sequencing Kit (Fermentas, St.-Leon-Rot) in an automated LI-COR 4200 sequencer (LI-COR, Bad Nauheim, Germany). All primers were 5'-labeled with IR800. Products were separated in a 6% polyacrylamide gel. Sequences were analysed with the eSEQ-program, version 2.0 (LI-COR).

Sequence identity among different PCV-2 samples was determined by Bootstrap analysis with ClustalX, version 1.81. Dendrograms were produced by Tree-View, version 1.6.6. Protein alignments were done by ClustalW program (<http://www.ch.embnet.org/software/ClustalW.html>).

Statistical analysis was done with the “Statistical Package for Social Sciences” (SPSS, version 11.0).

3.6. Nucleotide sequence accession number

The complete genomic sequence of one PCV-2 sample reported in this paper was deposited in the GenBank database, and given the accession number AY713470.

4. Results

4.1. Amplification and sequencing of one complete wild pig PCV-2 genome

From the 238 wild pigs investigated, 43 pigs, i.e. 18.1% were positive for PCV-2. Using a combination of primers (Table 1), seven DNA fragments representing the complete PCV-2 genome from one wild pig sample (#214), have been amplified (Fig. 1) and sequenced to compare with published sequences of PCV-2 samples from domestic pigs. The length of the genomic DNA was 1767 nucleotides. In comparison with most of the already published sequences, one nucleotide was missing from position 1042/1043 of the PCV-2 genome (Fig. 2). The shortening of the genomic sequence was due to a deletion of one of three

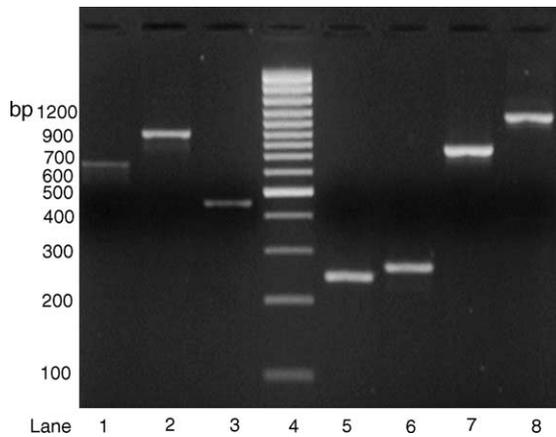


Fig. 1. PCR amplification of fragments representing the complete PCV-2 genome of sample #214. For details see text and Table 1. Lane 1: 632 bp product (bases 1–632; primers F1/Porcirc3); lane 2: 879 bp product (bases 1322–433; primers PCV-2IS/433r); lane 3: 446 bp product (bases 1322–1767; primers PCV-2IS/1768r); lane 4: 100 bp DNA-ladder; lane 5: 241 bp product (bases 501–741; primers Porcirc1/Porcirc2); lane 6: 264 bp product (bases 1322–1585; primers PCV-2IS/PCV-2IAS); lane 7: 742 bp product (bases 844–1585; primers PCV-2IAS/nPCRfwd); lane 8: 1085 bp product (bases 501–1585; primers Porcirc1/PCV-2IAS).

thymidines at position 1040–1042 of the 1768 nucleotide strand. The deletion caused a shift in the ORF2 with the stop-codon being displaced one

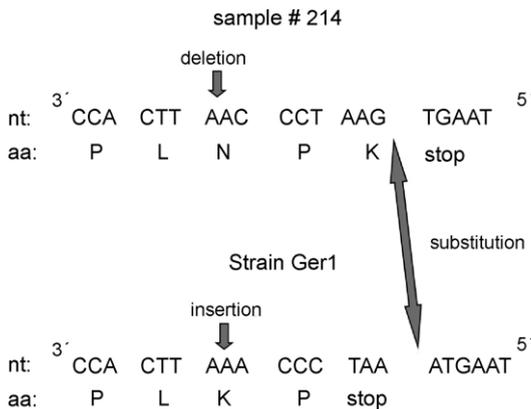


Fig. 2. Typical nucleotide (nt) and protein sequence (aa) at the N-terminus of the capsid protein of PCV-2: P-L-K-P-stop (lower sequence, e.g. GER1). This sequence is changed by a deletion of an adenine (upper sequence), which leads to the shortening of the PCV-2 genome in sample #214 (1767 nucleotides) and to a prolongation of the capsid protein (234 amino acids) due to a shift close to the stop-codon of ORF2. This shift causes an amino acid substitution at position 232 (K → N, lysine → asparagine) and an amino acid insertion (K, lysine) at position 234.

position towards the 3'-untranslated region, allowing one lysine to be added at position 234 of the capsid protein sequence, and thus leading to a product of 234 instead of 233 amino acids (aa) (Fig. 2). Overall nucleotide homologies between the wild pig sample and published sequences were between 95.5 and 97.8%. Highest sequence-convergence existed between the wild pig sample and Chinese samples (e.g. Shandong, accession number AY181947). There were clear divergences between wild pig samples and samples from German domestic pigs.

4.2. Genetic characterization of PCV-2 nucleic acid fragments from wild pigs

To investigate variability of PCV-2 samples from wild pigs of different hunting grounds, a 742 nucleotide fragment including nucleotides 844–1585 of the PCV-2 genome (Fig. 1, lane 7) was sequenced from 16 PCV-2-positive animals. Homologies of samples ranged between 98.9 and 100%. Eight of the samples, originating from different hunting areas of region 1, were identical in nucleotides. Their common sequence will be called the “consensus” in the following. Two samples, originating from regions 1 and 2 (#108 and #214), had one nucleotide change, each. Three samples from region 4 (#311, #312, #315) had 2–8 nucleotide changes compared to the consensus. The numbers of nucleotide changes between samples correlated significantly with the geographic distances between the sample regions ($r = 0.871$; $p = 0.000$). Three further samples, #138 (region 3), #44 (region 1), and #75 (region 2) showed 17, 30, and 35 nucleotide changes compared to the consensus sequence, i.e. homologies with the consensus of 97.7, 96.0, and 95.3%, respectively. A phylogenetic tree based on the 742 nucleotides, including a number of already published PCV-2 sequences from domestic pigs of different countries, grouped the consensus together with samples of 1–8 nucleotide changes and #138 together with mainly Chinese PCV-2 strains (Fig. 3). Sample #44 was grouped within an array of mainly French samples and sample #75 was assorted into a group of PCV-2 samples from mainly German and Austrian domestic pigs. The deduced amino acid sequences of the 16 samples, together with the sequences of already published PCV-2 samples of three domestic German and one domestic Chinese

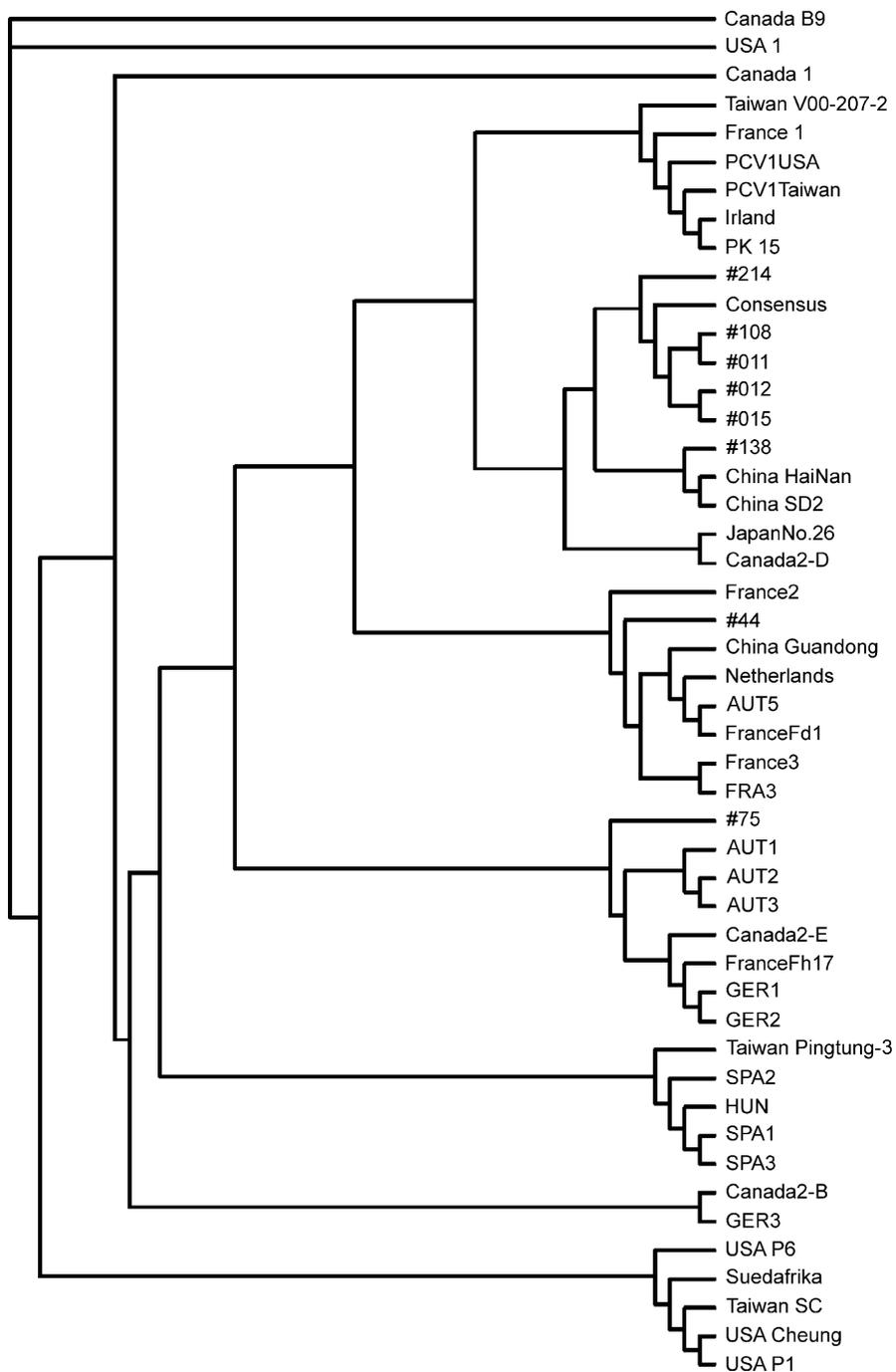


Fig. 3. Phylogenetic tree based on DNA sequence data of the capsid region (nt 938–1461) of 16 wild pig PCV-2 samples from German hunting grounds and on already published DNA sequences of domestic pig strains. Samples #44, #75, #108, #138, #214, #311, #312, and #315 are indicated. Identical sequences of the wild pigs (representing samples #9, #14, #19, #23, #36, #37, #50, and #55) are indicated as “consensus”. For different countries, the following abbreviations have been used: AUT: Austria; CAN: Canada; FRA: France; GER: Germany; SPA: Spain.

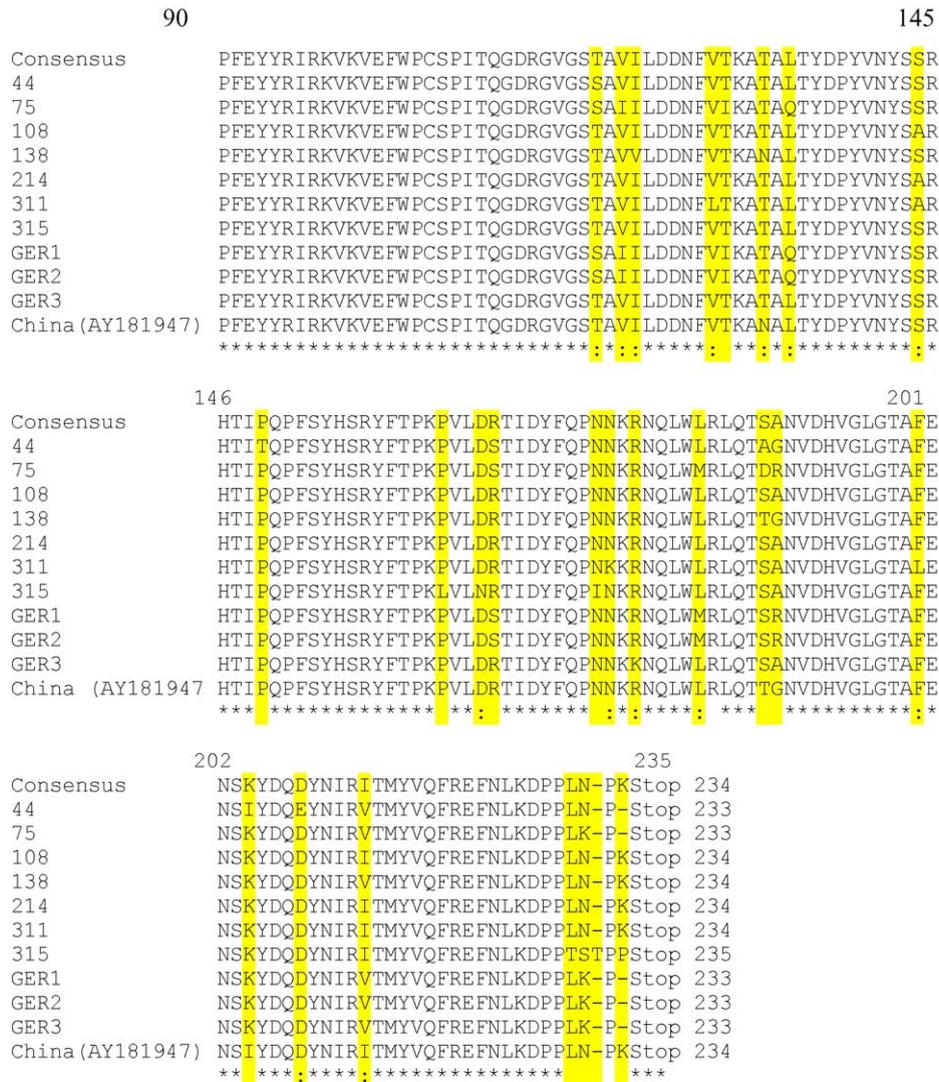


Fig. 4. Multiple amino acid (aa) sequence alignment of ORF2 (capsid protein, aa 91–233/234), obtained by ClustalW (1.74). Sequence differences are highlighted in grey, deletions are indicated by hyphens. “Consensus” names the identical sequence of samples #9, #14, #19, #23, #36, #37, #50, #55, and #312. #44, #75, #108, #138, #214, #311, and #315 differ from the consensus in at least one nucleotide.

strain is shown in Fig. 4; amino acid changes relative to the consensus were obvious at 25 positions. Protein-sequence homologies of wild pig samples ranged from 93.6 to 100%. A closer look at the situation of amino acid substitutions identified sets of amino acid substitutions which could be found in samples of different regions. One set, representing substitutions at amino acid positions 188, 189, and 213 was present in samples #44, #75, and #138. A second set showed substitutions at positions 120, 167, 146, and 148. This

set substantiates the close relation between samples #44 and #75. A substitution at amino acid 144 existed in samples #214, #108, and #311.

5. Discussion

PCV-2 infection is now recognized as the major cause of PMWS, one of the major health problems in today's swine industry world-wide (Allan and Ellis,

2000; Segales and Domingo, 2002). Additionally it has been linked to the chronic respiratory disease complex (Pesch et al., 2000; Harms et al., 2002; Segales et al., 2004), and reproductive disorders (Pensaert et al., 2001; Cariolet et al., 2001), including sow abortion, and represents one of the factors in the porcine dermatitis and nephropathy syndrome (Segales et al., 1998; Wellenberg et al., 2004). Although evidence for a connection between PCV-2 and PMWS was first reported in 1991, retrospective studies have identified PCV-2 infections dating back to the 1980s (Tischer et al., 1986). Thus it appears that the PCV-2 infection of pigs was wide spread even years ago, and that the predominant host–virus relationship is that of a subclinical, persistent infection (Krakowka et al., 2003). In this context, the question has to be addressed, whether wild pigs might play a role as hosts, carriers and potential sources for the PCV-2 infection of domestic pigs. It has been shown, that wild pigs can be infected by PCV-2, and that they can develop PMWS (Tischer et al., 1986; Sanchez et al., 2001; Segales et al., 2002, 2003; Ellis et al., 2003; Vicente et al., 2004; Schulze et al., 2003; Exel et al., 2004). Prevalences in wild pig populations have been found to range between 22.7 and 47% (Vicente et al., 2004; Exel et al., 2004; Knell et al., in press). But the relationship between domestic and wild pig PCV-2 samples and the variability of wild pig samples within and between populations of differing geographical locations is still unclear.

According to Fenaux et al. (2000) all PCV-2 samples are 1768 nucleotides in length and the ORF2 encodes 233 amino acids. In contrast, we found 1767 nucleotides and 234 amino acids in sample #214 which was completely sequenced. The shortening of the genome and the prolongation of the protein were both due to a deletion of one of the three thymidines in the range of position 1040–1042 of the 1768 nucleotide strand. The deletion was evident in 14 of the 16 wild pig PCV-2 samples and in the published domestic Chinese sample AY181947. Only two samples of our study (#44 and #75) were concordant with the majority of published domestic pig samples.

Based on a 742 nucleotide fragment of ORF2 which codes for the N-terminus of the capsid protein, our results indicate further variability in wild pig PCV-2 samples. Even in the same hunting ground, homologies between samples can be as low as 95.3%

based on nucleotides and 93.6% based on protein sequences. Fifty percent of our PCV-2 samples were identical in this fragment. A further 31% of the samples varied in 0.13–1.1% of the nucleotides and in 0.7–5% of the amino acids, compared to the consensus. These PCV-2 samples have the highest homologies not with German or European strains, but with Chinese samples (Fig. 3). Within this group, a strong correlation between substitution rates and geographic distances was evident ($r = 0.871$; $p = 0.000$). Two further samples (#44 and #75) differed from the consensus in 4–4.7% of amino acids and in 7–10.5% of the nucleotides, respectively. This variation is much higher than that described by Fenaux et al. (2000). As calculations have been made, based on a part of the highly variable putative antigenic epitope of the capsid, the data may not be generally applicable to the PCV-2 genome, but might play a role considering the immunogenicity of PCV-2 strains. The results of Kim and Lyoo (2002) revealed putative epitopes in the range of amino acids 117–131 and 169–183 of the capsid protein. The present study shows variability within these regions in wild pigs also. Samples #44 and #75 were homolog to the German strains, and the other samples, including the consensus, were homolog to the Chinese strains. This homology of most of our PCV-2 samples with Chinese strains is surprising. It remains unclear, if the three strains have evolved in the wild pigs, or if they have been introduced from external, maybe domestic sources. Further research should focus on the characterization of PCV-2 sequences from domestic pigs neighbouring the hunting grounds of this study, to improve the understanding of this relation, and on associations between PCV-2 infection and PMWS in wild pigs.

Acknowledgements

We wish to thank Mrs. Muehlhaeuser and Mrs. Franke for perfect assistance.

References

- Allan, G.M., Ellis, J.A., 2000. Porcine circoviruses: a review. *J. Vet. Diagn. Invest.* 12, 3–14.

- Cariolet, R., Blanchard, B., Le Dimna, M., Mahe, D., Keranflech, A., Julou, P., Beaurepaire, B., De Boissesson, C., Truong, C., Jestin, A., 2001. Consequences of PCV-2 experimental infection of non immune SPF sows using the intrauterine route. In: Proceedings of the International Conference on ssDNA Viruses of Plants, Birds and Primates, Saint-Malo, p. 129.
- Ellis, J., Krakowka, S., Lairmore, M., Haines, D., Bratanich, A., Clark, E., Allan, G., Konoby, C., Hassard, L., Meehan, B., Martin, K., Harding, J., Kennedy, S., Mc Neilly, F., 1999. Reproduction of lesions of postweaning multisystemic wasting syndrome in gnotobiotic piglets. *J. Vet. Diagn. Invest.* 11, 3–14.
- Ellis, J., Spinato, M., Yong, C., West, K., McNeilly, F., Meehan, B., Kennedy, S., Clark, E., Krakowka, S., Allan, G., 2003. *Porcine circovirus* 2-associated disease in Eurasian wild boar. *J. Vet. Diagn. Invest.* 15, 364–368.
- Exel, B., Steineck, W., Sipos, W., Schuh, M., Schmoll, M., 2004. Evidence of *porcine circovirus* type 1, type 2 and *porcine parvovirus* in wild boars in Austria. In: Proceedings of the 18th IPVS Congress, vol. 1, Hamburg, Germany, p. 106.
- Fenaux, M., Halbur, P., Gill, M., Toth, T.E., Meng, X.-J., 2000. Genetic characterization of type 2 *porcine circovirus* (PCV-2) from pigs with multisystemic wasting syndrome in different geographic regions of North America and development of a differential PCR-restriction fragment length polymorphism assay to detect and differentiate between infections with PCV-1 and PCV-2. *J. Clin. Microbiol.* 38, 2494–2503.
- Hamel, A.L., Lin, L.L., Nayar, G.P., 1998. Nucleotide sequence of *porcine circovirus* associated with postweaning multisystemic wasting syndrome in pigs. *J. Virol.* 72, 5262–5267.
- Harding, J.C., 1996. Post-weaning multisystemic wasting syndrome (PMWS): preliminary epidemiology and clinical presentation. In: Proceedings of the 27th Ann. Meet. West. Can. Assoc. Swine Pract., Saskatoon, p. 21.
- Harms, P.A., Halbur, P.G., Sorden, S.D., 2002. Three cases of porcine respiratory disease complex associated with *porcine circovirus* type 2 infection. *J. Swine Health Prod.* 10, 27–30.
- Kim, J.H., Lyoo, Y.S., 2002. Genetic characterisation of *porcine circovirus*-2 field isolates from PMWS pigs. *J. Vet. Sci.* 3, 31–39.
- Knell, S., Willems, H., Hertrampf, B., Reiner, G., 2005. Prävalenz porciner Circoviren in ausgewählten deutschen Wildschwein-Populationen. *Tieraerztl. Prax.*, in press.
- Krakowka, S., Ellis, J.A., McNeilly, F., Meehan, B., Rings, D.M., McCullough, K., Botner, A., Nauwynck, H., Charreyere, C., Allan, G., 2003. The pathogenesis of PCV-2-associated post-weaning multisystemic wasting syndrome in swine. In: Proceedings of the 4th International Symposium on Emerging and Re-emerging Pig Diseases, Rome, June 29–July 2, pp. 143–147.
- Larochelle, R., Bielanski, A., Muller, P., Magar, R., 2000. PCR detection and evidence of shedding of *porcine circovirus* type 2 in boar semen. *J. Clin. Microbiol.* 38, 4629–4632.
- Meehan, B.M., Creelan, J.L., Mc Nulty, M.S., Todd, D., 1997. Sequence of *porcine circovirus* DNA: affinities with plant circoviruses. *J. Gen. Virol.* 78, 221–227.
- Meehan, B.M., McNeilly, F., Todd, D., Kennedy, S., Jewhurst, V.A., Ellis, J.A., Hassard, L.E., Clark, E.G., Haines, D.M., Allan, G.M., 1998. Characterization of novel circovirus DNAs associated with wasting syndromes in pigs. *J. Gen. Virol.* 79, 2171–2179.
- Morozov, I.T., Sirinarumit, S.D., Sorden, P.G., Halbur, M.K., Morgan, K., Yoon, J., Paul, P.S., 1998. Detection of a novel strain of *porcine circovirus* in pigs with postweaning multisystemic wasting syndrome. *J. Clin. Microbiol.* 36, 2535–2541.
- Niagro, F.D., Forsthoefel, A.N., Lawther, R.P., 1998. Beak and feather disease virus and *porcine circovirus* genomes: intermediates between the geminivirus and plant circoviruses. *Arch. Virol.* 143, 1723–1744.
- Pensaert, M.B., Sanchez Jr., R.E., Nauwynck, H.J., 2001. Transmission of *porcine circovirus* type 2 from the sow to the litter. In: Proceedings of the International Conference on ssDNA Viruses of Plants, Birds and Primates, Saint-Malo, p. 84.
- Pesch, S., Schmidt, U., Ohlinger, V.F., 2000. Proliferative necrotizing pneumonia (PNP) is a result of coinfection with porcine reproductive and respiratory disease virus (PRRSV) and *porcine circovirus* type 2 (PCV-2). In: Proceedings of the 16th International Congress on Pigs Veterinary Society, p. 581.
- Sanchez, R., Nauwynck, H., Pensaert, M., 2001. Serological survey of PCV-2 antibodies in domestic and wild pig populations in Belgium. In: Proceedings of the ssDNA viruses of plants, birds, pigs and primates, St. Malo (France), p. 122.
- Segales, J., Domingo, M., Latimer, K.S., 1998. *Porcine circovirus* is present in cases of porcine dermatitis and nephropathy syndrome (PDNS). In: Proceedings of the 15th Int. Pig Vet. Soc. Congr., Birmingham, p. 215.
- Segales, J., Marco, I., Calsamiglia, M., 2002. Evidence of *porcine circovirus* type 2 infection in wild boar (*Sus scrofa*) in Spain. In: Proceedings of the 17th IPVS-Congress, Ames, Iowa, USA, p. 397.
- Segales, J., Gortazar, C., Vicente, J., Domingo, M., 2003. First description of post weaning multisystemic wasting syndrome in the will boar (*Sus scrofa*) in Europe. In: Proceedings of the 4th International Symposium on Emerging and Re-emerging Pig diseases, Rome, June 29–July 2, pp. 177–178.
- Segales, J., Domingo, M., 2002. Postweaning multisystemic syndrome in pigs. A review. *Vet. Q.* 24, 125–180.
- Segales, J., Rosell, C., Domingo, M., 2004. Pathological findings associated with naturally acquired *porcine circovirus* type 2 associated disease. *Vet. Microbiol.* 98, 137–149.
- Schulze, C., Neumann, G., Grutze, I., Engelhardt, A., Mirlé, C., Ehlert, F., Hlinak, A., 2003. Case report: *Porcine circovirus* type 2 infection in an European wild boar (*Sus scrofa*) in the state of Brandenburg Germany. *Dtsch. Tierarztl. Wochenschr.* 110, 426–428.
- Tischer, I., Rasch, R., Tochtermann, G., 1974. Characterization of papovavirus- and picornavirus-like particles in permanent

- pig kidney celllines. *Zentralbl. Bakteriolog. Org. A* 226, 153–176.
- Tischer, I., Miels, W., Wolff, D., Vagt, M., Griem, W., 1986. Studies on epidemiology and pathogenicity of *porcine circovirus*. *Arch. Virol.* 91, 271–276.
- Vicente, J., Segales, J., Hofle, U., Balasch, M., Plana-Duran, J., Domingo, M., Gortazar, C., 2004. Epidemiological study on *porcine circovirus* type 2 (PCV-2) infection in the European wild boar (*Sus scrofa*). *Vet. Res.* 35, 243–253.
- Wellenberg, G.J., Stockhofe-Zurwieden, N., de Jong, M.F., Boersma, W.J., Elbers, A.R., 2004. Excessive *porcine circovirus* type 2 antibody titres may trigger the development of porcine dermatitis and nephropathy syndrome: a case-control study. *Vet. Microbiol.* 99, 203–214.