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BRIEF REPORT

Homologous recombination within the capsid gene of porcine circovirus type 2 subgroup viruses via natural co-infection

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Abstract Several studies had reported homologous recombination between two porcine circovirus (PCV) type 2 subgroup viruses, PCV2a and PCV2b. The recombination events described thus far mapped either within the Rep gene sequences or the sequences flanking the Rep gene region. Previously, the presence of both PCV2a and PCV2b DNA sequences in tissues of the same infected swine from the 2005 United States PCV-associated disease outbreak was reported, which indicates that the animal was co-infected with both PCV2 subgroup viruses. Here, two naturally occurring chimeric genomes were identified that exhibited homologous recombination within the capsid gene sequences, and infectious viruses were recovered from both chimeric genomes after transfection into tissue culture cells.

Porcine circovirus (PCV) is a member of the genus *Circovirus* of the family *Circoviridae*, which includes a group of diverse animal viruses with a small, closed-circular, single-stranded DNA genome [12, 14]. Two genotypes of PCV have been identified, PCV type 1 (PCV1) and PCV type 2 (PCV2), and their genomes share 68–76% sequence homology. PCV1 is non-pathogenic, while PCV2 has been associated with several swine diseases [1, 4]. Based on phylogenetic analysis, PCV2 can be further divided into two subgroups: PCV2a (previously designated PCV2-group 2) and PCV2b (previously designated PCV2-group 1) [13]. While PCV2a and PCV2b viruses have been reported in Europe and in Asia, PCV2b viruses were not detected in North America prior to the 2004 and 2005 PCV-associated disease (PCVAD) outbreaks in Canada [3, 8] and in the United States [6], respectively. The sudden appearance of PCV2b viruses in diseased swine from widely separated locations in Canada and in the United States suggests that the new PCV2b viruses were more pathogenic than the previously circulating PCV2a viruses. The relative pathogenic capability of these two PCV2 subgroup viruses remains a subject of investigation [2, 7, 10, 15].

The genomic nucleotide (nt) sequences of PCV2a and PCV2b viruses differ by ~5%. It has been noted that different amino acid (a.a.) residues or motifs are preferred at certain locations of the Rep, capsid and ORF3 proteins of their respective genomes [6].

Recently, several studies have reported recombination between PCV2a and PCV2b viruses [9, 11, 13]. Interestingly, the break points for the recombination events described thus far have been mapped either within the Rep gene nt sequences or the sequences flanking the Rep gene region.

In the 2005 United States PCVAD outbreak, PCV2a and PCV2b DNA sequences were detected in the same animal [6]. In this work, experiments were carried out to investigate whether homologous recombination can occur within the capsid DNA sequences during PCV2a and PCV2b co-infection of the same animal. Total DNA isolated from the lymph node of a North Carolina pig (number 10) [6] was

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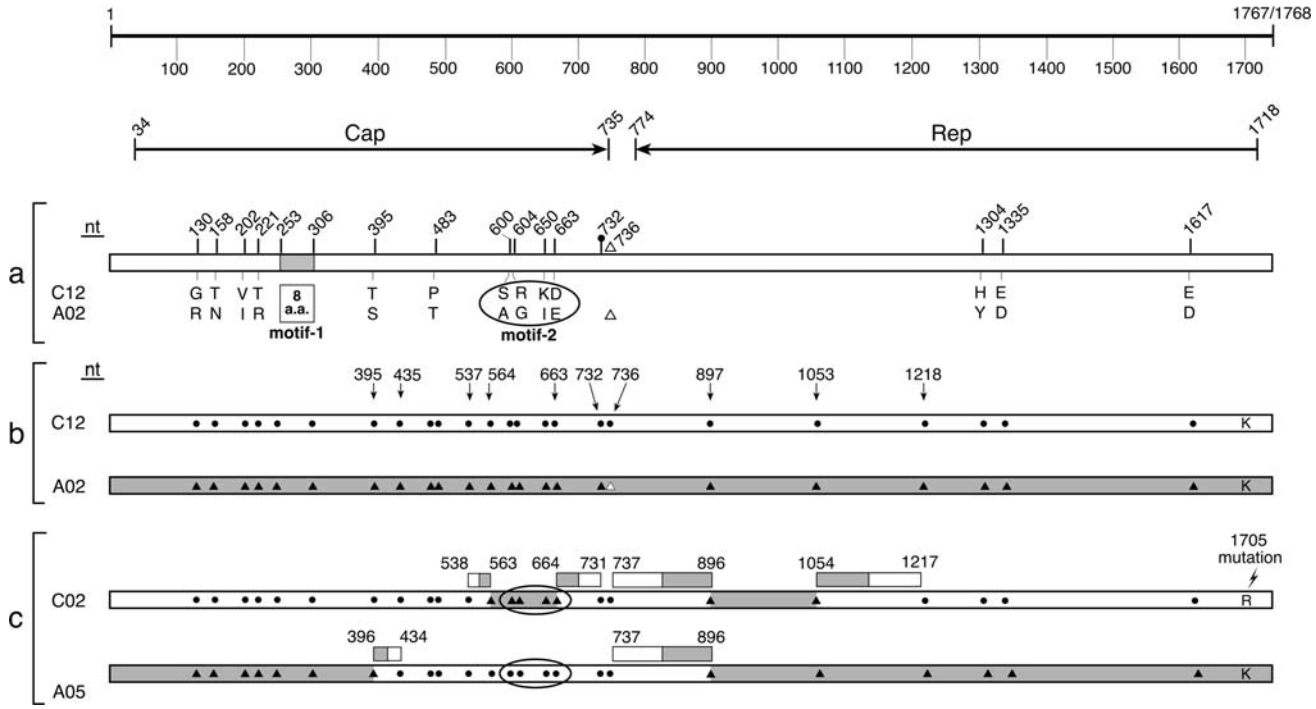

Fig. 1 Schematic representation of the parent viral genomes (C12 and A02) and the chimeric genomes (C02 and A05). **a** Locations of nucleotide changes that resulted in amino acid (a.a.) residue changes between C12 and A02. *Open triangle* indicates a nucleotide deletion. **b** Parent viral genomes. The C12 genome is represented by an open box with selected nucleotides indicated by *filled circle*, and the A02 genome is represented by a shadded box with selected nucleotides indicated by *filled triangle*. **c** Chimeric genomes of C02 and A05. The half-open/half-shaded boxes denoted with nucleotide locations on top of the viral genome indicate the regions within which cross-over occurred

subjected to PCR amplification, and the PCR products were inserted into a TA-cloning vector (Invitrogen, Carlsbad, CA) for sequence determination. The oligonucleotide primer set used, 1010BF (GCATGGATCCATCACTTCGTA ATGGT) and 1010BR (GCATGGATCCAAAAAAGAC TCAGTAA), yielded full-length viral genome PCR products. Initially, only the capsid gene open reading frame was sequenced. Of the 47 independent clones obtained, 42 were PCV2a (clone C12), three were PCV2b (clone A02) and two were PCV2a-PCV2b recombinant sequences (clones C02 and A05). Subsequently, the complete nt sequences of C12 (GenBank accession number FJ218001), A02 (GenBank accession number FJ218000), C02 (GenBank accession number FJ218002) and A05 (GenBank accession number FJ388889) were determined.

A schematic diagram aligning the full-length nt sequences of C12, C02, A02 and A05 is shown in Fig. 1, and the capsid gene sequences are shown in Fig. 2. The nt sequences are presented with the capsid gene open reading frame in the sense orientation. The genome of C12 (a PCV2a virus) is 1,768 nts long, and the genome of A02 (a PCV2b virus) is 1,767 nts long. There are 76 nt differences between C12 and A02, and 21 nt changes resulted in a.a. residue changes. Eighteen of the a.a. residue differences reside in the capsid protein, while only three a.a. residue differences reside in the Rep protein. As described previously [6], eight of the a.a. residue changes located between nt 255 and 306 (designated motif-1) within the capsid protein are unique to either PCV2a or PCV2b isolates. The 76-nt difference between C12 and A02 are scattered throughout the viral genomes and renders the two genome sequences easily discernable. Further downstream from motif-1 are four a.a. residues (designated motif-2) which differ between the A02 clone and the C12 clone sequences. In our DNA recombination analysis, cross-over was invoked as little as possible to account for the chimeric viruses.

For the chimeric C02 genome, the major parent is C12, and the sequences between nt 564–663 and nt 897–1,053 were replaced by the A02 sequences. The recombination event of nt 897–1,053 is tentative because it only involved the beginning (nt 897) and ending (nt 1,053) nts. These two nt changes did not result in any a.a. residue changes. Alternatively, the nt 897 and nt 1053 changes may also be the results of two point mutations. Incidentally, C02 had also acquired a point mutation (not from A02) at nt 1705, which resulted in a conservative a.a. residue change (Arg in C02 and Lys in C12) in the Rep protein. In Fig. 1, C02 was depicted as a double recombinant genome of C12 and A02 with a mutation at the

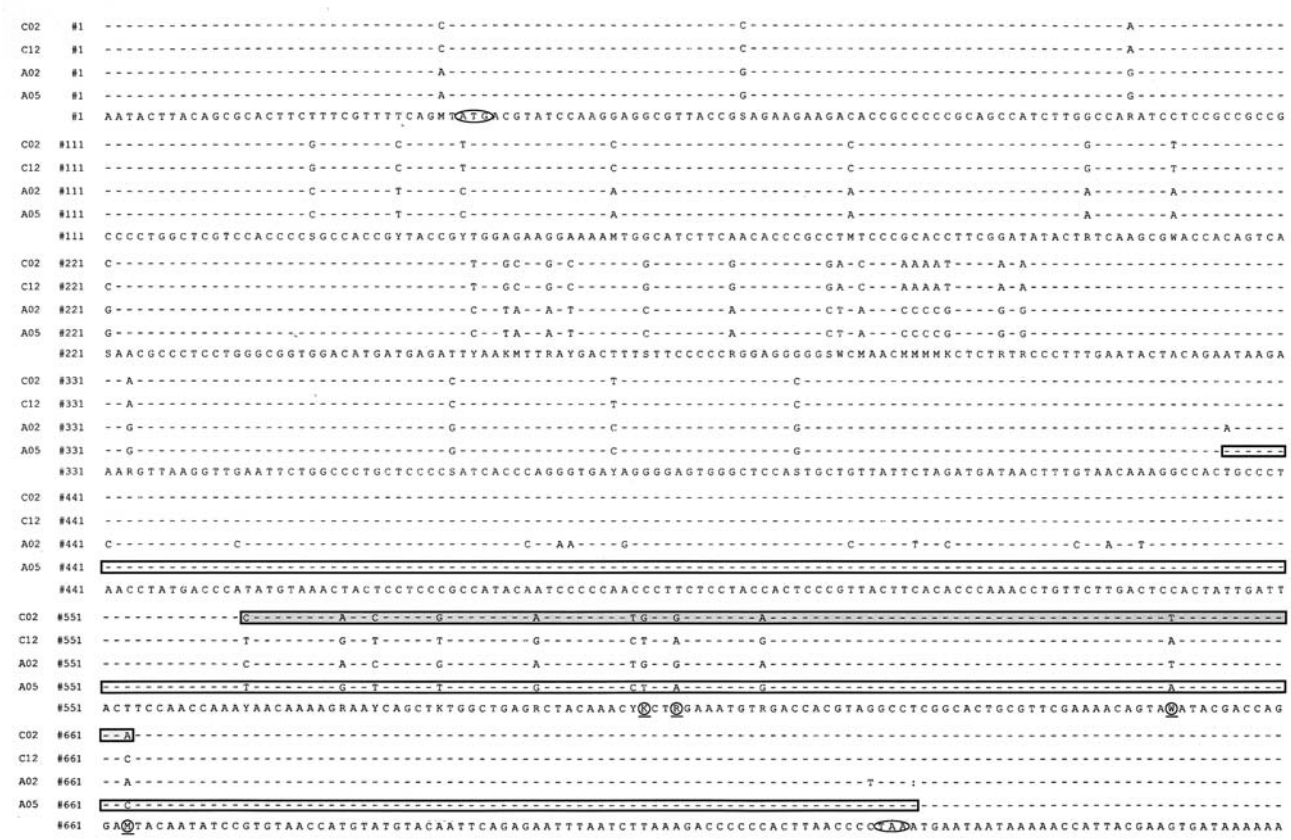

Fig. 2 Recombinant capsid sequence. The capsid gene sequences of C02, C12, A02 and A05 are presented. The capsid gene initiation and termination codons are enclosed in *oval boxes*. The single nucleotide deletion in A02 is indicated by (:). The A02 nucleotide sequence present in the C02 clone is enclosed in a *shaded box* and the C12 nucleotide sequence in the A05 clone is enclosed in an *open box*. The nucleotide that denotes motif-2, in the consensus sequence, are *circled* and *underlined*

Rep gene. The first crossover from C12 to A02 was between nt 538 and 563 and then back to C12 between nt 664 and 731. The second crossover from C12 to A02 was between nt 737 and 896 and then back to C12 between nt 1054 and 1217. With respect to a.a. sequence, C02 is a recombinant genome with a PCV2a-C12 backbone and the PCV2b-motif-2.

For the chimeric A05 genome, the major parent is A02, and the sequence between nt 435 and 736 came from C12. A05 can be generated from A02 and C12 with crossover from A02 to C12 between nt 396 and 434 and from C12 back to A02 between nt 737 and 896 (Fig. 1). Within the nt 435–736 sequence, there are 26 nt differences and a nt deletion between C12 and A02; however, only four nt differences resulted in a.a. residue (motif-2) changes. Thus, with respect to a.a. sequence, A05 is a recombinant genome with a PCV2b-A02 backbone and the PCV2a-motif-2.

Transfection experiments were conducted to recover infectious viruses from C12, C02, A02 and A05. The viral genomes were excised from the TA-cloning vector with Bam HI, ligated with T4 DNA ligase and then introduced by transfection into PK15 cells as described previously [5]. At 7 days, the transfected cultures were harvested, freeze-thawed three times, and then assayed for infectious viruses by inoculation onto fresh PK15 cells. The presence of infectious progeny viruses was observed by immunochemical staining [5] in all four cultures transfected with each of the cloned DNA (data not shown).

In this work, we detected two naturally-occurring recombinant viruses (C02 and A05) from a pig co-infected with a PCV2a (C12) and a PCV2b (A02) isolate. Nucleotide sequence analysis demonstrated that C02 is a PCV2a major parent recombinant and the A05 is a PCV2b major parent recombinant. Incidentally, the recombination events for both C02 and A05 occurred in a region containing the four a.a. residues (motif-2) between the parent sequences. The results unequivocally demonstrated that homologous recombination can occur within the capsid protein sequences to produce viable chimeric viruses when a host is co-infected with both the PCV2a and PCV2b subgroup viruses.

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