Emergence of a new type of porcine circovirus (PCV1/2a) in swine in Canada

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Introduction
Between September 2008 and January 2009, three cases (FMV08-1114252, FMV08-1133505 and FMV09-1134568) were submitted to the Veterinary diagnostic service of the University of Montreal. None of the three submitted cases were related to “Porcine circovirus associated diseases” (PCVAD). Several diagnostic assays were performed including a multiplex real-time quantitative PCR assay (mrtqPCR) for the detection and differentiation of porcine circovirus (PCV) type 2a and 2b genotypes in the lung and lymph nodes(1). Odds results were obtained with the mrtqPCR assay, suggesting genomic mutations in the viral genome.

Materials and methods
To confirm the genomic nature of the three PCV positive cases, specific PCR assays were performed to detect the ORF1 of PCV1 and the ORF2 of PCV2 genes. A third PCR assay was selected to amplify both ORF1 of PCV1 and ORF2 of PCV2 genes simultaneously. The entire PCV genome was amplified by PCR that produced 2 overlapping PCR products. Both strands of the purified DNA PCR products were sequenced(2). Virus isolation was conducted using the PK15A cell line as previously described(2) and an immunofluorescence assay (IFA) was performed to confirm the presence of PCV using an anti-PCV2 pig serum as described previously(3).

Results
All the obtained results converge to demonstrate the emergence of a new PCV. In addition, infectious viruses were isolated from 2 out of the 3 submitted cases and expression of the viral Cap protein encoded by the ORF2 gene was confirmed by IFA in PCV infected cells. The results indicate the presence of both PCV1 ORF1 and PCV2 ORF2 genes in the PCR products. To confirm the genomic nature of the new PCV, its viral genome was sequenced. The ORF1 was more genomically related to the ORF1 of PCV1 with a nucleotide (nt) identity of 99.7% compared to ORF1 of PCV2a and 2b with nt identities of 82.5% and 82.8%, respectively. On the other hand, the ORF2 was more genomically related to the ORF2 of PCV2a with a nt identity of 98.5% compared to ORF2 of PCV1 and PCV2b with nt identities of 66.7% and 92.5%, respectively. The nt identity of the entire viral genome of the new PCV was 86.4%, 88.7% and 86.5% compared to PCV1, PCV2a and PCV2b, respectively.

Discussion
It is proposed to name this new PCV by taking into account previously described nomenclature(4) and by indicating the origin of the ORF1 at first and the origin of the ORF2 in second. Consequently, the name proposed for this new PCV is PCV1/2a. The prevalence of PCV1/2a seems to be very low in Quebec, Canada (2.5% of PCV positive cases), and its origin is now in debate. Two hypotheses could explain the emergence of the PCV1/2a in swine: (a) the natural genetic recombination between PCV1 and PCV2a in infected animals; or (b) a human made virus originating from a chimeric killed vaccine strain. Still, even if PCV1 and PCV2a are found in low amount in Quebec swine, the genetic recombination between PCV1 and PCV2a is a possible explanation. In early 2008, a new vaccine was commercially available in Canada (Suvaxyn® PCV2). This vaccine uses a chimeric killed or inactivated PCV(5, 6). This chimeric PCV genome is composed of the ORF1 of PCV1 and the ORF2 of PCV2a. The nt sequences of the PCV1/2a strains were found to possess 5 to 6 nt differences compared to the chimeric PCV vaccine strain with a nt identity of 99.6–99.7% and all nt differences were located in ORF1.

References
5.  Gillespie et al., 2008 (Vaccine 26, 4231–4236).

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