

**P.006****PHYLOGENETIC ANALYSIS OF PORCINE CIRCOVIRUS TYPE 2 (PCV-2) IN FETUSES AND STILLBIRTH WITH NATURAL INFECTION**

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**Introduction**

Porcine Circovirus Type 2 (PCV2), the infectious agent of PCVAD (PCV2 associated diseases) is also implicated in reproductive failures in swine (1,2). Vertical transmission seems to be an important route of infection by PCV2, which has been detected by nested-PCR in organs of fetuses naturally infected (1,3).

The PCV2 isolates can be classified in PCV-2a, PCV-2b and PCV-2c genotypes based in its genome diversity. The genotype 2b has been more commonly associated with outbreaks of PMWS in North America and some European countries (4).

This study aims to perform a genetic analysis of the genome of PCV-2 viruses identified by PCR in aborted or stillborn fetus from a previous study (2).

**Materials and methods**

Samples from field cases, as aborted fetuses, mummified, stillborn and fragile piglets were collected and processed in order to detect PCV2 DNA and antigens. Samples were collected from 21 farms previously identified with occurrence of reproductive losses, and a total of 169 fetuses were necropsied. Fragments of organs, including heart, lungs, liver, kidney, lymphoid organs and nervous tissues were processed for viral and histopathological diagnostic. For viral isolation, DNA was extracted and nested-PCR reactions were performed using specific primers to detect sequences of PCV2 (5). Sequencing from the positive tissues was performed as previously described method and primers (6). The sequencing reactions employed BigDye Terminator chemistry and the products were run on an Applied Biosystems 3130xl Genetic analyzer. Consensus sequence was generated using the SeqScape v2.5 software (Applied Biosystems). A phylogenetic tree from partial genome sequencing of PCV-2 was constructed using the neighbor-joining method, using Kimura 2-parameter model, with the 500 reps of bootstrap in the MEGA 5.01 software based on nucleotide sequences.

**Results**

From 169 fetus examined by PCR, 29 were positive (17.1%). Virus sequences were found in several tested organs. From the positive ones, it was possible to perform partial sequencing of DNA from 5 fetuses. The sequence analyses determined that these viruses have a similar sequence and they were classified in PCV2a genotype as it is shown in Figure 1.

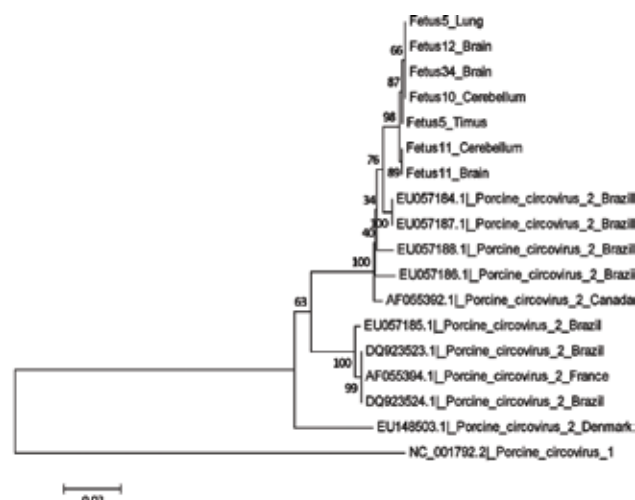


Figure 1: Phylogenetic tree, all 7 samples from this study were classified as PCV-2a genotype together with other Brazilian PCV-2a isolates from a previous work. Other Brazilian isolates were grouped with PCV-2b. PCV-1 genome was used as an out group.

**Discussion**

The results of this work are corroborating with the reports of association between PCV-2 and reproductive failure (1, 3). In a preview study it was shown the genetic diversity of Brazilian isolates (7), which also can be seen here in the Figure 1. The analysis of the partial sequenced genome revealed that all 7 samples of this work are grouped together with PCV-2a (4). In contrast, European isolates from reproductive failures include also PCV-2b (8). Herein, the findings might be related to the strains which are been successfully transmitted in Brazilian swine herds.

Also, the sequencing of the whole genome of these samples – which is underway - will help to make a definitive classification of these viruses as PCV-2a.

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**References**

- West, K.H. et al. (1999). *J Vet Diagn Invest* 11, 530-532.
- Ritterbusch, G. (2010). *Proc. IPVS Congress*. p.162.
- Maldonado, J. et al. (2005). *Vet J* 169, 454-456.
- Segalés, J. et al. (2008). *The Vet Rec* 162, 867-868.
- Kim, J. et al. (2001). *J Virol Meth* 98, 25-31.
- Mankertz, A. et al. (2000). *Virus Res* 66, 65-77.
- Ciacci-Zanella J. (2009). *Res Vet Sci* 87, 157-160.
- Hansen MS (2010). *Vet Mic* 144, 203 – 209.