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# **VOLUME II**



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### PCV2b genotype is predominant in captive wild boars in Brazil

JPH Sato<sup>1</sup>, D Gava<sup>2</sup>, R Schaefer<sup>2</sup>, NL Simon<sup>2</sup>, ME Cantão<sup>2</sup>, <u>JR Ciacci-Zanella<sup>2</sup></u>, DESN Barcellos<sup>1</sup>. Veterinary Medicine School, Federal University of Rio Grande do Sul/UFRGS, Porto Alegre, Brazil<sup>1</sup>, Embrapa Swine and Poultry Research Center, Animal Health Laboratory, Concórdia, Brazil<sup>2</sup>
janice.zanella@embrapa.br

#### Introduction

Porcine circovirus type 2 (PCV2) is an important infectious agent affecting both domestic and wild pigs (8). Phylogenetically, PCV2 is divided into two major groups, PCV2a (2A, 2B, 2C, 2D, 2E) and PCV2b (1A, 1B, 1C) which differ from each other in some nucleotides of the capsid protein (ORF2). A third genotype (PCV2c) was detected only in Danish pigs (6). Porcine circovirus associated disease (PCVAD) has been characterized in many pork producing countries worldwide (8). In Brazil, PCVAD was identified in 2000 for the first time, although PCV2 was detected in archived material dated from 1988 (2). Since its first detection, different genotypes (PCV2a and PCV2b) have been identified in Brazilian swine population. A single report have identified PCV2a in captive wild boar in 2012 (1). The aim of this study was to investigate the presence of PCV2 in captive wild boars.

#### **Materials and Methods**

Bronchial and mesenteric lymph nodes of 129 captive wild boars from two farms were collected at slaughter from January to July, 2012. Captive wild boars were slaughtered with seven months old and 32kg in average. Viral DNA was extracted from pools of lymph nodes of each animal using DNeasy Blood & Tissue Kit (Qiagen). The nested PCR (5) and PCR for sequencing (6) were performed using specific primers for PCV2 (3). The positive samples were tested by quantitative real time PCR (qPCR) (8). The PCR products were gel-purified using BigDye XTerminator Purification Kit (Qiagen) and nucleotide sequences were determined using an ABI3130xI Genetic Analyzer. The obtained sequences analyzed and assembled Phred/Phrap/Consed software (4). Phylogenetic analyses were performed using the Maximun Likelihood and Neighbor Joining methods in the MEGA 6.0 software (10). The nucleotide and amino acid sequences of ORF2 and the complete viral genome were compared with other PCV2 sequences from NCBI database to determine the genotype and cluster classification.

### Results

In the total, thirty-eight out of 129 (29.5%) pools of lymph nodes were positive for PCV2 by nested PCR. Seventeen out of eighty samples (21.3%) from farm #1 and twenty-one out of forty-nine samples (42.8%) from farm #2 were positive. Six out of 38 positive DNA samples were selected and qPCR analysed. All six samples were PCV2 qPCR positive, with viral copies ranging from 8.19x10<sup>6</sup> to 1.19x10<sup>9</sup> copies/uL.The genome sequence of all six samples were composed of

1,767 nucleotides (nt) which 699 nt codified ORF2 gene. All six samples showed 99% of nucleotide identity with PCV2b genotype. Although there were nucleotide differences on ORF2 sequences, the 233 amino acid analysis showed no difference among those sequences. Phylogenetic tree analyses classified all six sequenced samples as closely related with a PCV2b isolated from pigs in Brazil in 2004 (KC835191) and in 2009 (DQ923523) as well as PCV2b from wild boars detected in Hungary in 2003 (AY874163) and from Romania in 2008 (JN006458), 2010 (JN006464) and 2011 (JN382182). All five samples from farm #1 belonged to cluster 1A and one sample from farm #2 was classified as cluster 1B.

#### **Conclusions and Discussion**

This is the first description of PCV2b (cluster 1A and 1B) in captive wild boar. PCV2a infection was reported in PCVAD captive wild boars in Brazil previously (1). In general, PCV2a mainly ocurred before 2003 and PCV2b after 2003 (7). The occurrence of PCV2 in wildlife boars has been reported in different countries (9). The PCV2 is ubiquous and is present in pigs with or without clinical signs (7) but a high viral load was correlated with PCVAD (8). In our study, although the PCV2 load was elevated, which could indicate acute infection or viremia, no clinical condition or gross lesions were found. These findings show that PCV2b clusters 1A and 1B circulate in Brazilian captive wild boars, without clinical signs but with high viral load in lymph nodes. The PCV2 occurrence should be better investigated to determine the viral dynamic in the wild boar population.

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