RESEARCH ABSTRACT
SWINE HEALTH

Title: Pathogenicity of the ORF3 gene-silence mutant of type 2 porcine circovirus in pigs: a study towards the development of a marker vaccine – **NPB #06-006**

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Date Submitted: August 3, 2007

Abstract:

The open reading frame (ORF) 3 of porcine circovirus type 2 (PCV2) reportedly encodes a novel protein that is involved in apoptosis. To characterize the role of ORF3 in PCV2 replication and pathogenesis, we first generated an anti-peptide ORF3-specific rabbit antibody and attempted to detect ORF3 protein expression by IFA and western blot from PCV2 infected cells. After repeated attempts, we could not detect any evidence of ORF3 protein expression in PCV2 infected cells, and thus could not confirm the earlier reports by Kwang's group. To evaluate the effect of ORF3, if any, on PCV2 replication and pathogenesis *in vivo*, we created an ORF3-null PCV2 mutant by site-directed mutagenesis. The ORF3-null mutant (muPCV2) infectious DNA clone and virus from transfected cells can initiate PCV2 infection when inoculated into pigs, indicating that the ORF3 is dispensable for PCV2 replication *in vivo*. Since the ORF3-deficient PCV2 was reportedly less pathogenic than PCV2 in BALB/c mice, in this study we compared the pathogenicity of an ORF3-null PCV2 mutant (muPCV2) and the wild-type PCV2 in the natural host, pigs. Thirty-one pigs were divided into 3 groups of 11, 10, and 10 each. The 11 pigs in group 1 were each inoculated with PBS buffer as negative controls, 10 pigs in group 2 were each intramuscularly inoculated with 200 µg of muPCV2 infectious DNA clone, and 10 pigs in group 3 each with 200 µg of PCV2 infectious DNA clone. Blood was collected prior to inoculation and weekly thereafter, and tested for PCV2 antibodies by ELISA and serum viral DNA loads by quantitative PCR. All pigs were necropsied at 35 days post-inoculation (DPI) and various tissues were collected and analyzed for gross and microscopic lesions. The results showed that there was no significant difference in the average scores of gross or histological pathological lesions between pigs inoculated with the ORF3-null PCV2 mutant and pigs inoculated with the wild-type PCV2, although pigs inoculated with muPCV2 did have a delayed appearance of seroconversion, and decreased serum viral DNA loads. Thus, the data from this study do not fully support the conclusion of the published report that ORF3-deficient PCV2 is less pathogenic in mice. Consequently, the use of ORF3-null PCV2 as a vaccine candidate is not justified.