Singular PCV2a or PCV2b infection results in apoptosis of hepatocytes in clinically affected gnotobiotic pigs

Avanti Sinha1 Kelly Lager2 Chong Wang1 Tanja Opriessnig1
1. Iowa State University, Ames, IA, USA; 2. National Animal Disease Center, USDA-ARS, Ames, IA, USA

Introduction
Systemic infection with porcine circovirus type 2 (PCV2) is often clinically associated with respiratory signs, failure to thrive and diarrhea (1). Currently, PCV2 can be further subdivided into two main genotypes, PCV2a and PCV2b which under experimental conditions result in very similar macroscopic and microscopic lesions in conventional pigs (2). Viruses are often associated with apoptosis of host cells but there are conflicting results of the role of PCV2 in this pathway (3,4). The objective of the present study was to assess the association of PCV2 antigen with apoptosis in liver tissues from gnotobiotic pigs experimentally inoculated with PCV2.

Materials and Methods
Forty-eight gnotobiotic pigs were separated into five groups based on inoculation status and development of clinical disease: (1) Non-inoculated, healthy (n=4), (2) PCV2a, healthy (n=10), (3) PCV2a, clinically affected (n=6), (4) PCV2b, healthy, (n=13) and (5) PCV2b, clinically affected (n=15). Formalin-fixed and paraffin-embedded sections of liver from all pigs were subjected to a CCaspase-3 (CCasp-3) immunohistochemistry (IHC) assay, a TUNEL assay, and a PCV2 IHC (5). For each slide, 10 random 40x fields were scored for presence of characteristic staining. The scoring scheme used ranged from 0 (no signal) to 3 (high degree of staining in the majority of the cells and fields examined). All sections of liver tissue were also scored for the presence of the following microscopic lesions: Presence of neutrophils and degree of necrosis, degeneration and inflammation. The sum scores were used for analysis. The following nine comparisons were made using the Fisher’s Exact Test for dependency between the categorical variables in paired situations: (1) TUNEL score versus disease status, (2) CCaspase-3 score versus disease status, (3) PCV2 IHC score versus disease status, (4) PCV2 IHC versus TUNEL score, (5) PCV2 IHC versus CCaspase-3 score, (6) Lesion sum versus CCaspase-3 score, (7) Lesion sum versus TUNEL score, (8) Lesion sum versus PCV IHC and (9) Lesion sum versus disease status. A statistically significant difference was defined as a P-value of less than 0.05.

Results
Sixteen of 21 (76.2%) clinically affected PCV2-inoculated pigs and 11/27 (40.7%) clinically uninfected PCV2 inoculated pigs had moderate-to-severe hepatic lesions characterized by severe diffuse lymphohistiocytic hepatitis associated with degeneration and loss of hepatocytes. Specific CCasp-3 staining and TUNEL labeling was detected in the nuclei of hepatocytes in PCV2a and PCV2b infected pigs with significantly (P < 0.05) higher levels of apoptotic cells in clinically affected pigs. Clinically affected pigs also had significantly (P < 0.05) higher levels of PCV2 IHC stain compared to non-infected pigs. The lesion sum score was also significant in the clinically affected pigs (P < 0.05).

Discussion
This experiment was conducted in order to delineate the role of PCV2a and PCV2b genotypes in apoptosis of hepatic cells in gnotobiotic pigs. According to the results obtained, the gnotobiotic pigs when experimentally inoculated with PCV2a or PCV2b developed clinical disease which was associated with severe hepatitis, abundant amount of PCV2 antigen in inflammatory cells and hepatocytes, and apoptosis occurred in hepatocyte-like cells detectable by CCasp3 stains and the TUNEL assay. The pig experiments were of limited duration and it is not known if the 11/27 infected, but healthy pigs that had hepatic lesions and minimal apoptosis, would manifest clinical disease with a significant increase in CCasp4 and TUNEL stained cells. In conclusion, PCV2 infection regardless of genotype resulted in apoptosis of hepatocytes in clinically affected gnotobiotic pigs and apoptosis appears to be one pathway by which PCV2 induces disease.

References