Correlation of semi-quantitative reverse transcription polymerase chain reaction results for Porcine Epidemic Diarrhea Virus and the presence of positive immunohistochemistry

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Statement of the problem

Porcine epidemic diarrhea virus (PEDV) is a coronavirus that causes severe enteric disease in neonatal piglets characterized by dehydration, watery diarrhea, vomiting, and high mortality. Fast and reliable diagnostic tests are important to detect PEDV and diagnose clinical disease. Currently, reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) are widely used diagnostic tests for the determination of PEDV infection. While RT-PCR proves to be a fast, relatively inexpensive, and accurate test to detect viral nucleic acid, its use in differentiating subclinical infections from overt clinical disease has yet to be systematically investigated. Immunohistochemistry is used to view viral antigen within enterocytes and is thus helpful for confirming clinical disease associated with PEDV infection

Objectives

1. To compare RT-PCR and IHC on samples from infected pigs to determine guidelines that diagnosticians and clinicians can use to help estimate the stage of clinical disease associated with a given level of viral detection by RT-PCR.

Materials and methods

In this study, 150 case submissions to the Iowa State University Veterinary Diagnostic Laboratory were evaluated that contained formalin-fixed intestinal tissues and fecal samples or intestinal homogenates positive by PEDV N gene-based real-time RT-PCR. Immunohistochemistry was performed on all formalin-fixed samples and each sample was given an IHC score of 0-3 where a score of 0 indicated that no viral antigen was detected, a score of 1 indicated that less than 10% of the enterocytes were positive for viral antigen, 10% to 50% were positive for a score of 2, and over 50% of enterocytes were positive for a score of 3.

Results

Comparison of the RT-PCR CT values with the IHC scores revealed a strong negative correlation (-0.7695, $P < 0.001$) between the presence of viral antigen and the CT value where increasing scores by IHC were associated with lower CT values by PCR. Using IHC as the gold standard for the presence of clinical disease, the corresponding RT-PCR CT values were used to assess the diagnostic sensitivity and specificity of potential threshold CT values as indicators of clinical disease. In the current dataset, a PCR CT value of 20.2 or less had a diagnostic specificity of 91% and a sensitivity of 71% for detecting animals with observable viral antigen.

Discussion

Accordingly, when using the specific N-gene-based RT-PCR under the laboratory conditions evaluated in this report, a CT value of 20 or less from a fecal sample likely indicates an active clinical infection whereas higher CT values may require additional diagnostics for determining the impact of the detected virus. These data may aid in interpreting results from feces only diagnostic submissions where positive PCR results are obtained and the clinical significance is in question.