PO-16

Differentiation between Porcine Epidemic Diarrhea Virus and Transmissible Gastroenteritis Virus in Formalin-fixed Paraffin-embedded Tissues by Multiplex RT-nested PCR and Comparison with *in situ* Hybridization

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Introduction

Porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV) infections are considered difficult to distinguish clinically and histopathologically. Prompt differentiation between PEDV- and TGEV-associated enteritis would greatly facilitate the management of disease in countries where PEDV and TGEV are epizootic. Rapid differential diagnosis and treatment are crucial to reducing mortality and morbidity from PEDV- and TGEV-induced enteritis in piglets. The objective for this study was to develop a protocol to differentiate between PEDV and TGEV directly from formalin-fixed, paraffin-embedded tissue, using a multiplex reverse transcription-nested polymerase chain reaction (RT-nPCR) assay.

Materials and Methods

Sixty colostrum-deprived piglets 3 days of age, were randomly divided into four groups of 15 pigs each. Each group was randomly assigned to one of four treatments. The four treatments included intraoral inoculation with cell culture containing 1 of 2 viruses, PEDV strain SNUVR971496 or TGEV strain SNUVR980473, with both PEDV and TGEV, or with uninfected cell culture medium.

Three pigs from each group were sacrificed and necropsied at 12, 24, 36, 48, and 60 hours postinoculation (hpi). Extraction of RNA from formalin-fixed paraffin embedded tissues was performed as described previously with slight modifications [1]. RT-PCR was performed as previously described [2].

Results

The sensitivity was determined by dividing the number of PEDV RT-nPCR positive jejuni by the number of PEDV-inoculated pigs; 30/30 = 100%. Similarly, the sensitivity of the TGEV RT-nPCR was determined by dividing the number of positive jejuni by the number of inoculated pigs; 30/30 = 100%. The specificity was determined by dividing the number of control samples that were negative for multiplex RT-nPCR by the number of mock-infected control pigs: 15/15 = 100%.

Tissues were fixed in formalin for 24 h before embedment. Each specific primer pair for PEDV and TGEV yielded a multiplex RT-nPCR product of the expected size from RNA extracted from formalin-fixed, paraffin-embedded tissues. PEDV cDNA was detected in 7/15 and 15/15 jejuni of PEDV-inoculated pigs by multiplex RT-PCR and multiplex RT-nPCR, respectively. TGEV cDNA was detected in 8/15 and 15/15 jejunal tissues of TGEV-inoculated pigs by multiplex RT-PCR and multiplex RT-nPCR, respectively. PEDV and TGEV cDNAs were detected simultaneously in 7/15 and 15/15 jejunal tissues from pigs inoculated with both viruses by multiplex RT-PCR and multiplex RT-nPCR, respectively. PEDV and TGEV were not detected in formalin-fixed, paraffin-embedded jejunal tissues from negative control pigs by multiplex RT-PCR and multiplex RT-nPCR.

Discussion

The multiplex RT-PCR assay used in this study employed a nested protocol that increased the sensitivity for the detection of viral RNA from formalin-fixed paraffin-embedded tissues compared to conventional PCR methods. The increased sensitivity enabled positive signals to be obtained from a high percentage of clinical cases. The use of multiplex RT-nPCR as a monitoring and diagnostic tool for confirmation of the presence of PEDV and TGEV from formalin-fixed paraffin-embedded tissues can be a tremendous asset for the management of PEDV and TGEV in swine herds where epizootic viruses are concerned.

References

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2. **Jung, K., Kim, J., Kim, O., Kim, B. and Chae, C.** J. Virol. Methods 2003. **108,** 41-47.