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Development of Nested RT-PCR for the Detection of Swine Hepatitis E virus in Formalin-fixed, Paraffin-embedded Tissues and Comparison with *in situ* Hybridization

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Introduction

Hepatitis E virus (HEV) has been recognized as a major cause of enterically transmitted non-A, non-B hepatitis in many developing countries. The taxonomy of HEV is not clear and the virus remains unclassified. The objective of this study was to optimize conditions and procedures to detect swine HEV in formalin-fixed, paraffin-embedded tissues by nested RT-PCR and compare this detection method with in situ hybridization.

Materials and Methods

Forty pigs from 40 different commercial swine herds were selected on the basis of positive RT-PCR results on fresh liver tissues by RT-PCR. All pigs were negative for porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus 2 by in situ hybridization.

To isolate genomic RNA from deparaffinized tissue, the following three procedures were used. Samples from each pigs was examined using extraction method A, B, and C. In method A, 400 µl of digestion buffer (20 mM Tris [pH 8.0], 1 M guanidium thiocyanate, 25 mM β-mercaptoethanol plus 0.5% sarcosyl) containing 6 mg/ml of proteinase K (Gibco BRL, Grand Island, NY, USA) was added to the dried deparaffinized samples. In method B, 400 µl of digestion buffer (50 mM Tris [pH 8.0], 5 mM ethylenediaminetetraacetic acid [pH 8.0], plus 0.5% SDS) containing 500 µg/ml of proteinase K was added to dried deparaffinized samples. In method C, 400 µl of digestion buffer (500 mM Tris [pH 8.0], 20 mM ethylenediaminetetraacetic acid [pH 8.0], 10 mM NaCl plus 1% SDS) containing 500 µg/ml of proteinase K and 10 mg/ml of aurintricarboxylic acid was added to dried deparaffinized samples. RT-PCR was performed as previously described [1]. *In situ* hybridization was performed as previously described [2].

Results

All samples that were found positive by PCR were always found positive by nested PCR regardless of RNA extraction methods. In method A, 5 and 40 of the 40 samples were found positive by RT-PCR and nested RT-PCR, respectively. All 5 samples that were found positive by RT-PCR were also found positive by nested RT-PCR. In method B, 4 of the 40 samples were found positive by RT-PCR but 23 samples examined were found positive by nested RT-PCR. In method C, 2 and 7 of 40 samples were positive by RT-PCR and nested RT-PCR, respectively. In situ hybridization gave similar results for serial sections from each of the 40 tissue samples.

Discussion

The ability to amplify viral RNA from formalin-fixed, paraffin-embedded tissues by RT-PCR has a profound impact on diagnostic pathology. The reliable detection of viral RNA in formalin-fixed, paraffin- embedded tissues, however, is subject to serious technical limitations so far, although previous studies have demonstrated that viral RNA may be extracted from formalin-fixed, paraffin-embedded materials. In the field of diagnostic pathology, RT-PCR procedures are widely used and have many advantages, including the use of small amounts or fragmented cDNA as templates for molecular analysis. Because such paraffin blocks usually are available in all pathology departments, the potential for retrospective molecular and diagnostic studies is high if RNA from formalin-fixed, paraffin-embedded tissues can be analyzed.

References

1. **Meng, X. J., Halbur, P. G., Shapiro, M. S., Govindarajan, S., Bruna, J. D., Mushahwar, I. K., Purcell, R. H. and Emerson, S. U.**, J. Virol. 2001. **72**, 9714-9721.
2. **Choi, C. and Chae, C.**, J. Hepatol. 2003. **38**, 827-832.