Development and optimisation of multiplex RT-PCR for rapid detection of swine diseases

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ABSTRACT

Introduction: Highly pathogenic zoonotic diseases that infect pigs due to viral infections can cause infections and deaths among animals as well as humans. To limit the effect and spread of severe illness epidemics, early detection is crucial. There is a strong demand for testing multiple types of swine diseases from a single sample, especially those involving molecular testing. However, the assays are costly, time-consuming, and labour-intensive to perform. **Objective:** This study describes the development and initial evaluation of a multiplex reverse transcriptase-polymerase chain reaction (mRT-PCR) assay for rapid and simultaneous diagnosis of Japanese encephalitis virus (JEV), Nipah virus (NiV) and swine influenza virus (SIV). **Materials and methods:** The method uses three primer sets, each one specific for the corresponding virus, amplifying RNA fragments different in length, allowing a gel-based differential detection of the PCR products. Each of the three target fragments produced a specific amplicon of 519 bp (JEV), 300 bp (NiV) and 200 bp (SIV) in a single RT-PCR (sRT-PCR). The optimal reaction conditions were explored and standardised by adjusting the annealing temperature and primer concentrations based on the sRT-PCR condition. mRT-PCR was used to analyse 70 samples that were previously received for surveillance and diagnostic testing for the detection of these three viruses, including plasma, nasal swab, brain, kidney, and pooled organ. **Results and conclusion:** The results indicated that the specificity of mRT-PCR was comparable to that of sRT-PCR. The mRT-PCR took 2 hours and 50 minutes to run and complete, whereas the sRT-PCR took 9 hours and 5 minutes to detect these three diseases. This approach is more convenient and reliable for routine swine disease diagnosis. The mRT-PCR developed in this study may thus pave the way for rapid and cost-effective detection of these important pathogens in a single reaction.