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Detection of Influenza A Virus in Clinical Samples by Taqman® Reverse Transcriptase Polymerase Chain Reaction

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Introduction

Current methods of detecting influenza A virus in swine pneumonia samples are the antigen enzyme-immunoassay test (such as Becton-Dickinson Directigen™ Flu A (DDFlu)), virus isolation (VI), or immunohistochemistry (IHC) [1]. With the DDFlu test, results are available in as few as 15 minutes, with reliable specificity but variable sensitivity [2]. VI is slow, costly and detects only viable virus. IHC requires significant preparation time, cannot be used on antemortem samples, does not allow for further characterization of the virus, and its sensitivity is influenced by sample size, number of lung sections examined and timing of tissue collection. In order to improve our existing diagnostic tests, we developed an influenza A virus TaqMan® reverse transcriptase polymerase chain reaction (TaqMan®) test.

Materials & Methods

The TaqMan® test was developed to detect influenza A virus nucleoprotein (NP) RNA. NP was chosen for amplification because it is the most conserved influenza gene, allowing detection of influenza A viruses from mammalian, human and avian samples. NP is a major structural protein and NP mRNA is the most abundant viral transcript in infected cells [3]. A positive result on the TaqMan® test indicates the presence of influenza A virus NP gene in the sample. Primers were developed by aligning available human, avian, and mammalian influenza NP sequences from GenBank. Areas of similarity were determined, and forward and reverse oligonucleotide primers that recognized a conserved portion of the gene were selected to amplify a segment of 72 base pairs.

Results & Discussion

The TaqMan® test detected swine H1N1, swine H3N2, human H1N1 and human H3N2 influenza A virus reference isolates. The TaqMan® test was sensitive and able to detect 473 $TCID_{50}$/ml influenza A virus particles, which correlated to 8.0 X 10^{-3} µg of viral RNA/ml. It was also found to be specific because it did not detect non-influenza viruses such as porcine reproductive and respiratory syndrome virus, bovine respiratory syncytial virus, avian paramyxovirus, avian pneumovirus, bovine viral diarrhea virus, infectious bovine rhinotracheitis virus, parainfluenza 3 virus, or porcine type 2 circovirus.

We used the TaqMan® test on bronchial swabs and lung tissue from 499 swine respiratory disease cases submitted to the Minnesota Veterinary Diagnostic Laboratory between September and December 2001. Each sample was tested by TaqMan® and DDFlu. If the test was positive by either TaqMan® or DDFlu, the results were corroborated by VI, histopathology, and/or IHC. Of the 499 samples, 48 were positive by TaqMan®, DDFlu and VI. The TaqMan® test was able to detect influenza viral RNA in 12 samples that were negative by DDFlu. All 12 of these TaqMan® positive samples were also positive by either VI or IHC or had microscopic lesions characteristic of influenza virus infection. 8 samples were positive by TaqMan® and negative by DDFlu, and all 8 were negative by IHC and had non-specific broncho- or interstitial pneumonia. Both the TaqMan® and DDFlu tests failed to detect influenza in 2 samples that were positive by IHC and had microscopic lesions characteristic of influenza virus infection. The remaining 429 samples were negative by both TaqMan® and DDFlu, with no additional VI or IHC test results. The diagnostic sensitivity and specificity for the TaqMan® test were 97% and 98%, respectively. The diagnostic sensitivity and specificity for the DDFlu test on the same samples were 77% and 100%, respectively.

Nasal swabs were also submitted to the MVDL during this time for influenza detection. The number of submitted with the necessary confirmatory tests (VI) completed in addition to TaqMan® and DDFlu were few (n=32). Nevertheless, the diagnostic sensitivity and specificity for the TaqMan® was 100% and 95%, respectively. The diagnostic sensitivity and specificity for DDFlu on the same samples was 73% and 100%, respectively. Further validation of the TaqMan® PCR test on nasal swabs is ongoing.

The influenza A virus NP TaqMan® RT-PCR test is a sensitive and specific method for detecting influenza virus from swine lung samples and bronchial swabs. This test provides increased sensitivity, timely results, and will facilitate additional attempts to subtype and sequence viral nucleic acids from samples.

References

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