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Real-Time RT-PCR Detection of Influenza A Virus in Asymptomatic Culture-Negative Cotton Rats

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Background: A highly sensitive and specific set of real-time RT-PCR (rRT-PCR) assays for point of care detection of influenza virus would facilitate patient care and decrease costly evaluations. In a pandemic setting, rapid detection of infected individuals would enhance intervention and prevent widespread dissemination of disease in the community. The cotton rat is an established model for the study of influenza pathogenesis that could be used to investigate the ability of rRT-PCR to detect influenza in a controlled setting. **Objective**: This study compared the sensitivity of 'gold standard' culture to the recently developed PrimeMix real-time RT-PCR System for the detection of influenza virus in cotton rats. **Methods**: Cotton rats (primary infected) were inoculated intranasally with 10^7 $TCID_{50}$ influenza A (H3N2) virus and housed with non infected (sentinel) rats. All animals were observed daily for general health, weight and temperature. Nasal and lung tissue homogenates obtained on post infection day 1 & 4 were analyzed using traditional culture and rRT-PCR analysis. **Results**: All primary infected cotton rats (n=15) became ill and culture-positive influenza virus was detected from all nasal (6/6) and lung (6/6) samples (12/12) on Day 1 post infection. On Day 4 only nose samples (9/9), and no matched lung samples (0/9) were influenza culture-positive. Using rRT-PCR, influenza was detected from all nose (15/15) and 14/15 lung homogenates from primary infected rats on Day 1 and 4. Sentinel animals (n=10) were asymptomatic and all nose (0/10) and lung samples (0/10) on Day 1 and 4 were influenza culture-negative. However, using rRT-PCR, influenza was detected (limit of detection $\leq$ 10 RNA copies) in 8/10 lung and/or nose specimens from sentinel animals. Sentinel rats had elevated influenza antibody levels by 28 days post infection. **Conclusions**: PrimeMix rRT-PCR can detect influenza A/B and H3/H1/H5 virus that is below culture detection limits in less than two hours. This could be valuable for early therapeutic intervention for high risk populations and to decrease spread of infection during a pandemic. Studies are currently underway to evaluate these diagnostic assays for point of care detection in children and families.