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QUANTITATION OF INFLUENZA A VIRUS FROM NASAL AND LUNG TISSUE OF COTTON RATS USING REAL-TIME RT-PCR AND CULTURE

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An influenza real-time RT-PCR (rRT-PCR) assay for point-of-contact detection would be faster than traditional culture and may enhance intervention and prevent widespread dissemination of virus. The cotton rat is an established influenza model that could be used to study transmission/dissemination of virus among primary infected and non-infected animals. This study compared quantitative sensitivities of culture and rRT-PCR for detecting influenza virus in lung and nasal tissue from primary infected and sentinel (non-infected) cotton rats. Cotton rats (primary infected) were inoculated intranasally with 10^7 $TCID_{50}$ influenza A (H3N2) virus and housed with non- infected (sentinel) rats. Nasal and lung tissue homogenates obtained on post infection day 1,4,10,21, and 28 were analyzed using quantitative culture and rRT-PCR. All primary infected cotton rats (N=36) became ill and culture-positive influenza virus was detected from all nasal (6/6) and lung (6/6) samples on Day 1 post-infection. On Day 4 primary infected nose samples (9/9) but no matched lung samples (0/9) were culture-positive, and thereafter (Day 10,21,28) all primary-infected lung and nasal samples were culture-negative. All sentinel (non-infected) rats (Day 1-28) were culture-negative. However, rRT-PCR detected influenza in lung and nasal tissue from primary-infected and non-infected animals at times when culture was negative and at levels < 10 viral copies. The rRT-PCR method described here is rapid (<2 hours), more sensitive than traditional culture and could be valuable for point-of-care patient influenza detection.