

## Isolation of avian influenza virus from reverse transcription polymerase chain reaction–negative cloacal samples of waterfowl

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Avian influenza virus (AIV) is one of the most important zoonotic pathogens because of its potential risk to cause severe disease outbreaks in avian and human hosts. Virus isolation in embryonated chicken eggs (ECEs) remains a gold standard technique for AIV detection. However, some laboratories prefer molecular methods such as qRT-PCR for initial sample screening because of their high throughput sample processing and rapid results. Samples found positive on qRT-PCR are then inoculated in ECEs for virus isolation and characterization. This approach is based on the premise that qRT-PCR will detect all AIV-positive samples. The current study aimed to determine if AIV can be isolated from cloacal samples of waterfowl that were initially found to be negative by qRT-PCR screening. Quantitative RT-PCR–negative cloacal samples (n=1,369) were inoculated for virus isolation in commercial non-specific pathogen-free ECE. After 4 days of incubation, the allantoic fluids were harvested and inoculated in fresh ECE for a second passage. Allantoic fluids from 147 samples were positive for hemagglutination (HA) with chicken erythrocytes. Of the 147 HA-positive allantoic fluids, 82 were AIV positive when confirmed with qRT-PCR. Ten isolates were subtyped as H7N2 (n = 7), H7N1, H1N2, and H2N2. In addition, N subtype could be determined for isolates from an additional 25 samples. These results highlight the fact that screening by qRT-PCR may result in some false-negative cloacal samples for AIV. Results of the current study indicate that it may not be necessary to use costly SPF eggs unless an AIV vaccine is used in the commercial flocks.

### Biography

Dr. Sagar M. Goyal is a professor of virology at the Veterinary Diagnostic Laboratory and the department of Veterinary Population Medicine, University of Minnesota. M. El Zowalaty is a Ph D student waiting for graduation at Veterinary Diagnostic Laboratory on a scholarship to pursue doctoral research and an assistant lecturer at department of microbiology, Zagazig University, Egypt.