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Development of Methods for Rapid Assessment of Influenza Virus in Avian Environmental Samples

E. S. Clark1, L. T. Daum2, E. W. Alm1;

[1]Central Michigan University, Mount Pleasant, MI, [2] Longhorn Vaccines and Diagnostics, San Antonio, TX.

Migratory waterfowl infected with Avian Influenza Virus (AIV) shed high titers of virus in their feces, and AIV shed into the environment can persist for substantial periods of time under certain conditions. Surface waters contaminated by infected birds are a major route of transmission within bird populations, and may act as a seasonal reservoir for the virus. Human use of recreational areas contaminated by infected birds raise concerns of potential human transmission. Therefore monitoring potentially impacted sites should be considered and methods for rapid assessment of AIV in environmental samples developed. The objective of this study was to develop and optimize viral RNA collection and extraction protocols specific for environmental samples for detection using real-time RT-PCR (rRT-PCR). Droppings from waterfowl were collected at migratory stopovers and human recreational sites along the west coast of Lake Huron. Viral RNA was extracted from samples and then subjected to rRT-PCR using a previously developed Influenza A matrix gene assay specific for all sixteen (H1-H16) influenza A hemagglutinin (HA) subtypes. A novel method of extraction from feces was developed to reduce PCR inhibition and improve sensitivity of detection. Samples were placed directly in viral transport media (VTM), vortexed, and centrifuged. The resulting supernatant was analyzed in downstream applications with improved detection compared to no centrifugation. Preliminary processing has indicated Influenza A virus in 23 of 95 bird droppings. Feces collected during the Fall migration period contained more type A Influenza positive samples than feces collected during other times of year. These results indicate that environmental sampling could be a cost effective method of monitoring circulating strains of Influenza within bird populations. All samples that are positive for type A Influenza will be further characterized with HA specific primers and subjected to sequence analysis.