

Improved detection of influenza A virus from blue-winged teals by sequencing directly from swab material

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Abstract: The greatest diversity of influenza A virus (IAV) is found in wild aquatic birds of the orders Anseriformes and Charadriiformes. In these birds, IAV replication occurs mostly in the intestinal tract. Fecal, cloacal, and/or tracheal swabs are typically collected and tested by real-time RT-PCR (rRT-PCR) and/or by virus isolation in embryonated chicken eggs in order to determine the presence of IAV. Virus isolation may impose bottlenecks that select variant populations that are different from those circulating in nature, and such bottlenecks may result in artifactual representation of subtype diversity and/or underrepresented mixed infections. The advent of next-generation sequencing (NGS) technologies provides an opportunity to explore to what extent IAV subtype diversity is affected by virus isolation in eggs. In the present work, we evaluated the advantage of sequencing by NGS directly from swab material of IAV rRT-PCR-positive swabs collected during the 2013-14 surveillance season in Guatemala and compared to results from NGS after virus isolation. The results highlight the benefit of sequencing IAV genomes directly from swabs to better understand

subtype diversity and detection of alternative amino acid motifs that could otherwise escape detection using traditional methods of virus isolation. In addition, NGS sequencing data from swabs revealed reduced presence of defective interfering particles compared to virus isolates. We propose an alternative workflow in which original swab samples positive for IAV by rRT-PCR are first subjected to NGS before attempting viral isolation. This approach should speed the processing of samples and better capture natural IAV diversity. OPEN RESEARCH BADGES: This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at <https://doi.org/10.5061/dryad.3h2n106>.