Environmental Monitoring of Avian Influenza Virus in Scotland: Insights into Molecular Evolution and One Health Dynamics

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Avian Influenza Virus (AIV) is a highly diverse and rapidly evolving pathogen with significant implications for animal and public health. Wild waterbirds, particularly Anseriformes and Charadriiformes, act as natural reservoirs, facilitating viral maintenance and transmission across ecosystems. However, traditional surveillance systems—largely based on direct sampling and opportunistic carcass detection—face logistical, financial, and biosecurity constraints, potentially limiting early detection and underestimating viral circulation.

Here, we evaluate environmental sampling as a complementary, non-invasive surveillance strategy to improve AIV detection in wild bird populations. Between 2024 and 2026, a total of 3,500 environmental samples (faecal, sediment, and water) were collected from 20 sites across Scotland, targeting key habitats of Anseriformes and Charadriiformes, alongside opportunistic carcass sampling. RNA was extracted and screened using a nested PCR assay targeting the AIV matrix gene (Segment 7), followed by Sanger sequencing. Selected positive samples were further analysed using whole-genome sequencing with Oxford Nanopore Technology.

AIV RNA was detected across multiple sites and environmental matrices, with detections occurring at different time points, including periods of increased viral activity. These findings highlight the ability of environmental surveillance to capture otherwise undetected viral circulation. Faecal sampling yielded the highest detection success and proved operationally efficient, while consistent longitudinal sampling was critical for viral recovery and downstream genomic analyses. Whole-genome sequencing enabled recovery of viral genomic data, although with variable segment coverage. Phylogenetic analysis showed that environmental sequences clustered with recently reported sequences in Scotland, indicating the presence of currently circulating reassortant viruses in environmental samples.

These findings demonstrate that environmental sampling can enhance existing AIV monitoring systems by providing a scalable, non-invasive early warning tool. Integrating environmental and wildlife surveillance within a One Health framework improves disease monitoring, supports phylogenetic and epidemiological analyses, and strengthens preparedness for emerging zoonotic threats at the human–animal–environment interface.