HPAI in Germany since 2016 - Sounding the power of subtype-specific RT-qPCR and the limits of molecular epidemiology

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Abstract

Repeated incursions of gs/GD clade 2.3.4.4b subtype H5 high pathogenicity avian influenza viruses (HPAIV) to Germany have caused the most severe epizootic outbreaks among wild birds and poultry in Germany since the 1930s. Different subtypes and multiple reassortants were identified triggering challenges for a rapid sub- and pathotype-specific diagnosis. While the H5 hemagglutinin (HA) and particularly its endoproteolytic cleavage site showed a substantial degree of conservation, there was a wide variation of neuraminidase (NA) subtypes (N1, N3, N4, N5, N6, and N8) among the circulating HPAIVs in Germany.

Based on previously developed real time RT-PCRs (RT-qPCR) for the detection of HA and NA subtypes of AIV (Hoffmann et al., 2016), and for H5 pathotype definition (Naguib et al., 2018) we extended and updated a set of RT-qPCRs covering all 16 HA and 9 NA subtypes (Hassan, Ahrens et al., in preparation). Block combination of up to six selected RT-qPCRs from these sets were used for examining more than 3,500 suspicious samples. The majority rapidly yielded reportable sub- and pathotype-specific results already in the first round of assays. Samples that harboured AIVs of non-H5 subtypes were subjected to the full set of RT-qPCRs.

Suspected lateral transmission among commercial poultry holdings required fast genome analysis to flank field epidemiological investigations of incursion and outbreak connections. Here, a sequenced-based approach (King et al., 2020) combined routine AIV sequencing with a bioinformatics workflow, including network analyses, phylogenetic and -geographic classification of H5Nx subtypes. Multiple regionally and temporally restricted phylogenetic clusters were identified, allowing the definition of a close epidemiological relationship of some of the poultry outbreak series. However, actual sources of incursions, modes and directions of transmission chains could not be unambiguously defined for individual holdings. In this respect, molecular epidemiology was more successful in excluding epidemiological links between neighbouring outbreak holdings.

References

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