A43 Modeling the ecology and evolution of H13 and H16 avian influenza A subtypes in black-headed gulls to understand influenza disease dynamics

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Wild birds of the orders Anseriformes (ducks, geese, swans) and Charadriiformes (gulls, terns, shorebirds) are the natural hosts for all avian influenza A viruses and act as the reservoir source for influenza viruses that can cause epidemics and even pandemics in mammalian hosts. The ecology, epidemiology, and evolution of influenza A viruses in wild migrating birds are still poorly understood due to extreme complexity resulting from numerous virus host species that are hard to study during part of their annual cycle and infection with multiple virus subtypes. To increase our basic understanding of avian influenza A virus (AIV) epidemiology, evolution, and ecology, we will use viruses of the H13 and H16 subtypes in black-headed gulls (*Chroicocephalus ridibundus*) as a model system. Black-headed gulls are an ideal model species to increase this understanding owing to the fact that they are fairly easy to study year-round, are only infected routinely with two subtypes (H13 and H16), and are affected by annual epidemics in breeding colonies. Since 2006, black-headed gulls have been intensely sampled for influenza A viruses during the breeding period in four breeding colonies in the Netherlands (Griend, Blauwe Stad, De Kreupel, Veluwemeer; >5,000 samples) and year-round out with the breeding colonies (>8,000 samples). This has provided evidence of annual peak prevalence spikes of H13 and H16 virus infections in first-year birds (mostly fledglings) on the breeding colonies but low prevalence outside of the breeding period in fledged birds and in adults. To date, of a total of 258 H13 and 129 H16 virus isolates, we have sequenced 125 viruses using next generation sequencing methods. We anticipate that the sequence data, ecological data, and additional metadata, along with state-of-the-art phylogenetic analyses will lead to the development of the first quantitative epidemiological models for AIV in gulls —a first step towards modeling influenza viruses in other wild bird species such as ducks.

A44 Determination of highly conserved sites by deep sequencing in avian influenza A virus H5N1

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Avian influenza A viruses (AIAV) can evolve rapidly. It is believed that the error-prone viral RNA dependent RNA polymerase generates random mutations that form a viral population known as the viral quasispecies. There is ample evidence that AIAV quasispecies can adapt quickly to selection pressures, e.g. host antibody mediated immunity. However, information of how an AIAV population is maintained in a non-selective environment is limited. Such information is important for understanding the viral endogenous factors that restrict the viral evolution. In this study, we applied next generation sequencing (NGS) technology to determine constraint genome positions of two H5N1 isolates cultured in chicken embryos. The two isolates were both highly pathologic to avian hosts, but displayed high or mild severities in mice. We firstly assembled the H5N1 genomes by using the NGS (Illumina Hi-Seq) short reads. After filtering sites based on read quality and coverage, we focused on analyzing conserved sites at both within-population and between-population levels. The two viral isolates displayed statistically significant differences in genome wide patterns of nucleotide conservation. Publically available H5N1 sequences were used to validate the nucleotide conservation at higher phylogenetic levels. In total, 23 sites in five segments (PB2, PB1, PA, HA, NA, and MP) were determined for their extreme constraint of nil polymorphism within-population and beyond. One of these sites has been described for playing a role the activity of polymerase complex. Their highly constrained characteristics highlighted their importance. Functions of the rest of the sites (22 out of 23) were not clear. It is unclear why these sites maintained their clonal integrity.

A45 Family clusters of avian influenza A H7N9 infection in Guangdong Province, China

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Since its first identification, the epizootic avian influenza A H7N9 has persisted in China. Two waves were observed during this outbreak. No cases were reported from Guangdong province during the first wave but this province became one of the prime outbreak sites during the second wave. In order to identify the transmission potential of this continuously evolving infectious virus, our research group monitored all clusters of H7N9 infections during the second wave of the epidemic in Guangdong province. Epidemiologic, clinical, and virological data on these patients were collected and analyzed. Three family clusters including six cases of H7N9 infection were recorded. The virus caused severe disease in two adult patients but only mild symptoms for all the four pediatric patients. All cases reported direct poultry or poultry market exposure history. Relevant environmental samples collected according to their reported exposures tested H7N9 positive. Virus isolates from patients in the same cluster shared high sequence similarities. In conclusion, although continually evolving the currently circulating H7N9 viruses in Guangdong province have not yet demonstrated the capacity for efficient and sustained person-to-person transmission.

A46 MERS-CoV in Arabian camels in Africa and Central Asia

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