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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Middle East Respiratory Syndrome Coronavirus (MERS-CoV) causing infections in humans is genetically indistinguishable from the virus found in Arabian camels (dromedaries) in the Middle East. Although no primary human case of MERS was reported outside the Arabian Peninsula, camel populations in Africa are known to have high prevalence of antibodies against MERS-CoV. We carried out surveillance for MERS-CoV in dromedaries in Africa and Central Asia. By MERS-CoV spike pseudoparticle neutralization assay we confirmed that camel serum samples from African countries have high prevalence of MERS-CoV antibodies. Using RT-qPCR we detected MERS-CoV positives in camel nasal swabs from all different African countries from which samples were collected. However, dromedary serum and swab samples from Kazakhstan in Central Asia were negative for MERS-CoV by these assays. Phylogenetic analysis of the spike gene revealed that MERS-CoVs from Africa formed a cluster closely related to but distinct from the viruses from the Arabian Peninsula. Results from this study suggest that MERS-CoV is actively circulating in dromedary populations in Africa and the virus in Africa is phylogenetically distinct from that in the Middle East.

A47 Origin and possible genetic recombination of the middle east respiratory syndrome coronavirus from the first imported case in china: phylogenetics and coalescence analysis

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The Middle East respiratory syndrome coronavirus (MERS-CoV) causes a severe acute respiratory tract infection with a high fatality rate in humans. Coronaviruses are capable of infecting multiple species and can evolve rapidly through recombination events. Here, we report the complete genomic sequence analysis of a MERS-CoV strain imported to China from South Korea. The imported virus, provisionally named ChinaGD01, belongs to group 3 in clade B in the whole-genome phylogenetic tree and also has a similar tree topology structure in the open reading frame 1a and -b (ORF1ab) gene segment but clusters with group 5 of clade B in the tree constructed using the S gene. Genetic recombination analysis and lineage-specific single-nucleotide polymorphism (SNP) comparison suggest that the imported virus is a recombinant comprising group 3 and group 5 elements. The time-resolved phylogenetic estimation indicates that the recombination event likely occurred in the second half of 2014. Genetic recombination events between group 3 and group 5 of clade B may have implications for the transmissibility of the virus.

A48 Inference of biological functionality in individual genomic secondary structural elements found within capulavirus genomes

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The seeming simplicity of the iconic DNA double helix is deceptive. The genomes of single-stranded DNA and RNA viruses often contain numerous nucleic acid secondary structures. Whilst a number of these secondary structural elements have been found to play crucial roles during the life cycles of these viruses, the majority have neither any identified function nor known impact on viral fitness and evolution. Secondary structures can be predicted using nearest neighbour free-energy parameters that quantify the stability of a given secondary structure. Using an array of bioinformatic techniques we investigated the influence of inferred secondary structures on the sequence evolution of capulaviruses, a diverse genera of single stranded DNA viruses. We detected a significant association between structured regions of the genome and selective constraints on synonymous substitutions in coding regions. This is suggestive of either natural selection acting to preserve these structures or a predisposition toward lower mutation rates in base-paired regions of the genome. In addition, coevolution analyses revealed a significant tendency for nucleotides that are base-paired in predicted structures to coevolve in a complementary manner. Combined, these results highlight the pervasiveness of conserved genomic secondary structures within capulavirus genomes and support the notion that natural selection is favouring the maintenance of these structures, providing compelling evidence of their likely biological relevance. This structure-first strategy for comparative analysis of genome-wide secondary structures can be broadly applied to understand the contributions of higher-order genome structures to viral replication and pathogenicity.

A49 Molecular evolutionary dynamics of respiratory syncytial virus group A in recurrent epidemics in coastal Kenya

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The characteristic recurrent epidemics of human respiratory syncytial virus (RSV) within communities may result from the genetic variability of the virus and associated evolutionary adaptation, reducing efficiency of pre-existing immune responses. We analyzed the molecular evolutionary changes in the attachment (G) glycoprotein of RSV-A viruses collected over 13 epidemic seasons (2000–12) in Kilifi ($n = 649$), Kenya, and contemporaneous sequences ($n = 1{,}131$) collected elsewhere within Kenya and 28 other countries. Genetic diversity in the G gene in Kilifi was dynamic both within and between epidemics, characterized by frequent new variant introductions and limited variant persistence between consecutive epidemics. Four RSV-A genotypes were detected in Kilifi: ON1 (11.9%), GA2 (75.5%), GA5 (12.3%), and GA3 (0.3%), with predominant genotype replacement of GA5 by GA2, then GA2 by ON1. Within these genotypes, there was considerable variation in potential N-glycosylation sites, with GA2 and ON1 viruses showing up to 15 different patterns involving eight possible sites. Further, we identified 15 positively selected and 34 genotype-distinguishing codon sites, with six of these sites exhibiting both characteristics. The mean substitution rate of the G ectodomain for the Kilifi dataset was estimated at 3.58×10^{-3} [95% HPD: 3.04–4.16] nucleotide substitutions/site/year. Kilifi viruses were interspersed in the global phylogenetic tree, clustering mostly with Kenyan and European