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Frequency and patterns of reassortment in natural influenza A virus infection in a reservoir host

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ABSTRACT

Influenza A viruses (IAV) can dramatically alter both genotype and phenotype at a rapid rate as a product of co-infection and reassortment. Avian IAV exhibit high levels of phylogenetic incongruence, suggesting high levels of reassortment in the virus reservoir. Using a natural-experimental system, we reconstructed relationships amongst 92 viruses across 15 subtypes from 10 Mallards in an autumn season. Phylogenetic analyses estimated that 56% of the isolated viruses were reassorted. Network analysis demonstrated different patterns of reassortment and limited exchange of segments between primary and secondary infections. No clear patterns of linkage between segments were found, and patterns within a season were likely the consequence of continued introduction of new constellations, high viral load and diversity in the wild bird reservoir, and co-infections. This is the first IAV study to implement multiple tools available for elucidating factors governing reassortment patterns in naturally infected Mallards.

Introduction

Influenza A viruses (IAV) belong to the family *Orthomyxoviridae*, and are enveloped viruses with a genome consisting of eight segments of negative-sense single-stranded RNA (Kawaoka et al., 2005; Webster et al., 1992). IAV exhibit high mutation rates due to an error prone RNA-dependent RNA polymerase and, consequently, a rapid rate of change in antigenic proteins. Avian IAV evolves at the same rate as mammalian influenza viruses (Chen and Holmes, 2006, 2009), and the eight segments have multiple evolving lineages (Bahl et al., 2009; Chen and Holmes, 2009, 2010; Dugan et al., 2008). Reassortment, the process in which new virus variants can arise through exchange of the RNA segments in co-infected cells, results in the propensity for IAV to dramatically alter both genotype and phenotype by combining segments of different origin in new combinations (Rambaut et al., 2008; Salomon and Webster, 2009; Webster et al., 1992). By reassortment, novel viral strains can appear rapidly, and if these introductions contain genomic segments determining antigenicity (particularly the surface proteins hemagglutinin [HA] and neuraminidase [NA]) from distantly related strains, it may provide new strains the opportunity to proliferate among immunologically naïve hosts (Webster et al., 1992). IAV have a broad host range, and viruses infecting humans, pigs, horses and wild birds form different phylogenetic clades across all segments (Olsen et al., 2006; Webster et al., 1992). In avian lineages, additional genetic distinction is evident, whereby viruses isolated from North America, Eurasia and South America have different genetic and antigenic lineages (Olsen et al., 2006; Pereda et al., 2008). Although these distinctions exist, it has been shown that the PB1, PA and H6 segments currently circulating in North American wild birds have an Eurasian origin (Bahl et al., 2009; zu Donha et al., 2009). Not only can IAV exchange segments within a host group, but host-specific viruses can also reassort following co-infection, providing the capacity to alter host range. For instance, reassortment between human, avian and swine lineages have resulted in viruses with pandemic potential, and have been implicated in the last three human IAV pandemics (Lindstrom et al., 2004; Rabadan et al., 2006; Scholtissek et al., 1978; Taubenberger et al., 2005).

Wild waterfowl and shorebirds (including gulls) are the main avian reservoir hosts for IAV. In these hosts there are many co-circulating HA/NA subtypes, high levels of co-infection in single animals (Sharp et al., 1993) and high level of reassortment (Bahl et al., 2009; Chen and Holmes, 2006, 2010; Dugan et al., 2008).

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However, neither the specific pattern, nor the scope of co-infection and reassortment has been adequately assessed. One hurdle has been the lack of automated tools and computational limitations to deal with high levels of phylogenetic complexity across eight segments. A second problem is the lack of robust data. Indeed, to date, studies assessing reassortment in wild bird populations largely utilize multiyear data, often collected across large spatial scales and across multiple host species (Bahl et al., 2009; Chen and Holmes, 2006, 2010; Dugan et al., 2008). Multiyear data does not allow the detailing of specific patterns of reassortment in the short term, nor does it illustrate which factors are mechanistically important. It has been shown that the likelihood of segments being incorporated through reassortment varies, and that genetic linkages between different segments do occur in human and swine IAV (Downie, 2004; Khiabanian et al., 2009; Varich et al., 2008). Unlike IAV dynamics in these hosts, very high levels of incongruence have been demonstrated in analyses of low pathogenic avian IAVs (Bokhari and Janies, 2010; Dugan et al., 2008; Lam et al., 2011). Further, despite investigations utilizing sequence data from field isolates combined with laboratory assessments using controlled infections of cell lines, results pertaining to the non-random nature of reassortment are not consistent in avian IAV.

In the present article, we address the process of IAV reassortment on a short time scale in natural infections in a reservoir host, the Mallard (*Anas platyrhynchos*). We studied the IAV infection patterns in 10 immunologically naïve sentinel Mallards in a wild setting in Sweden over the course of a full autumn season. The birds were kept in contact with wild waterfowl; the sentinels shared water with, and were exposed to aerosols generated by wild Mallards entering and feeding within a duck trap. Hence, these 10 ducks represent a probe to illustrate the major patterns of introduction, extinction, reassortment, and maintenance in the viral reservoir. Using this experimental set up, with a daily sampling regime, we aimed to assess the (a) the order of infections and reinfections, (b) overall magnitude and scope of reassortment, (c) the carry-over of segments and viruses between primary and secondary infections, (d) disentangle the involvement of antigenic HA and NA combinations, (e) linkage between different segment combinations, and (f) the putative role of co-infection in our dataset.

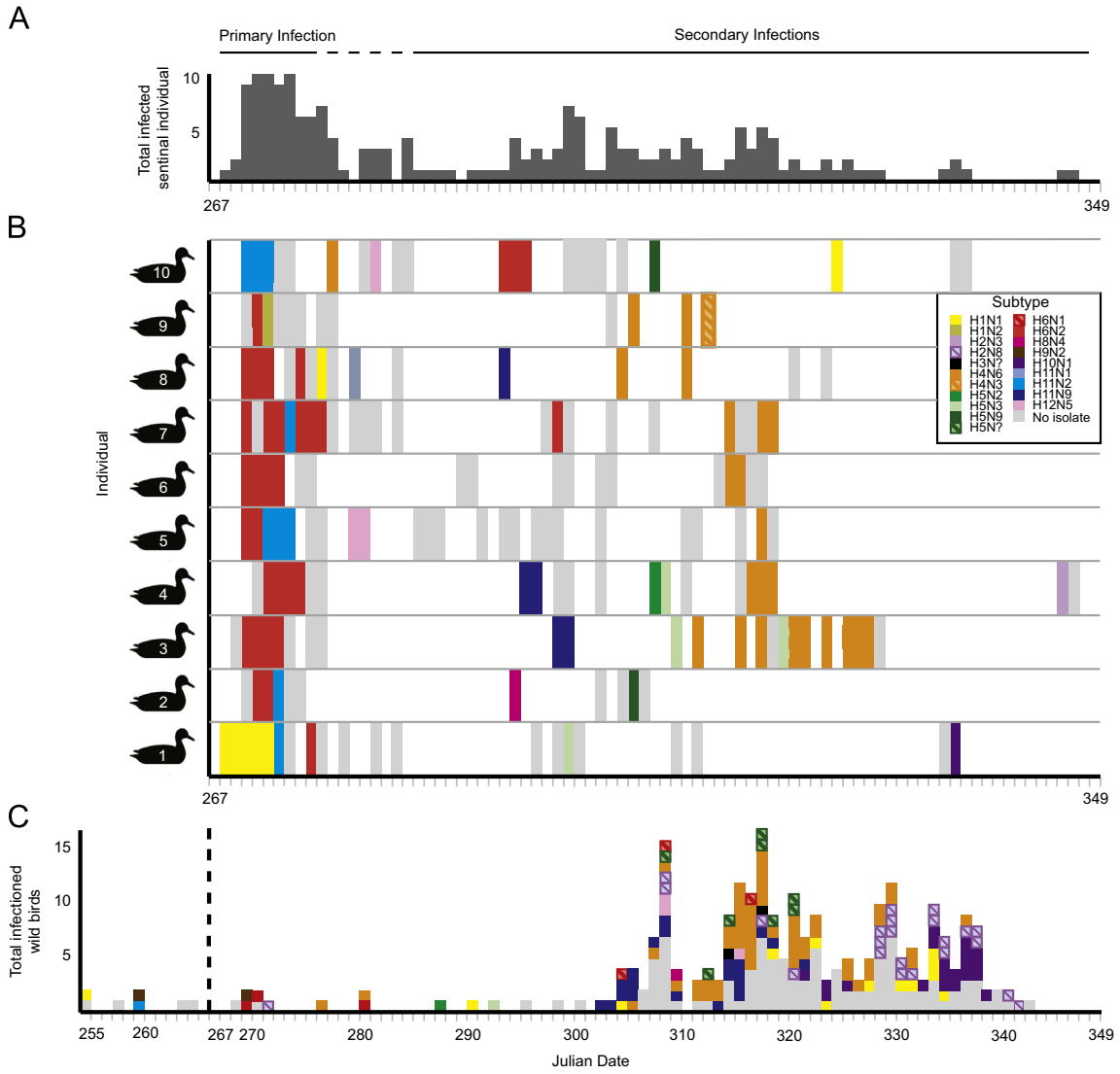

Fig. 1. Influenza A viruses detected and isolated from Mallards between 24 September and 15 December 2009 (Julian day 267–349). (A) Total number of infections per day by determined by rRT-PCR in the 10 sentinel ducks placed in the trap. (B) Detailed infection history for each sentinel duck. (C) Total number of viruses detected and isolated from the wild ducks during the period of study. Days where no infection was detected are in white, where infection was detected by rRT-PCR but no virus was isolated in culture are in gray, and where virus was detected and isolates grew in culture are colored by subtype.