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Evolutionary pathway for the 2017 emergence of a novel highly pathogenic avian influenza A(H7N9) virus among domestic poultry in Tennessee, United States

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ABSTRACT

In March 2017, a novel highly pathogenic avian influenza A(H7N9) virus was detected at two commercial broiler breeder facilities in Tennessee, United States. In this study, a wild bird low pathogenic avian influenza A virus, A/blue-winged teal/Wyoming/AH0099021/2016(H7N9), was shown to be the probable precursor of the novel H7N9 virus; this low pathogenic virus has eight possible progenitor genes sharing > 99% sequence identity with the novel H7N9 virus. Phylogeographic analyses showed that viral gene constellations that formed and circulated among dabbling ducks contributed to the emergence of the novel H7N9 virus. This is in contrast to the virus that caused the 2016 H7N8 outbreak, which had more genetic contributions from viruses circulating among diving ducks. Study findings support the need for ongoing wild bird surveillance to monitor circulating viruses and to understand possible evolutionary pathways of virus emergence in poultry.

1. Introduction

Influenza A viruses (IAVs) have been recovered from variety of bird species, including at least 105 species of 26 different families (Olsen et al., 2006). Among wild birds, those of wetland and aquatic environments, such as birds in the order *Anseriformes* (particularly ducks, geese, and swans) and those in the order *Charadriiformes* (particularly gulls, terns, and waders) constitute the major natural IAV reservoir (Webster et al., 1992). These birds maintain a large IAV genetic pool, which contributes to the appearance of new IAVs in humans, lower mammals, and domestic poultry and other birds. Many *Anseriformes* and *Charadriiformes* birds perform regular long-distance migrations (Anonymous, 1996), and they are distributed globally, except in the most arid regions of the world (Anonymous, 1996). Wild bird migration facilitates virus transmission and genetic diversity through reassortments and adaptations among IAVs from different geographic locations.

It is well documented that wild birds have been the predominant source of IAVs detected in domestic poultry, and introductions of IAVs from wild birds to domestic poultry occasionally causes outbreaks among domestic poultry. Understanding the evolutionary pathway for emerging IAVs detected in domestic poultry, especially learning which wild bird species are involved in these introductions, will help us understand the natural history of IAVs and provide potential evidence to support the development of strategies for avian influenza surveillance among wild birds. Through retrospective analyses, Krauss and colleagues proposed shorebirds, gulls and dabbling ducks as the source of IAV introduction into domestic poultry in North America (Krauss et al., 2004, 2015). Through phylogeographic analyses, Xu et al. (2017) suggested that viral gene constellations circulating among diving ducks can contribute toward the emergence of H7N8 IAVs that affect poultry, and Li et al. (2018) indicated that, from 2001 to 2017, dabbling ducks and geese/swans could have facilitated emergences of the H5 IAVs associated with 18 individual introductions into domestic poultry in the United States. These studies suggested that certain species of wild bird may serve an important role in the maintenance, diversification, and transmission of IAVs in the wild bird reservoir.

After being introduced into domestic poultry, subtype H5 and H7 low pathogenic avian influenza (LPAI) viruses can mutate into highly pathogenic avian influenza (HPAI) viruses, often through acquisition of basic amino acids in the cleavage region of the HA protein by insertion or substitution (Garcia et al., 1996) or through insertion of a long peptide sequence in the cleavage region of the HA protein from

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recombination with another gene segment(s) or host genome (Suarez et al., 2004; Pasick et al., 2005; Maurer-Stroh et al., 2013). However, whether and how a subtype H5 or H7 IAV would mutate from LPAI virus to HPAI virus cannot yet be predicted.

In March 2017, subtype H7N9 HPAI virus was detected in a commercial broiler breeder facility in Lincoln County, Tennessee, United States, with a single event of secondary spread within company. Subsequent surveillance detected H7N9 LPAI virus in Tennessee, as well as neighboring states of Alabama, Kentucky, and Georgia (Lee et al., 2017; USDA-APHIS, 2017). Nomenclature introduced in a previous study referred to the first identified HPAI virus as Commercial Poultry Farm (CPF)-TN-2017(H7N9) (Li et al., 2018). Through genomic analyses, a prior report showed that it is probable that a H7N9 LPAI virus mutated to H7N9 HPAI virus after being introduced from wild birds to the commercial broilers in Tennessee (Lee et al., 2017; USDA-APHIS, 2017). However, the origin and evolution of wild bird virus precursors to CPF-TN-2017(H7N9), and which wild bird species contributed to such emergence is unclear. The aim of our study was to use phylogenetic and phylogeographic analyses to identify progenitor genes associated with CPF-TN-2017(H7N9), to illustrate the evolutionary pathway for the emergence of this virus, and to identify probable wild bird species that may contribute to its emergence.

2. Results

2.1. H7N9 HPAI viruses detected in Tennessee originated from IAVs in North American wild birds

In this study, a total of eight H7N9 viruses were analyzed. Of these, three were LPAI isolates [A/chicken/Tennessee/17-007429-7/2017(H7N9), A/chicken/Tennessee/17-007429-3/2017(H7N9), and A/chicken/Tennessee/17-007431-4/2017(H7N9)], and five were H7N9 HPAI isolates representing the two affected premises: A/chicken/Tennessee/17-007147-3/2017(H7N9), A/chicken/Tennessee/17-007147-2/2017(H7N9), A/chicken/Tennessee/17-007147-5/2017(H7N9), A/chicken/Tennessee/17-007147-1/2017(H7N9) from the first premise, and A/chicken/Tennessee/17-008279-4/2017(H7N9) from the single event of secondary spread]. Sequence analyses showed that there are 98.13–100.00% nucleotide sequence identity between HA genes of the H7N9 LPAI viruses and those of H7N9 HPAI viruses, 99.58–100.00% nucleotide sequence identity between the NA genes of the H7N9 LPAI viruses and those of H7N9 HPAI viruses, and 99.72–100.00% nucleotide sequence identity between six other genes of H7N9 LPAI viruses and those of H7N9 HPAI viruses. These viruses were collectively referred to as H7N9/TN.

Consistent with findings in a prior study (Lee et al., 2017), our phylogenetic analyses of the HA, NA, and six internal genes suggested that these H7N9/TN viruses belong to the North American lineage and share high nucleotide sequence identity (> 99%) with A/blue-winged teal/Wyoming/AH0099021/2016(H7N9) across all eight genes. Further analyses showed that the HA gene of H7N9/TN belongs to sublineage 3 (Fig. S1), which also includes contemporary H7 viruses that are circulating among wild birds in North America and that have caused sporadic infections among US domestic poultry since 1996 (Xu et al., 2017). These H7N9/TN viruses are not genetically related to the enzootic H7N9 viruses in China nor to the enzootic H7N3 viruses in Mexico (Fig. S1).

Molecular clock analyses suggest that the mean time to most recent common ancestor for H7N9/TN was during the 2016–17 fall–winter wild bird migration season (October 1, 2016–January 14, 2017) (Table 1). The mean evolutionary rate of the H7 gene was 6.19×10^{-3} substitutions per site per year (subs/site/year) (95% highest posterior density = 5.62×10^{-3}–6.80×10^{-3} subs/site/year), which is significantly higher than that for other gene segments (range, 2.58×10^{-3}–4.00×10^{-3} subs/site/year) (Xu et al., 2017).

2.2. Evolutionary pathways for emergence of H7N9/TN

Our genotype analyses identified a single genotype for H7N9/TN HPAI viruses, and, as described above, A/blue-winged teal/Wyoming/AH0099021/2016(H7N9) had the same genotype; thus, this LPAI virus is a probable precursor virus for H7N9/TN. We further investigated possible genetic events that contributed to generation of this H7N9 precursor virus in wild birds. Examination of the eight gene segment–specific phylogenetic trees indicated that a group of H11N9 viruses isolated from dabbling ducks (referred to as H11N9–DD) in 2015 have three or four genetic segments, including the N9 gene, that are genetically close to those of the emerging A/blue-winged teal/Wyoming/AH0099021/2016(H7N9) virus. Furthermore, a group of H7N3 viruses isolated from dabbling ducks (referred to as H7N3–DD) in 2015 have three to four genes, including the HA gene, that are genetically close to genes of the emerging H7N9 virus. Thus, the genetic makeup of A/blue-winged teal/Wyoming/AH0099021/2016(H7N9) seems to have been derived from H7N3-DD and H11N9-DD (Figs. 1 and 2).

We performed phylogenetic analyses to identify possible genetic connections between the H7N9/TN viruses and two H7N9 low pathogenic avian influenza viruses, A/mallard/Minnesota/AI09-3770/2009(H7N9) (abbreviated as H7N9/MN) and A/goose/Nebraska/17097-4/2011 (H7N9) (abbreviated as H7N9/NE), from two most recent cases [BYP-MN-2009(H7N9) and BYP-NE-2011(H7N9)] detected in domestic poultry of the United States. Results showed only four gene segments of H7N9/MN and that H7N9/NE HA are genetically associated with H7N9/TN. Specifically, HA, NA, and MP genes of both H7N9/MN and H7N9/NE, PB1 of H7N9/MN, and PB2 of H7N9/NE belong to the same genetic clade for the corresponding gene segment of H7N9/TN (Fig. 3). Molecular characterization showed that the sequence identities between HA and NA genes of H7N9/TN and those of H7N9/MN were 95.54% and 97.10%, respectively and that the sequence identities between HA and NA genes of H7N9/TN and those of H7N9/NE were 96.25% and 97.96%.

Spatial analyses from 2011 to 2016 of genetic clusters associated with H7N9 viruses suggested the progenitor gene segments were widely spread across all four North American migratory bird flyways (i.e., the Atlantic, Mississippi, Central, and Pacific Flyways (Fig. 4), and the data are consistent with those of a previous publication that reported a lack of knowledge regarding the geographic patterns for different subtypes of IAV HA (except H5) and NA genes and for internal IAV genes (Li et al., 2018).

2.3. The IAVs from dabbling ducks contributed to emergence of H7N9/TN

To identify the specific wild bird species that contributed to the emergence of viruses associated with H7N9/TN viruses, we characterized the diffusion pathways of IAVs from different host groups. Results from phylogeographic analyses suggested that all gene segments except NP are significantly associated with one functional group of bird species: dabbling ducks (Table 2). Specifically, very strong support (Bayes factor = 189.32) was shown for the transmission of PA gene from dabbling ducks to the chickens in which H7N9/TN virus was detected; strong support was shown for transmission of PB2, PB1, HA, and NA genes (Bayes factor = 12.09, 36.53, 18.29, and 21.56, respectively) from dabbling ducks to the chickens; and support was shown for transmission of MP and NS genes (Bayes factor = 6.79 and 7.88, respectively) from dabbling ducks to the chickens. However, the Bayes factor was not significant to support that the NP gene was transmitted from dabbling ducks or any other wild bird species to the chickens in which H7N9/TN virus was detected.

2.4. H7N9 LPAI virus evolved into an HPAI strain in chickens and has unique insertions from historical outbreaks

With both the LPAI and HPAI H7N9 isolates detected in chicken