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Genetic evidence supports sporadic and independent introductions of subtype H5 low pathogenic avian influenza A viruses from wild birds to domestic poultry in North America

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Genetic evidence supports sporadic and independent introductions of subtype H5 low pathogenic avian influenza A viruses from wild birds to domestic poultry in North America

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Running title: Introduction of H5 LPAIVs into US domestic poultry

Key words: low pathogenic avian influenza; subtype H5; wild birds; domestic poultry; backyard poultry; live bird market; dabbling duck; goose; swan; evolutionary network; reassortment; phylogenetic; United States

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Abstract

Wild bird–origin influenza A viruses (IAVs or avian influenza) have led to sporadic outbreaks among domestic poultry in the United States (US) and Canada, resulting in economic losses through the implementation of costly containment practices and destruction of birds. We used evolutionary analyses of virus sequence data to determine that 78 H5 low pathogenic avian influenza viruses (LPAIVs) isolated from domestic poultry in the US and Canada during 2001–2017 resulted from 18 independent virus introductions from wild birds. Within the wild bird reservoir, the hemagglutinin gene segments of H5 LPAIVs exist primarily as two co-circulating genetic sublineages, and our findings suggest the H5 gene segments flow within each migratory bird flyway and among adjacent flyways, with limited exchange between the non-adjacent Atlantic and Pacific Flyways. Phylogeographic analyses provided evidence that IAVs from dabbling ducks and swans/geese contributed to emergence of viruses among domestic poultry. H5 LPAIVs isolated from commercial farm poultry (i.e. turkey) were descended from a single introduction typically remain a single genotype, whereas those from live bird markets sometimes led to multiple genotypes, reflecting the potential for reassortment with other IAVs circulating within live bird markets. H5 LPAIV introduced from wild birds to domestic poultry represent economic threats to the U.S. poultry industry, and our data suggest that such introductions have been sporadic, controlled effectively through production monitoring and a stamping-out policy, and are, therefore, unlikely to result in sustained detections in commercial poultry operations.

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Importance

Integration of viral genome sequencing into influenza surveillance for wild birds and domestic poultry can elucidate evolutionary pathways of economically costly poultry pathogens. Evolutionary analyses of H5 LPAIVs detected in domestic poultry in US and Canada during 2001–2017 suggest that these viruses originated from repeated introductions of IAVs from wild birds, followed by various degrees of reassortment. Reassortment was observed where biosecurity was low and there were opportunities for more than one virus to circulate existed (e.g. congregations of birds from different premises such as live bird markets). None of the H5 lineages identified were maintained long term in domestic poultry, suggesting that management strategies have been effective in minimizing the impacts of virus introductions on US poultry production.

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72 **Introduction**

73 Influenza A viruses (IAVs) are single-stranded, negative sense RNA viruses with
74 eight genomic segments. Wild waterbirds, especially migratory waterfowl, such as geese
75 and ducks, along with gulls and shorebirds are purported to be the natural reservoir for
76 IAVs of the hemagglutinin (HA) subtypes H1–16 and neuraminidase (NA) subtypes N1–
77 9. IAVs are maintained in wild waterbirds, and those of low pathogenicity result in
78 enteric infections with rare evidence of clinical illness. In contrast, IAVs typically result
79 in respiratory disease in gallinaceous birds, such as chickens and turkeys; clinical signs
80 and disease severity vary and are strain-dependent. Subtypes H5 and H7 low pathogenic
81 avian influenza A viruses (LPAIVs) demonstrate the potential to evolve from low to
82 highly pathogenic avian influenza A viruses (HPAIVs) through increased HA cleavability
83 by acquiring multiple basic amino acids (1-3) or insertions (4-8) at the cleavage site
84 during replication (8, 9). H5 and H7 HPAI causes high mortality among domestic poultry
85 leading to large economic losses.

86 Reassortment is another mechanism that can contribute to the generation of novel
87 and economically costly IAVs in domestic birds. For example, H7N9 and H10N8 IAVs
88 recently identified in China appear to have wild bird–origin HA and NA genes and
89 internal genes from IAVs circulating among domestic poultry (10, 11). Both of these
90 reassortant viruses have been associated with human disease that may have resulted from
91 contact with infected birds at live bird markets (LBMs). A H7N9 IAV that most likely
92 resulted from reassortment of H7, N9, and H9N2 avian IAVs has caused >1,566 human
93 infections and at least 613 deaths in China (11, 12); a H10N8 avian-origin IAV also

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originated from reassortant of H10N8 and H9N2 IAVs in LBMs and caused human infections (10, 13).

In the United States (US) and Canada (collectively referred to as North America for the purposes of this study), poultry production systems include large commercial poultry farms (CPFs), backyard poultry operations (BYPs), game bird poultry farms (GBPs), and LBMs. CPFs are defined as large-scale commercial poultry farms with >1,000 domestic birds per year. BYPs are defined as residential farms raising small flocks of domestic birds; these, typically produce ≤1,000 birds per year. GBPs are poultry operations raising small flocks of game birds, such as pheasants and quail, often released for sport harvest. LBMs are operations that typically supply live birds for on-site slaughter to consumers. Some, such as botanicas, may sell live birds as well. LBMs may acquire birds from both non-CPF and CPF sources. In the US, CPFs are located primarily in the southern and midwestern regions, while many LBMs are located in urban areas of the western and northeastern regions; and BYPs and GBPs are present in all regions (14-16).

Detections of IAVs in domestic poultry are not uncommon (17); wild bird–origin IAVs are usually identified as the source of virus across a variety of North American poultry production systems (3, 9, 17-21). In rare cases, LPAIVs circulating in LBMs have been associated with outbreaks at commercial farms (22). However, there is limited information regarding the evolutionary patterns of IAVs detected in North American poultry. Such information is necessary to improve our collective understanding of the natural reservoirs (location and wild bird taxa) in which viruses that ultimately lead to poultry outbreaks are maintained, how evolutionary mechanisms for IAVs might vary

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between poultry production systems, and the frequency with which IAVs are shared among CPFs, BYBs, GBPs, and LBMs.

In this study, we genetically characterized and compared inferred evolutionary pathways of 78 H5 LPAIVs from the US and Canada during 2001–2017. This study seeks to explore the evolutionary pathways of IAVs that move from wild birds into domestic poultry. Better understanding of viruses at this interface will help improve influenza surveillance and management strategies, reduce economic and animal losses, and decrease opportunities for the generation of novel pathogens including those that can infect humans and cause pandemic influenza threats.

Results

H5 LPAIVs were sporadically detected in CPFs and LBMs. In this study, an isolate is defined as an IAV recovered from wild birds or domestic poultry, and an introduction is defined as a case of IAV infection in domestic poultry; one or more isolates may be recovered from the same introduction. A total of 78 H5 LPAI isolates from domestic poultry in North America during 2001–2017 were included in the study. Isolates were recovered from CPFs (n = 11), BYPs (n = 3), GBPs (n = 2), and LBMs (n = 62) (Table 1). H5 isolates were identified on CPFs in turkey (n = 11, commercial turkey growers are indoor operations with curtain-sides); from BYPs in a mallard duck, a domestic duck and a guinea fowl; from GBPs in a pheasant and a quail; and in LBM samples collected from domestic ducks (n = 42), chicken (n = 7), guinea fowl (n = 6), turkey (n = 1), quail (n = 2), pheasant (n=1), and unknown species (n = 3) (Table 1). Based upon the inferred phylogenetic relationships and nucleotide identities for the HA gene segment, these 78

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H5 poultry isolations result from 18 independent introductions (details in Materials and Methods) (Figure 1A; Figure S1. Among the 18 introductions, 5 were detected in CPFs, 9 in LBMs, 2 from a BYP, and 2 from a GBP (Figure 1A). Each introduction event was identified by the operation type, state, year, and HA/NA subtype from the first isolate; for example 'CPF-WI-2017(H5N2)' denotes an H5N2 event in commercial turkeys from Wisconsin during 2017. Where more than one event occurred in a state within one year, the events are distinguished by a letter; for example 'LBM-NJ-2006(H5N2)a' and 'LBM-NJ-2006(H5N2)b' represent two independent H5N2 events in New Jersey LBMs during 2006.

H5 LPAIVs from US and Canadian domestic poultry are of North American lineage and share genetic ancestry with wild bird–origin IAVs. Geographic lineage was assigned based upon phylogenetic analyses of the HA gene for the 78 H5 LPAIVs; we considered this lineage to be comprised primarily of four distinct sublineages (Figure 1B). A total of 70 of the 78 domestic poultry isolates characterized in this study, as well as the majority of the wild bird–origin H5 LPAIVs identified in the US and Canada from 2001 to 2017 clustered within sublineages 1 and 2. Viruses causing enzootic outbreaks of IAVs among domestic poultry in Mexico are clustered in sublineage 3, whereas IAVs circulating in both wild birds and domestic poultry in the US and Canada during 1966–1990 were grouped in sublineage 4 (Figure 1B). Eight additional H5 subtype IAVs identified in poultry from the US and Canada, along with a relatively small number of wild bird–origin IAVs isolated from samples collected during 1987–2008, clustered in clades not assigned to these four sublineages (Figure 1B).

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We used the relationships inferred by phylogenetic and nucleotide sequence identities to further distinguish genetic groups within sublineages 1 and 2. The 78 H5 isolates from North American poultry clustered into 18 distinct genetic groups. Five genetic groups contained isolates detected in domestic birds sampled on CPFs, nine genetic groups contained isolates detected in birds sampled from LBMs, two genetic groups contained isolates detected in birds sampled from BYPs, and two genetic groups contained isolates detected in birds sampled from GBPs. For most poultry isolates (67%), nucleotide sequence identity at the HA gene segment of H5 LPAIVs was >99% similar to that for wild bird–origin viruses (Figure 1B; Figure S1; Table S1); however, within a genetic group, the poultry isolates were most similar to each other, suggesting a common introduction event with subsequent spread (Figure S1). Based upon this analysis, each of the 18 genetic groups was determined to represent discrete introduction events of IAVs from wild birds to domestic poultry. Additionally, only one production system was affected in each of the 18 inferred introduction events (e.g., CPFs, BYPs, GBPs, or LBMs) (Table 1).

Based upon phylogenetic analyses, the NA and internal gene segments of the H5 LPAI from poultry (including CPFs, LBMs, GBPs and BYPs) were genetically diverse (Figure 2). Phylogenetic analyses also identified potential precursor strains for 15 of the 18 purported introductions; three events were excluded: CPF-CA-2002(H5N2) had an incomplete genome, LBM-NY-2016(H5N2) lacked wild bird progenitor genes and LBM-NY-2007(H5N2) lacked wild bird precursors (Table 2). For 13 of the inferred introductions, potential wild bird progenitor strains shared similar phylogenetic positions in tree topologies with high nucleotide sequence similarity for 3 to 4 gene segments

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(Table 2). Potential wild bird progenitor viruses across all eight gene segments were identified for two purported introductions: a Canada goose (*Branta canadensis*) virus for CPF-MO-2016(H5N1) and an American wigeon (*Mareca americana*) for BYP-OR-2006(H5N2) (Table 2).

Geographic and temporal patterns of H5 LPAIV lineages in North America. Fifteen of the 18 introductions were detected in only one flyway: seven within the Atlantic Flyway (one CPF and six LBM detections), three within the Mississippi Flyway (two CPF and one BYP detection); one in the Central Flyway (a CPF detection); and four within the Pacific Flyway (one CPF, one BYP, and two GBP detections). The remaining three introductions were LBM-associated detections in the Atlantic and Mississippi Flyways. Of the LBM events, eight were genetically related other IAVs detected in Canadian provinces or US states (Table 1).

To further evaluate gene flow for the H5 HA gene segment, we performed phylogeographic analyses for viruses in sublineages 1 and 2. Viruses in sublineage 1, which included viruses associated with 13 introduction events, were predominantly from the Central (5.36%), Mississippi (31.07%), and Atlantic (63.57%) Flyways. Most (81.03%) of the viruses in sublineage 2, associated with three introduction events, were from wild birds sampled within the Pacific Flyway (Figure 1C). Two events were not associated with either lineage 1 or 2: CPF-CA-2002(H5N2), and LBM-NY-2007(H5N2), the latter of which involved seven LPAIVs detected during 2007–2009. Bayesian analyses of viruses in sublineage 1 and 2 indicated unilateral or bilateral state transitions suggestive of H5 HA gene flow among the Atlantic, Mississippi, and Central Flyways

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and among the Mississippi, Central, and Pacific Flyways (Bayes factor >3). However, no state transitions suggestive of H5 HA gene flow were supported between the Atlantic and Pacific Flyways (Bayes factor <3, no significant transition was observed) (Table S2; Figure S2). In summary, phylogeographic analyses suggested H5 HA gene flow across adjacent or nearby flyways but not between the Atlantic and Pacific Flyways.

H5 LPAIVs detected in domestic poultry are likely descendant from those in dabbling ducks or geese/swans. To understand whether specific hosts were associated with virus introductions detected in domestic poultry, we categorized bird host species into 9 functional groups based on taxonomic and ecologic attributes: dabbling duck, diving/sea duck, goose/swan, gull/tern/seabird, raptor, shorebird, other avian, unknown, and poultry (see Materials and Methods section for details). Phylogeographic analyses were performed to estimate Bayes factors between the domestic poultry viruses and viruses from each functional group. A combination of Bayes factor ≥3 and mean indicator ≥0.5 was used as the threshold of statistical significance; a larger Bayes factor indicates a higher probability that a specific functional group is associated with an introduction event (3, 23).

Using phylogeographic analyses, we assessed the genetic origins for 16 of the 18 purported introduction events; two events were excluded: CPF-CA-2002(H5N2) had an incomplete genome, and LBM-NY-2016(H5N2) lacked related wild bird samples. Our results suggest that IAVs in wild waterfowl (dabbling ducks, swans/geese, or diving/sea ducks) were the probable progenitor for at least one viral gene segment for 15 of 16 investigated introduction events; no probable wild bird IAV progenitor was identified for

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LBM-NY-2007(H5N2) (Tables S3 and S4). Neither gulls/terns/seabirds nor raptors were supported as probable sources of gene segments for H5 IAVs detected in domestic poultry, and shorebirds were supported as a probable progenitor for only one gene segment for a single introduction event [MP gene of CPF-VA-2007(H5N1)]. Dabbling ducks, in particular, were associated with numerous gene segments from North American poultry viruses, including all gene segments of viruses involved in the following outbreaks: CPF-WI-2017(H5N2) (Bayes factor 6.59 to 35.12), CPF-MB-2010(H5N2) (Bayes factor 3.24 to 189.74), LBM-NJ-2015(H5N1) (Bayes factor 4.65 to 138.71), LBM-NY-2006(H5N2) (Bayes factor 6.89 to 1,244.25), and LBM-NJ-2006a(H5N2) (Bayes factor 12.21 to >10,000) (Table S4). For recent introductions (2015–2017): CPF-WI-2017(H5N2) was associated with dabbling duck origin viruses across all eight segments (Bayes factor 6.59 to 35.12) (Table S3); CPF-MO-2016(H5N1) was associated with goose/swan origin viruses across six segments (PB2, PB1, PA, HA, MP, and NS) (Bayes factor 15.69 to 149.91) (Figure 3; Table S3); and LBM-NJ-2015(H5N1) was associated with dabbling duck viruses (HA and NA; Bayes factor 53.98 and 8.12, respectively), diving/sea duck virus (NP; Bayes factor 169.25), and goose/swan virus (NS; Bayes factor 9.55) (Table S3). Functional groups associated with all eight gene segments were identified for only four of the 18 purported introductions into North American poultry during 2001–2017. Among them, all gene segments were associated with dabbling duck, goose/swan, or unknown functional groups (Tables S3 and S4).

H5 LPAIVs in LBMs have potential opportunities for reassortment. The opportunity for reassortment exists whenever two or more viruses circulate simultaneously. For CPFs,

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only a single genotype was associated with each introduction event, whereas, multiple genotypes were identified among viruses associated with two of the LBM introductions [LBM-NY-2006(H5N2) and LBM-NJ-2001(H5N2)]; (Figure 2). Neither LBM virus was found to be related to other poultry introduction events in this study. Reassortment may have played a role in the evolutionary pathways of viruses associated with LBM-NY-2006(H5N2) and LBM-NJ-2001(H5N2). Phylogeographic analyses suggest that viruses from several functional groups may have contributed to the evolution of viruses detected in LBMs (Table S4). The viruses from the unknown group could include other reservoirs (e.g. wild bird) for which we lack data or uncontrolled viruses in the LBMs. Thus, these H5 viruses can be associated with a single introduction of H5 gene, but NA or internal genes could be associated with viruses (all HA/NA subtypes rather than only H5 subtype) that may circulate in LBMs or with introductions from other reservoirs (e.g. wild birds).

Compared with H5 viruses detected on CPFs, those detected in LBMs were more temporally and spatially diverse. For example, four LBM introductions events included detections over several years and/or multiple states [LBM-NJ-2007(H5N2), LBM-NY-2006(H5N2), LBM-NJ-2006(H5N2)b, and LBM-NJ-2001(H5N2)], whereas CPF introduction events did not occur across years.

Temporal gaps exist between emergence and detection of H5 LPAIVs in domestic poultry in North America. The temporal gap between the time of H5 virus introduction into poultry and flock detection was estimated. Molecular clock analysis was used to determine the time of most recent common ancestor (TMRCA) between inferred wild bird progenitors and poultry isolates, and documented detection dates were obtained for 9

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introduction events (Table 3). Estimated temporal gaps for three CPF introductions were 146, 80, and 142 days (average 123 [±37 standard deviation] days) (Table 3). The temporal gaps for seven LBM introductions varied from 97 to 487 days (average 231 [±137 standard deviation] days) (Table 3).

Discussion

We investigated evolutionary pathways for 78 H5 LPAIVs detected in North American domestic poultry across CPFs, BYPs, GBPs, and LBMs during 2001–2017; our data suggest that the events were the result of 18 discrete virus introductions from wild birds. The H5 LPAIVs from North American poultry in this study share ancestry with viruses circulating among wild waterfowl, a finding generally consistent with those from other investigations of outbreaks in US poultry production systems (3, 9, 17-21). Our findings support that H5 LPAVs maintained in certain wild bird species represent an ongoing threat to domestic poultry in North America.

A previous study investigating the ancestral origins of H7 HPAIV reported among turkeys in Indiana, US, suggested that IAVs from diving ducks were associated with evolution of the virus ultimately introduced into the CPFs resulting in an outbreak (3). Our results show limited evidence for diving duck–associated IAVs contributing to the ancestry of H5 IAVs detected in North American poultry; instead, we found proportionally more evidence for contributions from dabbling ducks and geese/swans (Figure 3; Table S4). These findings suggest multiple potential evolutionary pathways for IAVs that are introduced to domestic poultry, and they highlight one of the challenges for avian influenza surveillance in wild birds: identifying the most pertinent wild bird species

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to target for sample collection to obtain meaningful reference information for better understanding the emergence of IAVs among poultry (24, 25).

We explored the time and location of H5 IAV introductions detected in various poultry production systems, including multiple introductions into CPFs and LBMs but did not identify any clear patterns for introductions. Instead, H5 IAV introductions appeared to be sporadic throughout the year and occur in states/provinces in multiple regions of the US and Canada. However, it is possible that the number of purported H5 introductions identified in this study was too small to detect patterns or that our data were of insufficient resolution to accurately identify the true times/locations of introduction events.

Our results suggest that sublineages of contemporary North American–origin H5 gene segments have different geographic distributions: sublineage 1 was predominantly detected in the Atlantic Flyway but was also detected at a lower frequency in other flyways, whereas sublineage 2 was most frequently detected in the Pacific Flyway but was also identified at lower relative abundance in other flyways. Recent genomic evidence indicates that viruses initially have modest fidelity to migratory bird flyways; however, over multiple years, IAV lineages tend to disperse flyways (26, 27). Sublineages 1 and 2 have been co-circulating in wild birds in the North America since 2002, so their restricted gene flow appears to be atypical. Post-hoc phylogeographic analyses did not show similar geographic patterns for other HA gene segment sublineages or for those of other genes (data not shown). Additional research is needed to identify genetic barriers in North America for these two H5 sublineages.

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Our results provide evidence that, compared with H5 IAV introductions in North American LBMs, those in each CPF introduction had a single genotype and more recent estimated TMRCA with viruses circulating among wild birds. This may indicate that poultry management activities in North America have successfully identified and quickly stamped out low pathogenic H5 IAVs in domestic birds raised on CPFs before the viruses have reassorted or become highly pathogenic. IAVs associated with introductions in LBMs sometimes had more than one genotype and, compared with CPF-associated IAVs, had longer mean TMRCAs with inferred wild bird predecessor viruses. Thus, viruses in North American LBMs may have a longer opportunity to co-circulate with other IAVs and subsequently form reassortants. Furthermore, the detection of genetically similar viruses associated with a single introduction event across multiple years and US states/Canadian provinces suggests that management activities relative to the detection and stamping out of H5 IAVs may be less efficient in LBMs than on CPFs.

On the basis of our results, we propose a conceptual model (Figure 4) describing generalized evolutionary pathways for low pathogenic H5 avian IAVs detected in North American domestic poultry. Our results suggest that H5 viruses detected in domestic poultry in the US and Canada are descended from IAVs circulating among wild birds, typically waterfowl, that are periodically introduced into poultry production systems. It appears that, on CPFs, H5 IAVs are detected relatively quickly through surveillance efforts and that the viruses are effectively eradicated through slaughter and additional preventative measures, as witnessed by apparent independent evolutionary pathways for viruses of a single genotype associated with each introduction event. In contrast, our study provides evidence that viruses in LBMs are not detected as rapidly as those on

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CPFs and, therefore, may have a greater potential for genetic reassortment with other wild bird–associated IAVs. The high mobility of birds sold in LBMs also facilitates the geographic spread of viruses in the US states and Canada.

In summary, the apparent repeated introduction of H5 IAVs from wild birds to domestic poultry in North America highlights the importance of avian IAV surveillance, particularly at the interface of wild birds and domestic poultry. Proactive and strategic surveillance covering multiple wild bird species and integrating genomic sequencing approaches is critical to understanding the evolutionary pathways of IAVs; such knowledge will be valuable in efforts to refine monitoring activities and optimize early warning systems. In addition, our findings support the premise that avian H5 IAVs introduced from wild birds present an ongoing threat to domestic poultry and can be controlled effectively through management practices, particularly those implemented in North American CPFs.

Materials and Methods

Data. To understand the genesis of low pathogenic avian H5 IAVs in North America, we sequenced 37 isolates recovered from domestic poultry samples collected during 2001–2017 (Table S5); 27 of the isolates were H5N2 viruses (including mixed viruses) from LBMs, five were H5N2 viruses from CPFs, one was H5N2 virus from a BYP, one was H5N2 virus from a GBP, and three were H1N1viruses from turkeys (n = 2) and a chicken (n = 1). Of note, if multiple isolates identified from the same case report had identical genomic sequences, only one of the isolates was selected for this study. We analyzed sequence data for these 34 poultry H5 isolates and 44 other North American poultry–

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origin IAVs deposited in public databases (total of 78 H5 isolates) (Table 1).In addition, we sequenced 127 H5 IAV isolates originating from wild birds (mostly during 2015 and 2016, n = 116) in 30 US states (Table S5); the isolates were from dabbling ducks (n = 94), shorebirds (n = 16), geese/swans (n = 10), gulls/terns/seabirds (n = 3), diving/sea ducks (n = 1), and unknown avian species (n = 3). Genomic sequencing and sequence assembly were performed as previously described (3); the GenBank accession numbers are listed in Table S5. The geographic, temporal, and host distribution of all viruses we sequenced are shown in Figure S3. In this study, the IAVs from commercial flocks and LBMs were isolated during poultry pre-movement monitoring activities, suspected IAV outbreaks or surveillance activities; the IAVs from wild birds were isolated during active influenza surveillance.

To perform systematic analyses, we retrieved the genomic sequences for all avian-origin IAVs from the following public databases in April 2017: the Influenza Virus Resources (28) (https://www.ncbi.nlm.nih.gov/genomes/FLU), the Influenza Research Database (29) (https://www.fludb.org), and GISAID (30) (https://www.gisaid.org). For those viruses for which we obtained redundant sequences, we only included the longest sequence contig per gene segment in analyses. In total, we used sequences from ~20,000 influenza viruses worldwide, including >9,000 IAVs of North American origin (Table S6). Because our preliminary phylogenetic analyses of these genomic sequences suggested that the low pathogenic H5 IAV isolates in domestic poultry were genetically related to North American lineage IAVs (data not shown), we focused subsequent data analyses only on sequences for IAVs detected in North America. The temporal and host

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391 distribution of all viruses of North American origin included in this study are shown in
392 Figure S4.
393
394 **Sequence alignment and phylogenetic analysis**. Multiple sequence alignments were
395 generated using MAFFT v7.273 (31). Phylogenic analyses were performed using an
396 approximate maximum-likelihood method with a generalized time–reversible substitution
397 model and 'CAT' approximation rate model by using FastTree v2.1 (32). These
398 preliminary trees provided initial inference regarding tree topology among all sequences
399 in the public databases, specifically to differentiate gene segment sequences of North
400 American lineages from those of Eurasian lineages. A refined phylogenetic tree for each
401 gene segment was then re-constructed using a maximum-likelihood method by running
402 RAxML v 8.2.9 (33). A gamma model of rate heterogeneity and a generalized time–
403 reversible substitution model were used for these phylogenetic analyses, and
404 bootstrapping was conducted using the same rate and substitution model. Phylogenetic
405 trees were visualized by ggtree v1.6.11 (34) and FigTree v1.4.3
406 (http://tree.bio.ed.ac.uk/software/figtree/). Topologies of phylogenetic trees were
407 validated using MrBayes 3.2.7 (35), PAUP* 4.0 (36), and PHYLIP 3.6 (37).
408
409 **Genotype analyses and assignment of possible progenitor gene and potential**
410 **precursor viruses for low pathogenic H5 avian IAVs.** Genotypes for IAVs were
411 assigned through analyses assessing genetic similarity among viral genome constellations.
412 Gene segments were considered to be genotypically similar if they co-occurred in clades

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with other gene segment sequences (as determined using inferred tree topology) with a minimum bootstrap value of 70 and shared nucleotide sequence identities ≥95% (18).

Possible progenitor gene segments and precursor viruses for H5 IAVs of North American poultry origin were also assessed using tree topology and sequence identities. Possible progenitor gene segments were inferred when the following criteria were met: 1) the candidate gene segment shared a phylogenetic clade with a minimum bootstrap value of 70 with a North American poultry gene segment; 2) the candidate progenitor gene segment and North American poultry gene segment shared ≥98% nucleotide sequence identity; 3) the candidate gene segment shared the highest nucleotide sequence identity with the poultry-origin IAV gene segment in its genetic cluster; and 4) the putative progenitor gene segment was detected prior to detection of the North American poultry IAV gene segment. A potential precursor virus was inferred when a virus had three or more possible progenitor gene segments from an H5 IAV identified in North American poultry.

Definition of purported H5 introduction into North American poultry. Multiple H5 IAVs detected in North American domestic poultry were inferred to have resulted from the same introduction event if the following criteria were met: 1) the genetic sequence for the H5 HA gene segment of these two viruses shared a common clade with a minimum bootstrap value of 70; 2) the H5 HA gene segment of these two viruses shared ≥98% nucleotide sequence identity; and 3) the nucleotide sequence identity shared with H5 HA gene segments from IAVs originating from North American poultry was greater than that shared with gene segments from wild bird–origin IAVs.

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436

437 **Phylogeographic analyses to infer transition of viruses between wild birds and**
438 **domestic poultry and between North American migratory bird flyways.** To enable
439 inference of wild bird hosts associated with H5 IAV introductions in North American
440 domestic poultry, we performed phylogeographic analyses to assess support for
441 associations between specific functional groups of wild bird IAV hosts and H5 IAV gene
442 segments detected in domestic poultry. Hosts of influenza viruses were categorized into 9
443 functional groups: dabbling duck, diving/sea duck, goose/swan, gull/tern/seabird, raptor,
444 shorebird, other avian (i.e., American coot, double-crested cormorant, red-necked grebe,
445 rock dove, and western grebe; n = 5), and unknown (i.e., hosts for whom the species was
446 unclear or for which the domestic status was ambiguous, including ducks, geese, feces,
447 fowl, and poultry; n = 22). To minimize sampling bias, we balanced the number of
448 sequences in each host group by resampling: for each year, we randomly selected a
449 maximum of 10 sequences from each functional group.

450 Phylogeographic analyses were performed as previously described (3, 23). An
451 asymmetric substitution model with Bayesian stochastic search variable selection and a
452 strict clock model were applied in the analyses. We used Markov chain Monte Carlo
453 methods, setting the chain length to 100 million with sampling every 10,000 states. The
454 convergence of each run was checked by Tracer v1.6 (http://beast.community/tracer)
455 before continuing to the next step. All poorly configured states were removed according
456 to a 10% burn-in rate. After that, maximum clade credibility phylogenetic trees were
457 generated using TreeAnnotator v1.8.4 (38) (http://beast.community/treeannotator). Bayes
458 factor was calculated to indicate the statistical support level. Significant transition was

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459 indicated by a combination of Bayes factor ≥3 and mean indicator ≥0.5. Statistical
460 support levels were interpreted from Bayes factors as follows: Bayes factor <3 indicates
461 no support; 3 ≤ Bayes factor <10 indicates support; 10 ≤ Bayes factor <100 indicates
462 strong support; 100 ≤ Bayes factor <1000indicates very strong support; and Bayes factor
463 ≥1,000 indicates decisive support. Maximum clade credibility phylogenetic trees were
464 visualized by FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

465 In addition to the above analyses on the potential sources of wild bird hosts for
466 the H5 IAV gene segments detected in domestic poultry, we also inferred the transition
467 patterns of influenza A viruses across North American migratory bird flyways. We first
468 designated each US state and Canadian province into either the Atlantic, Mississippi,
469 Central, or Pacific Flyway based on the administrative definition of North American
470 migratory bird flyways (https://www.fws.gov/birds/management/flyways.php) (Figure
471 1C). Phylogeographic analyses were then performed to assess support for transitions of
472 IAVs between among migratory bird flyways. To minimize the biases for sample
473 selection, we included all viruses in sublineages 1 and 2 (identified from phylogenetic
474 tree of H5 gene) in the phylogeographic analyses. Phylogeographic analyses and data
475 interpretation are the same as described above.

476

477 **TMRCA estimation**. The Bayesian Markov Chain Monte Carlo method implemented in
478 BEAST v1.8.4 (38) was used to estimate the substitution rates and TMRCA between
479 inferred predecessor HA gene segments of IAVs identified in the wild bird reservoir and
480 H5 IAVs detected in poultry for which specific dates of detection were available. SRD06
481 partitioned substitution model, uncorrelated lognormal relaxed clock model, and

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Bayesian skyline coalescent tree prior were implemented in the molecular clock analyses. Two independent runs with 100 million chain length (sampling frequency = 10,000) were combined by LogCombiner v1.8.4 (http://beast.community/logcombiner) and then analyzed by Tracer v1.6 using a 10% burn-in rate (http://tree.bio.ed.ac.uk/software/tracer/).

Accession number(s). Sequence were deposited in GenBank under accession numbers CY235315 to CY235322, KU310460 to KU310475, KY131302 to KY131333, KY131357 to KY131364, KY550909 to KY550916, MF046190, MF046211, MF046222, MF046227, MF046237, MF046275, MF046276, MF046280, MF046298, MF046299, MF046306, MF046330, MF046351, MF046361, MF046366, MF046376, MF046389, MF046403, MF046406, MF046412, MF046415, MF046416, MF046434, MF046442, MF046448, MF046452, MF046468, MF046472, MF046481, MF046490, MF046496, MF046504, MF046507, MF046509, MF046514, MF046523, MF046531, MF046536, MF046544, MF046547, MF046556, MF046567, MF046570, MF359819 to MF359890, MF613674, MF613685, MF613713, MF613716, MF613724, MF613731, MF613745, MF613747, MF613753, MF613755, MF613762, MF613766, MF613772, MF613776, MF613777, MF613792, MF613809, MF613812, MF613817, MF613823, MF613869, MF613881, MF613903, MF613905, MF613918, MF613924, MF613928, MF613937, MF613940, MH341739 to MH341906, and MH546139 to MH547031.

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ASB, TJD, SK, MKT, AMR, DES, RJW, and X-FW initiated and designed this study; ASB, TJD, MLK, SK, JMN, MKT, AMR, ABR, and DES collected data; LL and X-FW performed experiments; LL and X-FW wrote the first draft of the manuscript; and ASB, TJD, SK, JMN, MKT, AMR, ABR, DES, RJW, and X-FW revised the manuscript.

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652 **Figure Legends**

653

654 **Figure 1.** Detections of low pathogenic H5 avian influenza A viruses in domestic poultry
655 in the United States and Canada (2001–2017). **A)** The temporal distributions of
656 introductions of H5 viruses detected on commercial poultry farms (CPFs), backyard
657 poultry (BYPs), game bird poultry (GBPs,) and in live bird markets (LBMs) during
658 2001–2017. An introduction was defined by a tree topology of the H5 gene with a
659 bootstrap value ≥70 (Figure S1) and shared nucleotide sequence identity ≥98%. **B)**
660 Simplified phylogenetic tree displaying general topology for North American sequence of
661 influenza A viruses at the H5 hemagglutinin gene. The distribution of functional groups
662 of wild bird hosts for influenza A viruses in the tree are summarized by pie charts. **C)** US
663 state and Canadian province of origin for H5 subtype influenza A virus isolates detected
664 in North American domestic poultry during 2001–2017 and geographic distribution of the
665 influenza A viruses of H5 sublineages 1 and 2 in wild birds based on the North American
666 administrative definition of migratory bird flyways
667 (https://www.fws.gov/birds/management/flyways.php). Circle sizes indicate the number
668 of H5 influenza A virus isolates in the corresponding US state/Canadian province. Based
669 on ecologic attributes, the hosts of influenza viruses were categorized into 9 different
670 groups: dabbling duck, diving/sea duck, goose/swan, gull/tern/seabird, poultry, raptor,
671 shorebird, other avian, and unknown. Viruses from avian species that did not fit into any
672 of these functional groups were categorized as 'other avian', and viruses for which the
673 species sampled were unclear or for which the domestic status was ambiguous were
674 categorized as 'unknown'.

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676 **Figure 2.** Summary of genotypic analysis of low pathogenic H5 avian influenza A
677 viruses detected in domestic poultry in the United States (2001–2017). Genotypes were
678 assigned by unique combinations of sublineages for each gene, which were determined
679 based on tree topology with a bootstrap value ≥ 70 and a nucleotide sequence identity ≥
680 95%. To simplify the illustration, only the representative viruses were selected for each
681 genotype including those with unique combinations of location of detection
682 (state/province) and year. Numbers on the tree indicate individual H5 low pathogenic
683 introductions and were linked to unique IDs of those introductions to the right.

684

685 **Figure 3.** Summary of analyses to assess transition of influenza A viruses from a specific
686 functional group of wild birds to the low pathogenic H5 avian influenza A viruses
687 detected in domestic poultry. In this figure, phylogeographical analyses showed one
688 recent and representative H5 low pathogenic introduction in North American poultry,
689 CPF-MO-2016(H5N1), which is an introduction of virus in Missouri turkey as an
690 example. The trees shown in the figure are constructed on the basis of the maximum
691 clade credibility phylogenetic trees. Phylogeographical analyses were performed using all
692 isolates for each inferred H5 introduction. Branches of the phylogenetic trees were
693 colored according to the estimated ancestral state of the functional group of wild birds
694 from discrete trait reconstruction. Arrow widths are based on the Bayes Factor support
695 levels. Statistical support is provided in greater detail in Table S3.

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Figure 4. Conceptual model summarizing the generalized inferred evolutionary pathways for low pathogenic (LP) H5 avian influenza A viruses (IAVs) detected on commercial poultry farms and in live bird markets (LBMs) in the United States and Canada during 2001–2017. H5 viruses introduced by wild waterfowl were inferred to circulate for a longer time in LBMs than on commercial poultry farms. Furthermore, we found evidence suggesting reassortment between H5 viruses and other influenza A viruses in LBMs, resulting in multiple genotypes associated with a single introduction event. The blue and red lines denote genetically distinct gene segments; in each virus, the segments were vertically sorted in the order of PB2, PB1, PA, HA, NP, NA, MP, and NS.

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Table 1. H5 LPAI viruses detected in domestic poultry in the United States and Canada (2001–2017).

Introduction[a]	Host	Region	Year	Isolate name [a]	Sample date	Subtype
BYP-MI-2015(H5N2)	mallard	Michigan	2015	A/mallard/Michigan/15-031493-1orig/2015	2015-10-01	H5N2
BYP-OR-2006(H5N2)	duck guinea fowl	Oregon	2006	A/duck/Oregon/459674-3/2006	2006-09-29	H5N2
				A/guineafowl/Oregon/459674-5/2006	2006-09-29	H5N2
GBP-CA-2014(H5N8)	quail	California	2014	A/quail/California/K1400794/2014	2014-04	H5N8
GBP-ID-2008(H5N8)	pheasant	Idaho	2008	A/pheasant/Idaho/08-002590-63/2008	2008	H5N8
CPF-WI-2017(H5N2)	turkey	Wisconsin	2017	A/turkey/Wisconsin/17-007146-1/2017	2017-03-02	H5N2
				A/turkey/Wisconsin/17-007146-2/2017	2017-03-02	H5N2
				A/turkey/Wisconsin/17-007146-3/2017	2017-03-02	H5N2
				A/turkey/Wisconsin/17-007319-3/2017	2017-03-03	H5N2
				A/turkey/Wisconsin/17-007981-6/2017	2017-03-09	H5N2
CPF-MO-2016(H5N1)	turkey	Missouri	2016	A/turkey/Missouri/16-014037-7/2016	2016-04-29	H5N1
CPF-MB-2010(H5N2)	turkey	Manitoba	2010	A/turkey/MB/FAV11/2010	2010-11-25	H5N2
				A/turkey/MB/FAV10/2010	2010-11-26	H5N2
CPF-VA-2007(H5N1)	turkey	Virginia	2007	A/turkey/VA/505477-18/2007	2007-07-11	H5N1
				A/turkey/Virginia/505477-17/2007	2007-07-11	H5N1
CPF-CA-2002(H5N2)	turkey	California	2002	A/turkey/CA/D0208651-C/02	2002	H5N2
LBM-NY-2016(H5N2)	duck muscovy duck	Ontario New York New Jersey	2016	A/domesticduck/ON/FAV-18CS46/2016	2016	H5N2
				A/duck/NewYork/16-020978-2orig/2016	2016	H5N2
				A/duck/NewYork/16-021467-1orig/2016	2016	H5N2
				A/duck/NewYork/16-021916-1orig/2016	2016	H5N2
				A/duck/NewYork/16-021920-1orig/2016	2016	H5N2
				A/muscovyduck/NewJersey/16-021456-4/2016	2016	H5N2
				A/muscovyduck/NewJersey/16-021457-2/2016	2016	H5N2
LBM-NJ-2015(H5N1)	chicken	New Jersey	2015	A/chicken/New_Jersey/15_002659_2/2015	2015-01-20	H5N1

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LBM-NJ-2011(H5N2)	duck	New Jersey	2011	A/duck/NewJersey/11-064045-002/2011	2011-12-20	H5N2
LBM-NY-2007(H5N2)	duck muscovy duck	New York	2007-2009	A/duck/NewYork/07-002127-001/2007	2007-10-30	H5N2
				A/muscovyduck/NewYork/08-000560-002/2008	2008-03-13	H5N2
				A/duck/NewYork/08-000759-001/2008	2008-04-22	H5N2
				A/duck/NewYork/08-000937-001/2008	2008-05-22	H5N2
				A/muscovyduck/NewYork/09-002670-002/2009	2009-03-04	H5N2
				A/duck/NewYork/09-005059-001/2009	2009-04-14	H5N2
				A/muscovyduck/NewYork/09-005059-002/2009	2009-04-14	H5N2
LBM-PA-2007(H5N2)	chicken duck guinea fowl muscovy duck pleasant	Pennsylvania New York New Jersey	2007-2008	A/duck/Pennsylvania/07-002198-003/2007	2007-11-09	H5N2
				A/muscovyduck/NewJersey/07-002376-001/2007	2007-12-05	H5N2
				A/guineafowl/NewYork/08-000170-003/2008	2008-01-07	H5N2
				A/pheasant/NewYork/08-000170-002/2008	2008-01-07	H5N2
				A/guineafowl/NewYork/08-000238-001/2008	2008-01-28	H5N2
				A/chicken/NewJersey/251-4/2008	2008-02-01	H5N2
				A/chicken/NewJersey/577-6/2008	2008-03-27	H5N2
				A/chicken/NewJersey/08-000640-006/2008	2008-04-03	H5N2
				A/guineafowl/NewJersey/08-000640-008/2008	2008-04-03	H5N2
				A/guineafowl/NewJersey/08-000841-001/2008	2008-05-14	H5N2
				A/muscovyduck/NewJersey/08-000912-001/2008	2008-05-28	H5N2
LBM-NY-2006(H5N2)	duck quail turkey	New York Pennsylvania	2006-2007	A/duck/NewYork/465571/2006	2006-10-23	H5N2
				A/duck/NewYork/465976/2006	2006-10-24	H5N2
				A/duck/NewYork/466787/2006	2006-10-31	H5N2
				A/turkey/NewYork/465977/2006	2006-10-31	H5N2
				A/duck/NewYork/470179/2006	2006-11-07	H5N2
				A/avian/NewYork/466812/2006	2006-11-09	H5N2
				A/duck/Pennsylvania/07-467189-1/2006	2006-11-09	Mixed
				A/duck/NewYork/469961/2006	2006-11-13	H5N2
				A/duck/NewYork/489761/2007	2007	H5N2

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				A/duck/NewYork/481172/2007	2007-01-23	H5N2
				A/duck/NewYork/483239/2007	2007-02-02	H5N2
				A/duck/NewYork/484057/2007	2007-02-06	H5N2
				A/duck/NewYork/484680/2007	2007-02-12	H5N2
				A/duck/NewYork/490722/2007	2007-03-21	H5N2
				A/duck/NewYork/492652/2007	2007-04-05	H5N2
				A/duck/NewYork/494165/2007	2007-04-18	H5N2
				A/quail/NewYork/07-501360-1/2007	2007-06-02	H5N2
				A/quail/NewYork/501360/2007	2007-06-12	H5N2
				A/duck/NewYork/504371/2007	2007-06-22	H5N2
				A/duck/NewYork/504372/2007	2007-06-22	H5N2
LBM-NJ-2006(H5N2)a	chicken duck muscovy duck	New York New Jersey Pennsylvania	2006	A/avian/NewJersey/437109/2006	2006-05-09	H5N2
				A/chicken/NewYork/439236/2006	2006-05-10	H5N2
				A/chicken/NewYork/439235/2006	2006-05-15	H5N2
				A/muscovyduck/NewYork/62095-1/2006	2006-05-15	H5N2
				A/duck/NewYork/440410/2006	2006-05-17	H5N2
				A/duck/NewYork/440409/2006	2006-05-23	H5N2
				A/duck/NewYork/445743/2006	2006-06-19	H5N2
				A/chicken/Pennsylvania/446080-7/2006	2006-07	H5N2
				A/duck/Pennsylvania/446080-6/2006	2006-07	H5N2
				A/duck/Pennsylvania/446080-7/2006	2006-07	H5N2
LBM-NJ-2006(H5N2)b	guinea fowl	New York New Jersey	2006-2007	A/avian/NewYork/448534/2006	2006	H5N2
				A/guineafowl/NewJersey/447114/2006	2006-07-19	H5N2
				A/guineafowl/NewJersey/07-002030-001/2007	2007-10-23	H5N2
LBM-NJ-2001(H5N2)	duck	Maine New Jersey	2001-2002	A/duck/NJ/117228-7/2001	2001-07-16	H5N2
				A/duck/ME/151895-7A/2002	2002-01-29	H5N2

[a]An isolate is defined as an avian influenza virus recovered from wild birds or domestic poultry. An introduction describes a case of avian influenza infection detected in domestic poultry, and one or multiple isolates can be recovered from the same introduction.

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Table 2. Potential precursor viruses for predominate genotype of low pathogenic H5 avian influenza virus introductions in domestic poultry in in the United States and Canada (2001-2017)

Representative isolate	Introduction	Potential precursor virus[a]	No.[b]	Sequence Identity (%)[c]							
				HA	NA	PB2	PB1	PA	NP	MP	NS
A/turkey/Wisconsin/17-007146-1/2017	CPF-WI-2017(H5N2)	A/northernpintail/Ohio/15OS5861/2015(H5N2)	4	99.14	98.81			98.26	98.96		
A/turkey/Missouri/16-014037-7/2016	CPF-MO-2016(H5N1)	A/canadagoose/DelawareBay/601/2016(H5N1)	8	98.82	99.29	99.43	99.56	99.35	99.53	99.47	99.52
A/turkey/MB/FAV10/2010	CPF-MB-2010(H5N2)	A/americangreen-wingedteal/Illinois/2975/2009(Mixed)	4		98.74		98.18		98.88		99.29
A/turkey/VA/50547718/2007	CPF-VA-2007(H5N1)	A/mallard/PA/454069-9/2006(H5N1)	4	98.74	99.39		98.31			99.40	
A/turkey/CA/D0208651C/02	CPF-CA-2002(H5N2)			ND[d]							
A/duck/NewYork/16-020978-2orig/2016	LBM-NY-2016(H5N2)			ND							
A/chicken/NewJersey/150026592/2015	LBM-NJ-2015(H5N1)	A/americangreen-wingedteal/Wisconsin/11OS3580/2011 (H11N2)	4					98.18	98.33	98.55	99.16
A/duck/NewJersey/11-064045-002/2011	LBM-NJ-2011(H5N2)	A/mallard/Ohio/11OS1961/2011(H5N2)	4	99.60	99.86		98.92			99.80	
A/duck/NewYork/08-000759-001/2008	LBM-NY-2007(H5N2)			ND							
A/chicken/NewJersey/2514/2008	LBM-NJ-2007(H5N2)	A/mallard/Maryland/07OS2433/2007(H5N2)	3	98.90	98.25						98.24
A/turkey/NewYork/465977/2006	LBM-NY-2006(H5N2)	A/americanwigeon/Iowa/463993/2006(H5N2)	4	99.08	98.54		98.53	98.29			
A/chicken/Pennsylvania/4460807/2006	LBM-NJ-2006(H5N2)a	A/mallard/Maryland/897/2004(H5N2)	4	98.63	98.75		99.01		99.29		
A/avian/NewYork/448534/2006	LBM-NJ-2006(H5N2)b	A/mallard/Ohio/468158/2006(H5N2)	4	98.34	98.63			98.14		98.30	
A/duck/NJ/1172287/2001	LBM-NJ-2001(H5N2)	A/mallard/Maryland/302/2001(H5N2)	3		98.58			98.48			99.42
A/mallard/Michigan/15-031493-1orig/2015	BYP-MI-2015(H5N2)	A/mallard/Ohio/11OS2156/2011(H5N2)	4	98.68	98.96				98.57		99.30
A/duck/Oregon/4596743/2006	BYP-OR-2006(H5N2)	A/americanwidgeon/Oregon/467919/2006(H5N2)	8	99.60	100.00	99.83	99.78	99.86	99.81	99.73	99.88
A/quail/California/K1400794/2014	GBP-CA-2014(H5N8)	A/mallard/California/1479/2013(mixed)	3		98.05			98.07		99.20	
A/pheasant/Idaho/08-002590-63/2008	GBP-ID-2008(H5N8)	A/ruddyturnstone/NewJersey/AI06-582/2006(H6N7)	3			98.40	98.78			98.40	

[a]A potential precursor virus was defined by the virus with at least three possible progenitor genes (see method section for details); [b] Number of possible progenitor genes; [c]Only sequence identity >98% are shown: [d]ND, not done because complete genome was not available.

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Table 3. Time to most recent common ancestor estimation of HA gene for low pathogenic H5 avian influenza A virus introductions from commercial farms (CPFs) and live bird markets (LBMs) in the United States and Canada (2001-2017).

Source	Introduction [a]	Mean TMRCA	95% HPD[b] low	95% HPD high	First detected[c]	Difference[d] (days)	Average (days)	Standard deviation
Commercial Farms	CPF-WI-2017(H5N2)	2016-10-07	2016-01-01	2016-06-18	2017-03-02	146		
	CPF-MB-2010(H5N2)	2010-09-06	2010-11-16	2010-06-02	2010-11-25	80		
	CPF-VA-2007(H5N1)	2007-02-19	2007-06-10	2006-10-09	2007-07-11	142	123	37
Live Bird Markets	LBM-NY-2007(H5N2)	2007-07-24	2007-10-18	2007-04-02	2007-10-30	97		
	LBM-PA-2007(H5N2)	2007-06-23	2007-09-23	2007-03-14	2007-11-09	138		
	LBM-NY-2006(H5N2)	2006-02-12	2006-06-17	2005-10-09	2006-10-23	253		
	LBM-NJ-2006(H5N2)a	2005-09-26	2006-01-18	2005-05-17	2006-05-09	225		
	LBM-NJ-2006(H5N2)b	2006-01-09	2006-06-22	2005-07-10	2006-07-19	191		
	LBM-NJ-2001(H5N2)	2000-03-16	2001-03-01	1999-01-23	2001-07-16	487	231	137

[a] For each introduction, all isolates with exact sampling date were included in analysis; [b]First detected date of an introduction was defined as the earliest sampling date among all isolates within this introduction; [c] HPD, highest posterior density; [d]Difference between date of first detection and mean time to most recent common ancestor.

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Figure 1

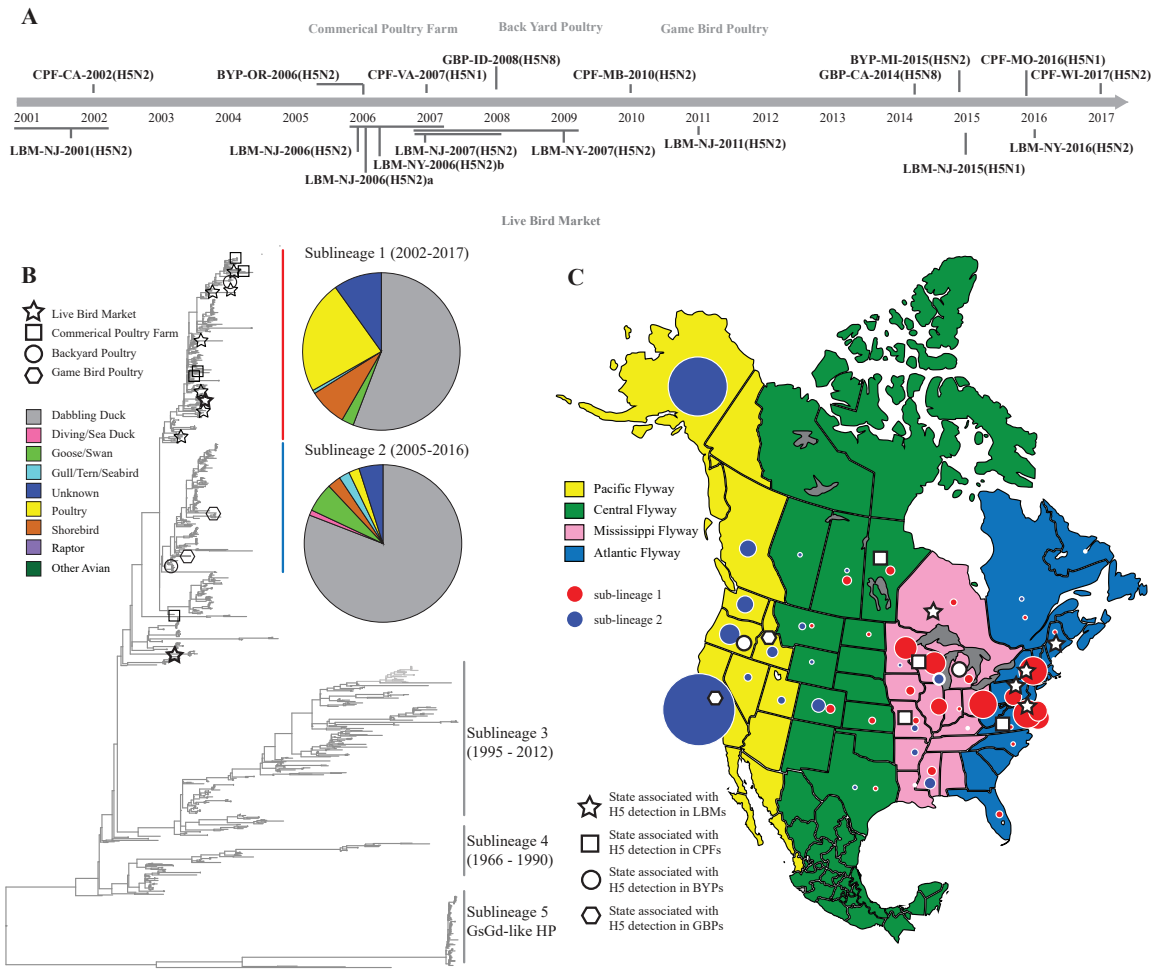

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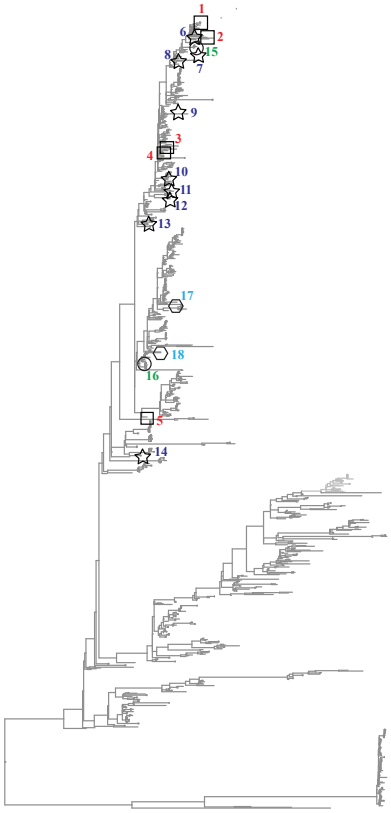
Figure 2



H5 IAVs detected in CPFs
H5 IAVs detected in LBMs
H5 IAVs detected in BYPs
H5 IAVs detected in GBPs

ID	Introduction Name	HA NA PB2 PB1 PA NP MP NS	Isolates(n)	Representative Isolate Name	Subtype	Year
		Commercial poultry farm	**11**			
1	CPF-WI-2017(H5N2)		5	A/turkey/Wisconsin/170071461/2017	H5N2	2017
2	CPF-MO-2016(H5N1)		1	A/turkey/Missouri/160140377/2016	H5N1	2016
3	CPF-MB-2010(H5N2)		2	A/turkey/MB/FAV10/2010	H5N2	2010
4	CPF-VA-2007(H5N1)		2	A/turkey/VA/50547718/2007	H5N1	2007
5	CPF-CA-2002(H5N2)		1	A/turkey/CA/D0208651C/02	H5N2	2002
		Live bird market	**62**			
6	LBM-NJ-2015(H5N1)		1	A/chicken/NewJersey/150026592/2015	H5N1	2015
7	LBM-NY-2016(H5N2)		7	A/duck/NewYork/16-020978-2orig/2016 A/domesticduck/ON/FAV-18CS46/2016 A/muscovyduck/NewJersey/16-021456-4/2016	H5N2	2016
8	LBM-NJ-2011(H5N2)		1	A/duck/NewJersey/11-064045-002/2011	H5N2	2011
9	LBM-NY-2006(H5N2)		17	A/turkey/NewYork/465977/2006 A/quail/NewYork/501360/2007	H5N2	2006 2007
			2	A/duck/NewYork/492652/2007	H5N2	2007
			1	A/duck/Pennsylvania/07-467189-1/2006	Mixed H5/H11 N2/N9	2007
10	LBM-PA-2007(H5N2)		11	A/duck/Pennsylvania/07-002198-003/2007 A/muscovyduck/NewJersey/07-002376-001/2007 A/chicken/NewJersey/2514/2008 A/guineafowl/NewYork/08-000170-003/2008	H5N2	2007 2008
11	LBM-NJ-2006(H5N2)a		10	A/chicken/Pennsylvania/4460807/2006 A/duck/NewYork/445743/2006 A/avian/NewJersey/437109/2006	H5N2	2006
12	LBM-NJ-2006(H5N2)b		3	A/guineafowl/NewJersey/447114/2006 A/guineafowl/NewJersey/07-002030-001/2007	H5N2	2006 2007
13	LBM-NJ-2001(H5N2)		1	A/duck/ME/1518957A/2002	H5N2	2002
			1	A/duck/NJ/1172287/2001	H5N2	2001
14	LBM-NY-2007(H5N2)		7	A/duck/NewYork/492652/2007 A/duck/NewYork/08-000759-001/2008 A/Muscovyduck/NewYork/09-005059-002/2009	H5N2	2007 2008 2009
		Backyard flock poultry	**3**			
15	BYP-MI-2015(H5N2)		1	A/mallard/Michigan/15-031493-1orig/2015	H5N2	2015
16	BYP-OR-2006(H5N2)		2	A/duck/Oregon/4596743/2006 A/guineafowl/Oregon/459674-5/2006	H5N2	2006
		Game bird flock poultry	**2**			
17	GBP-CA-2014(H5N8)		1	A/quail/California/K1400794/2014	H5N8	2014
18	GBP-ID-2008(H5N8)		1	A/pheasant/Idaho/08-002590-63/2008	H5N8	2008

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Figure 3

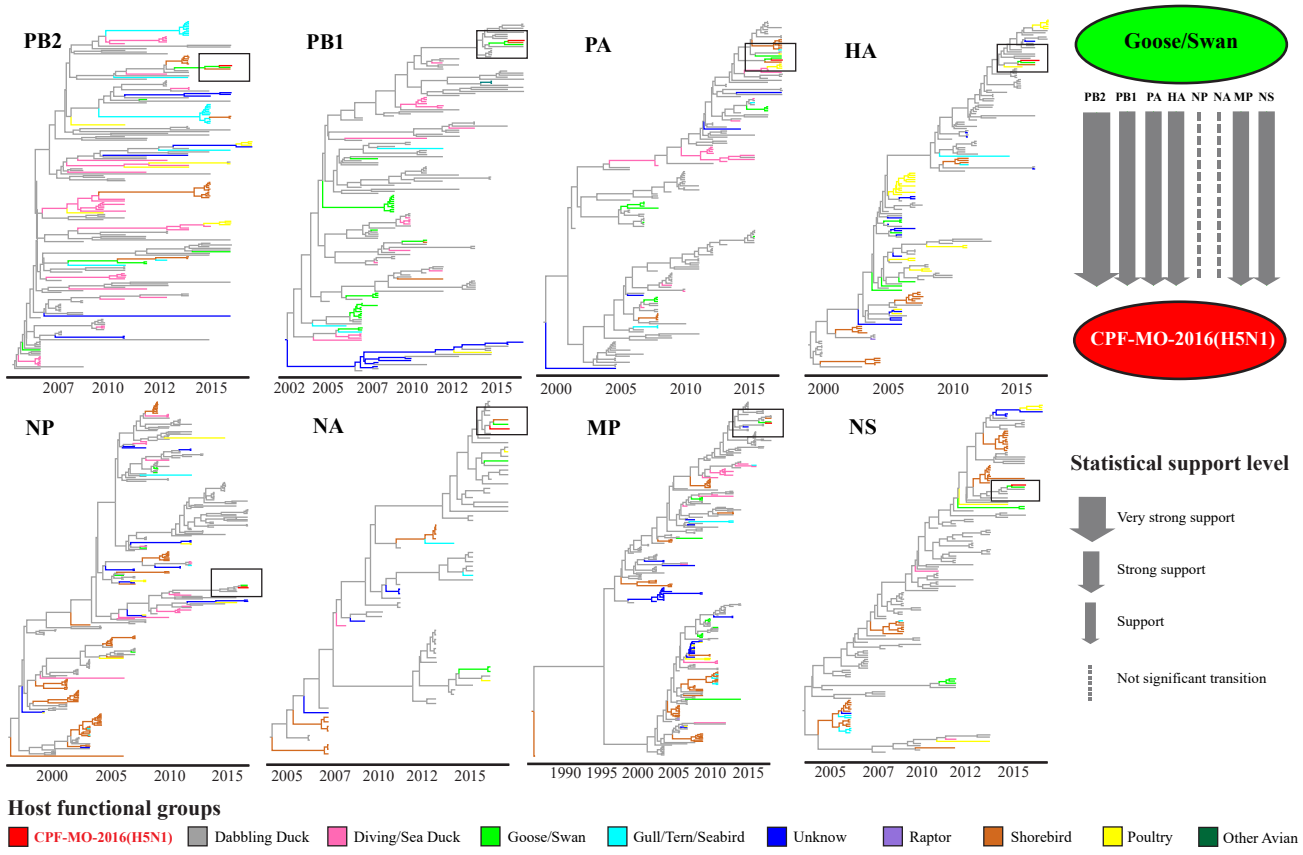

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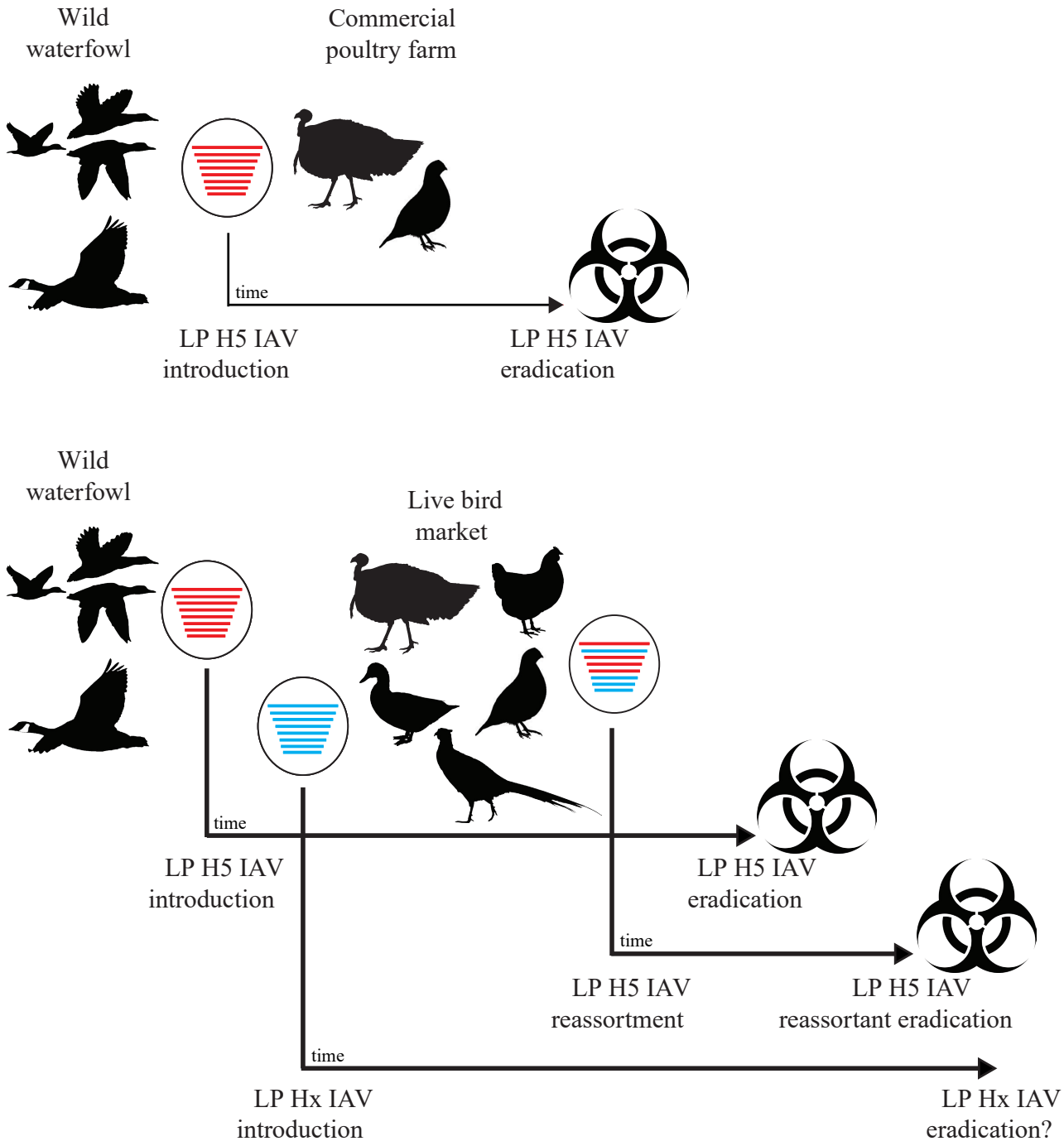
Figure 4



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