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Assessment of Introduction Pathway for Novel Avian Influenza Virus into North America by Wild Birds from Eurasia

(2013)

Northern Pintails (*Anas acuta*) migrating on the Bosque del Apache National Wildlife Refuge, New Mexico.
Photo Credit: Silas Bourne

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ABSTRACT: A critical question surrounding emergence of novel strains of avian influenza viruses (AIV) is the ability for wild migratory birds to translocate a complete (unreassorted whole genome) AIV intercontinentally. Virus translocation via migratory birds is suspected in outbreaks of highly pathogenic strain A(H5N1) in Asia, Africa, and Europe. As a result, the potential intercontinental translocation of newly emerging AIV (e.g. A(H7N9) from Eurasia to North America via migratory movements of birds) remains a concern. An estimated 1.48 to 2.91 million aquatic birds, principally *Anseriformes* (ducks, geese, and swans) and *Charadriiformes* (gulls, terns, and shorebirds) move annually between Eurasia and North America. AIV prevalence in Alaskan waterfowl populations shared between Eurasia and North America has been reported to range from <0.1 to 32.2 percent. Here we present an assessment to address the likelihood of whole (unreassorted) genome translocation of Eurasian strain AIV into North America. The scope of this assessment was limited specifically to assess the weight of evidence to support the movement of an unreassorted AIV intercontinentally by migratory aquatic birds. We reviewed high impact scientific publications to assess the evidence related to intercontinental movement of avian influenzas by aquatic birds, specifically between Eurasia and North America. In addition, we reviewed the available information for bird populations and movements (migratory and other) between North America and all other continents. We then identified common factors described across the publications related to the pathway of introduction of a novel AIV intercontinentally into North America. In our assessment of the scientific published literature, there appear to be at least four factors that may contribute to the potential for introduction of a specific AIV intercontinentally into North America by wild birds. These factors, in aggregate, may provide a framework for evaluating the likelihood of new forms of AIV from Eurasia to be introduced through aquatic birds and become established in North America.

Keywords: avian influenza, introduction, North America, pathway, wild bird, movement

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Acknowledgements

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ABBREVIATIONS

Acronym	Meaning
A(H5N1)	highly pathogenic Asian strain H5N1
A(H7N9)	low pathogenic Asian strain H7N9 currently circulating in China
AIV	avian influenza viruses
ARS	Agricultural Research Service
HP	highly pathogenic
HPAIV	highly pathogenic avian influenza viruses
LP	low pathogenic
LPAIV	low pathogenic avian influenza viruses
NCBI	National Center for Biotechnology Information
RNA	ribonucleic acid
SEPRL	Southeast Poultry Research Laboratory
USGS	United States Geological Survey
WHO	World Health Organization

EXECUTIVE SUMMARY

A critical question surrounding emergence of novel strains of avian influenza viruses (AIV)—both highly pathogenic (HP) and low pathogenic (LP)—is the ability for wild migratory birds to translocate a complete (unreassorted whole genome) AIV intercontinentally. Virus translocation via migratory birds contributed in part to the spread of HP Asian strain H5N1 (here referred to as A(H5N1)) out of Southern China and Southeast Asia across Central Asia, into Europe and Africa (Kilpatrick et al., 2006; Salzberg et al., 2007; Prosser et al., 2009; Gaidet et al., 2010; Gilbert et al., 2010; Takekawa et al., 2010). Recent phylogenetic analysis of A(H7N9) indicates that migratory birds from two distinct and distant flyways (Mediterranean-Black Sea and East Asian) may have contributed to the emergence of the virus (Gao et al., 2013; Kageyama et al., 2013; Liu et al., 2013). As a result, the potential dissemination of newly emerging AIV within Eurasia to North America via migratory movements of birds remains a concern (Feare, 2007; Gauthier-Clerc et al., 2007).

North America has an estimated 62.3 million migratory waterfowl (USFWS, 2012). During the summer breeding season the northern margins of North America (i.e. Alaska and Northern Atlantic region) receive species from six continents: North America, South America, Asia, Africa, Australia, and Antarctica (Winker and Gibson, 2010). This results in the northern margins of North America having direct annual connections with aquatic birds from many parts of the world. Eurasian birds are common in northern regions of North America (specifically Alaska) during the summer breeding period (Conant and Groves, 2005; Eldridge et al., 2005; Winker et al., 2007; Winker and Gibson, 2010).

An estimated 1.48 to 2.91 million waterfowl (ducks, geese, and swans), shorebirds, gulls, and terns move annually between Eurasia and North America with 13 to 20 percent being waterfowl (Winker and Gibson, 2010). AIV prevalence in these populations shared between Eurasia and North America has been reported to range from <0.1 to 32.2 percent depending on location, species, age, and sex (Ip et al., 2008; Hill et al., 2012b). Populations of AIV are genetically divergent across large geographic scales, resulting in Eurasian, North American, and South American gene pools of viruses (Gorman et al., 1990a; Gorman et al., 1990b; Ito et al., 1991; Ito et al., 1995; Widjaja et al., 2004). In geographic locations where migratory aquatic bird flyways overlap, greater evidence has been found for mixed lineage viruses (e.g. viruses composed of gene segments from multiple virus populations) (Pearce et al., 2009; Pearce et al., 2011; Lam et al., 2012). This genetic reassortment is thought to be the reason that to date no completely Eurasian viruses (whole genome) have been observed in North America (Krauss et al., 2007; Dugan et al., 2008).

The scope of our assessment was limited specifically to assess the weight of scientific evidence to support the potential movement of a complete AIV (whole genome) intercontinentally by aquatic birds. In our assessment of the scientific published literature, there appear to be at least four factors that may contribute to the potential for introduction of a specific AIV intercontinentally into North America by aquatic birds.

1) **Location and timing—**The location and timing of the virus emergence in relationship to North American migratory bird breeding grounds and flyways may influence AIV spread to new areas.
2) **Host species affected—**Host species ecology (particularly with regard to breeding, migration, and ability to migrate long distances, largely intercontinentally, while infected) is important to the spread of AIV between continents.
3) **Viral characteristics and pathogenicity—**Viral pathogenicity, susceptibility of host species to particular strains of AIV, and the fitness of the virus (i.e. its ability to 'compete') are important factors in potential transport and subsequent establishment of the viruses to new areas.
4) **Unforeseen stochastic events—**Unforeseen stochastic events have contributed to the long distance movement of AIV, particularly A(H5N1), virus across continental barriers.

Aggregate assessment of potential—Together these four factors provide a framework for evaluating the likelihood of a whole-genome introduction of a specific strain of AIV, such as influenza A(H7N9), from Eurasia into North America through migratory aquatic birds. For such an event to occur, a series of conditions must be met, including:

1) Sufficient proximity of the emergent virus to North America, or North American wild bird flyways, that it doesn't reassort before reaching the continent.
2) Exposure of a susceptible species to a sufficient viral dose that the bird becomes infected and carries the unreassorted virus while the bird is still healthy – without the virus reassorting – across the Pacific Ocean (most likely at the Bering Strait).
3) If the bird does not reach North America, it must shed the virus in a location where it persists in the environment long enough for another susceptible bird to be infected and then transport the virus – unreassorted – intercontinentally.
4) Once the virus reaches North America, it must survive, remain intact (i.e., unreassorted), infect another susceptible species, and be carried unreassorted from northern breeding grounds to other parts of the flyway, or to other flyways.
5) The virus must be robust enough to compete with other AIV strains infecting aquatic birds in North America.

While possible, to meet all these conditions (even for multiple exposures) reduces the likelihood of intercontinental translocation of unreassorted AIV from Eurasia into North America by aquatic birds.

Currently, influenza A(H7N9) is affecting provinces in Eastern China including a region which has a large potential AIV reservoir population of migratory waterfowl, domestic ducks, and chickens (Martin et al., 2011). This region has been identified by two studies as high risk for influenza A(H5N1) (Fang et al., 2008; Martin et al., 2011). The available data indicate that the potential for introduction of A(H7N9) into North America through aquatic migratory birds is possible. However, the likelihood ranges from extremely low to low due to:

1) The distance and potential ecological and geographic barriers between Asian and North American flyways;
2) Preliminary pathogenicity studies which suggest gallinaceous poultry are highly susceptible to A(H7N9); there is evidence that some poultry adapted strains of AIV are less efficiently transmitted in aquatic birds;
3) Lack of evidence (to date, on the basis of limited surveillance) that migratory aquatic bird species have yet been affected by influenza A(H7N9).

There is a large degree of uncertainty in our assessment. Currently there are few data available describing which species are affected, the extent of surveillance in aquatic bird species, and the susceptibility of aquatic birds, specifically waterfowl, to A(H7N9); these limitations hinder our ability to make definitive statements about the likelihood of A(H7N9) spreading into and through aquatic bird populations to North America. As new information emerges, the potential for introduction of A(H7N9) could change rapidly and stochastic events that may influence the movement of the virus are possible.

There is strong evidence that aquatic birds seasonally introduce Eurasian origin AIV genetic material into North America. Consequently, there is a demonstrated and continual emergence of new AIV resulting from reassorted Eurasian and North America AIV strains among aquatic birds, especially in Alaska (Pearce et al., 2010; Ramey et al., 2010a; Ramey et al., 2010b; Pearce et al., 2011; Lam et al., 2012). Furthermore, recent phylogenetic analysis of A(H7N9) indicates that migratory birds from two distinct and distant flyways (Mediterranean-Black Sea and East Asian-Australasian) may have contributed to the emergence of this virus (Liu et al., 2013).These new strains of AIV are generally well tolerated by their natural avian hosts but have unknown potential for pathogenicity to poultry and humans. Several recent studies have suggested that surveillance of AIV using genetic markers (changes in the viral genome) of adaptation could help identify which new strains of AIV may be of greater concern (Pepin et al., 2010; Tejeda and Capua, 2011; Flanagan et al., 2012).

BACKGROUND

Chinese authorities reported to the World Health Organization (WHO) on March 31, 2013 the identification of a novel avian influenza virus (AIV) A(H7N9) isolated from humans with severe respiratory disease (WHO, 2013c). Surveillance has found the novel virus in chickens, pigeons (captive and feral), ducks, and in environmental samples collected from live poultry markets (FAO, 2013). To date the novel virus has not been found on chicken farms, but was found on one pigeon farm (OIE, 2013). As of May 8, 2013 Chinese authorities had reported a total of 131 laboratory-confirmed cases of human infection with AIV A(H7N9), including 32 deaths (WHO, 2013b). Cases have been detected in eight provinces (Anhui, Fujian, Henan, Hunan, Jiangsu, Jiangxi, Shandong, and Zhejiang), and two municipalities (Beijing and Shanghai) in eastern and northern China (**Figure 1**). Taiwanese authorities reported one human case that had recently traveled to China (Chang et al., 2013). The specific animal reservoir(s), mode of transmission to humans, and scope of the spread of this virus among animal and human populations is unclear at this time (WHO, 2013d). The reported human cases are not known to be epidemiologically linked. Close contacts of cases are being monitored and to date none have been confirmed infected; therefore, human-to-human transmission is not known to be occurring at this time. For cases with exposure information, most reported exposure to animals (mostly poultry), but many cases have no known animal exposure (ECDPC, 2013). Infection of humans with other AIV A(H7) subtypes has typically resulted in mild illness and has been reported in the past from various countries, but this is the first report of human infection with AIV A(H7N9) (WHO, 2013d).

Figure 1. Provinces in China currently affected by avian influenza A(H7N9), 24 April 2013 (ECDPC, 2013). Inset map shows affected provinces in comparison to the latitude of San Diego, California, USA.

Chinese authorities have increased surveillance in animals with tens of thousands of samples collected from various surveillance streams, including live poultry/bird markets, poultry farms, pig farms, swine and poultry slaughter facilities, and aquatic birds and their habitats (because aquatic birds, particularly waterfowl, are known reservoirs for avian influenza (Swayne and Halvorson, 2008). To date the virus has not been found in wild migratory birds in China (FAO, 2013). Sequence data and preliminary infectivity studies suggest A(H7N9) exhibits low pathogenicity in poultry and is of avian origin. Low pathogenic A(H7N9) viruses of North

American lineage have been detected in commercial poultry in the United States, but these strains are genetically distant from those in the Eurasian H7 lineage.

The origin of this novel strain remains unknown as it was not previously found during existing surveillance activities in China (Shi et al., 2013b). However, initial phylogenic analysis suggested that the six internal genes from avian A(H9N2) viruses were previously isolated in China (Kageyama et al., 2013; Shi et al., 2013b). The origins of the major epitopes hemagglutinin (HA) and neuraminidase (NA) were further elucidated in a recent phylogenetic analyses that reported the core proteins of A(H7N9) most likely resulted from a reassortment of avian influenza viruses of at least four origins. The H7 gene segment is most closely related to the haemagglutinin gene from H7N3 viruses isolated from ducks in China's Zheijang province, whereas the neuraminidase gene is most closely related to that from H7N9 isolates from Korean ducks and wild birds (Gao et al., 2013; Liu et al., 2013). Furthermore, NA genes were related to wild duck European AIV lineages. The NA genes of A(H7N9) were similar to H11N9 strain of mallard (*Anas platyrhynchos*) from Czech Republic (2010) and a H7N9 strain of common teal (*Anas crecca*) from Spain (2008)(Liu et al., 2013). This indicates that the NA gene fragment of the novel H7N9 virus possibly originated from AIV carried by wild birds. The internal genes of the virus appear to be inherited from two different groups of H9N2 virus circulating in chickens; but, a 2012 (H9N2) from a wild passerine bird (*Fringilla montifringilla*) is the closest relative for the PB2, PB1, and PA segments (Gao et al., 2013; Liu et al., 2013).

These analyses indicate that ducks and chickens probably acted as the intermediate hosts leading to the emergence of A(H7N9) (Gao et al., 2013; Kageyama et al., 2013; Liu et al., 2013). Genotypic and potential phenotypic differences imply that the isolates causing this outbreak form two separate subclades (Liu et al., 2013). Adaptation by the novel A(H7N9) virus to mammalian hosts is suggested by mutations that are associated with the recognition of receptors present in the upper respiratory tract of mammals and improved replication of avian influenza virus in mammals (ECDPC, 2013; WHO, 2013a). These mutations could favor high-affinity interaction with human receptors in the upper respiratory tract, a prerequisite for virus transmission by the aerosol route (Liu et al., 2013; Van Ranst and Lemey, 2013).

Response activities by health authorities have been quite costly to the Chinese poultry industry and have included culling of live birds, closure of live bird markets and bird trades, prohibition of live bird vendor stands, and prohibited importation of live birds. By April 16, 2013 it was estimated that the loss to the poultry industry was more than 10-billion yuan (or over $1.6 billion US dollars) (Anonymous, 2013).

Preliminary reports from the USDA Agricultural Research Service (ARS) Southeast Poultry Research Laboratory (SEPRL) pathogenicity studies suggest that gallinaceous poultry are highly susceptible to this novel H7N9 strain, shedding the virus in high amounts and are likely a potential source of infection for humans. ARS also reports that this virus demonstrates tropism for the respiratory tract, which contrasts with the traditional LPAIV gastrointestinal tract target. Therefore, respiratory transmission from the birds is more likely, with high amounts of virus detected in oral-pharyngeal swabs as compared to the cloaca. So far, no clinical signs in the quail, chickens, turkeys, or pigeons have been reported from the ongoing studies. This is expected, as low pathogenic AIV strains of AIV generally present with mild or no clinical signs (Swayne and Halvorson, 2008). This has led to some concern of the silent spread of the novel A(H7N9) virus to new areas in apparently healthy birds.

INTRODUCTION

Two independent questions have been raised in the scientific literature related to intercontinental dissemination of avian influenzas. First, can infected aquatic birds that are shedding virus be healthy enough to migrate (Flint, 2007; Fries et al., 2013)? Second, is there direct intercontinental movement of infected individuals, or contact between Eurasia and North American migrants, which results in transfer of viruses between continents (Kilpatrick et al., 2006)?

Here we present an assessment to address these questions and describe factors that may be involved in the potential intercontinental movement of a complete AIV (whole genome) into North America. Our assessment had four goals.

1) We conducted a review of high impact English scientific publications (n = 130) to assess the evidence related to intercontinental movement of avian influenzas by aquatic birds, specifically between Eurasia and North America. More specifically, we evaluated the reported phylogenetic and phylogeographic studies as they relate to intercontinental relationships of avian influenza in aquatic birds.
2) We assessed the ability of avian influenza infected birds to migrate or move long distances, specifically intercontinentally. This included assessment of the experimental infection studies in addition to case reports.
3) We evaluated other factors such as environmental persistence of AIV, which may contribute to intercontinental movement.
4) We reviewed the available information for bird populations and movements (migratory and other) between North America and all other continents. This included assessment of the entire bird banding record for all bird movements, evaluation of available studies describing movements, and review of available population data.

We then identified common factors described across the publications related to the aquatic bird pathway of introduction of a novel AIV intercontinentally into North America.

METHODS

Literature Review

We used a structured literature review to identify and characterize studies on the status of intercontinental movement of AIV in aquatic birds (Khan et al., 2003; Okoli, 2012). Our review was generally restricted to literature published since 1990, but focused on more recent scientific advances in knowledge of AIV. All literature relating to aquatic birds and avian influenza for intercontinental movement, phylogenetics, and immunology were considered eligible and are reflected in the use of the broad search terms. The review focused only on the scientific peer reviewed literature in the public domain. We expect that this search will have captured the majority of high impact contemporary scientific publications on intercontinental exchange of AIV via aquatic birds.

The literature review involved three steps. First, we identified keywords for use in the search process. Second, we conducted a systematic review of PubMed, Scopus, and Web of Science according to the search terms. Finally, once all relevant sources were identified and retrieved, we reviewed and categorized studies. Categories were identified a-priori and included:

1) Phylogenetic and phylogeographic studies as they relate to intercontinental relationships of avian influenza in aquatic birds;
2) Studies assessing the susceptibility of water birds to avian influenza;
3) Environmental persistence of AIV particularly as it relates to transmission;
4) Studies assessing the ability of avian influenza infected birds to migrate or move long distances;
5) Studies documenting the long distance movement of avian influenza via aquatic birds; and,
6) Studies documenting the long distance movement of aquatic birds between North America and other continents.

To assess the evidence related to intercontinental movement of AIV, data describing the rates of genetic exchange of virus were enumerated in table format for easy comparison. Studies describing the ability of avian influenza infected birds' ability to migrate or move long distances were summarized and reported. The frequency with common themes across all studies was assessed, tabulated, and tallied. We reported these common themes. Due to differing methodologies and often incomplete reporting of results, meta-analysis was not applicable for assessment of pathway factors. We formulated a subset of factors that may determine the possibility of intercontinental movement of AIV. These factors were used as a framework for assessing the potential for a complete AIV (whole genome) to move intercontinentally, and for the current A(H7N9) to move from China to North America via aquatic birds. We then provide an assessment of these factors using the likelihood terminology described in Appendix C.

Wild Bird Movement Data

In order to evaluate the documented evidence for intercontinental movement of aquatic birds between North America and other continents, the bird banding data for all observed movements between North America (United States, Mexico, Canada, and Hawaiian Islands) and other continents was acquired from the United States Geological Survey (USGS) Patuxent Bird Banding Laboratory data (USGS, 2013). We used data describing birds moving both into and out of North America because data describing movements of birds banded in Asia, and subsequently recovered in North America, are very limited. In doing this we assume that the majority of these movements are migratory and movements out of North America also represent a subsequent movement back into North America from a similar location on the other continent. Our assessment of these data then represents a determination of the extent to which North American origin birds may contact Asian birds but does not reflect the converse – Asian birds migrating to North America and subsequently returning to Asia. Specifically, we used data for all species for the extent of the banding record (1913 to present) (n=150,902). These data represent birds that are banded in North America and subsequently encountered on other continents (n=149,791). Conversely, the data also represent birds that were banded on other continents and recovered in North America (n=2,121). These data were aggregated by subfamily for *Anseriformes Anatidae* (ducks), which is thought to be the principal natural host for AIV. However, differing prevalence rates have been reported by subfamily. The order *Charadriiformes* (gulls, terns, and shorebirds) were aggregated by family and all other species were grouped into a single category.

The data were also aggregated by continent and continent sub-region. Encounter locations greater than five miles from a continent's coast were eliminated from the analysis. The frequency of movements between continent sub-regions and North America by aggregated species was calculated. This metric was used as an indication of the potential movements of aquatic birds between North America and other continents. Additionally, data describing the estimated populations of species known to migrate or move between Asia and North America were acquired and summarized. The resulting aquatic bird movement data (banding and reported populations), and the systematic literature review, were used in aggregate to assess the potential pathway of aquatic birds introducing novel AI from Eurasia into North America.

RESULTS

Wild Bird Movement Data

Bird Banding Data

There were 13,038 documented movements of aquatic birds between North America and other continents between 1920 and 2013. Species (251) from 15 orders and 40 families were observed moving between North America and other continents (USGS, 2013). The majority (75.3%) of these movements were within the Americas (Caribbean, Central America, and South America) (**Table 1**). An even larger majority of movements (83.2%) between North America and other continents were largely characterized by species which are considered the primary natural host for AIV – *Anatidae* and *Charadriiformes*. Movements between Asia and North America accounted for 19.2% of observations. These movements were largely (94.2%) for swans, geese, and ducks (*Anatinae* and *Anserinae*) (**Table 2**). The majority (96.4%) of these movements were between Asiatic Russia; however, movements were also documented between Eastern Asia, Australia, New Zealand, Melanesia, and Micronesia. For birds banded in Asia and recovered in North America the majority (91.0%) where in the Pacific Americas flyway however birds were also recovered in the Mississippi Americas flyway (5.5%) and Atlantic Americas flyway (3.4%).

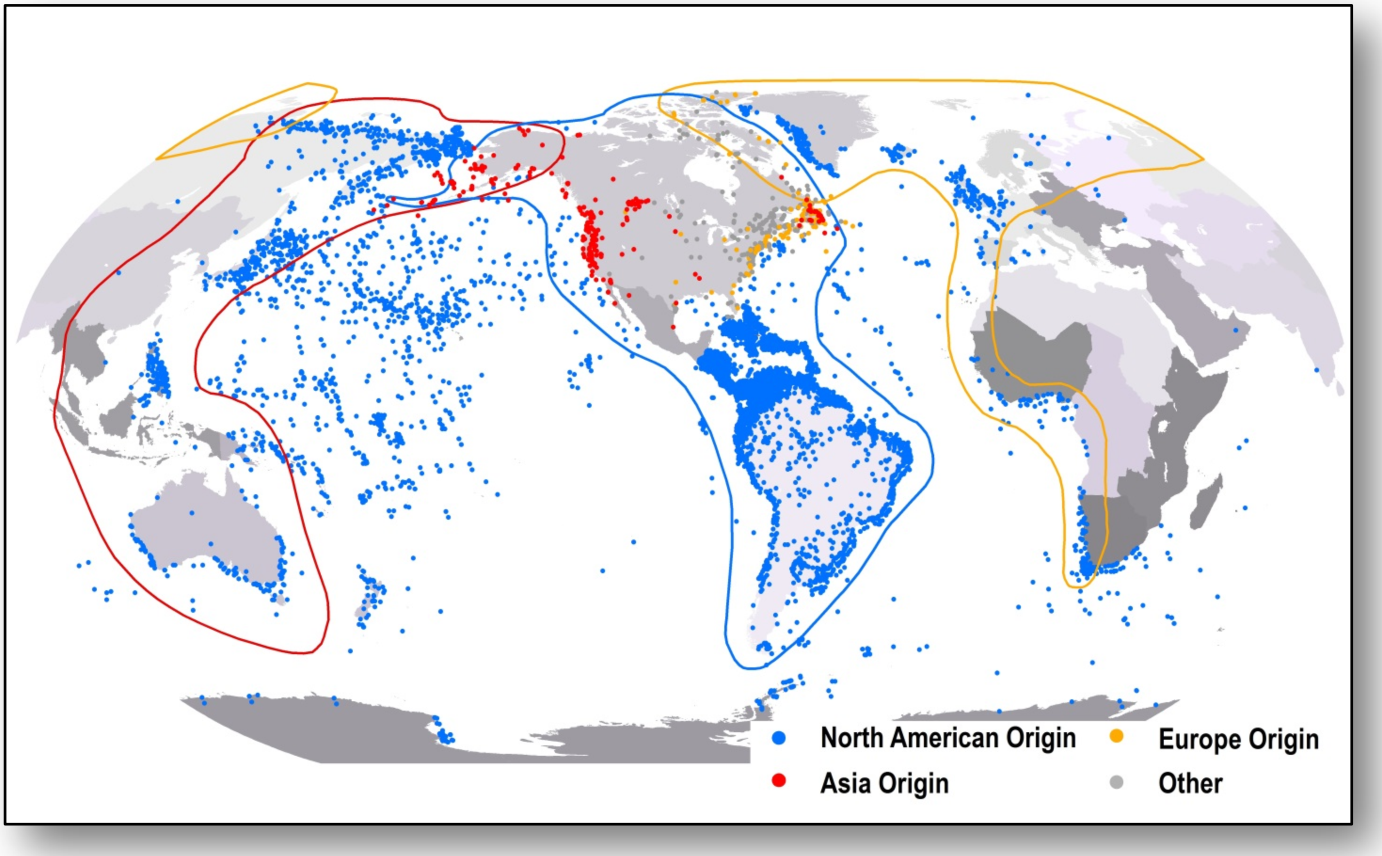

Figure 2. Distribution of bird bands for birds banded in North America and recovered in other continents (blue) and for birds banded in Asia (red) and Europe (orange) and recovered in North America between 1913 to present. Crude representations of the Americas flyway (blue polygon) and the East Asian/Australia flyway (red polygon) and East Atlantic (orange polygon) are included (USGS, 2013).

Table 1. Observed individuals moving between North America and other continents.

	Africa	Asia	Australia	Caribbean	Central America	Europe	Greenland	Oceania	South America	Total	Percent
Anatidae											
Anatinae		382		1431	661	16	102		1541	4133	31.7
Anserinae		2035			2	20	118	2		2177	16.7
Dendrocygninae				10						10	0.1
Total		2417		1441	663	36	220	2	1541	6320	48.5
Charadriiformes											
Charadriidae		1		4				1	4	10	0.1
Laridae	5	39		1272	628	15	26	5	1911	3901	29.9
Scolopacidae		34	10	81	7	21		15	445	613	4.7
Total	5	74	10	1357	635	36	26	21	2360	4524	34.7
Other	2	8	13	692	819	8	311	26	315	2194	16.8
Total	7	2499	23	3490	2117	80	557	49	4216	13038	
Percent	0.1	19.2	0.2	26.8	16.2	0.6	4.3	0.4	32.3		

Table 2. Observed individuals moving between North America and Asian, South Pacific, and Australia.

	Asiatic Russia	Australia New Zealand	Eastern Asia	Melanesia	Micronesia	Total	Percent
Anatidae							
Anatinae	372		10			382	14.9
Anserinae	2035				2	2037	79.4
Total	2407		10		2	2419	94.2
Charadriiformes							
Charadriidae	1			1		2	0.1
Laridae	38			1	4	43	1.7
Scolopacidae	21	11	13	2	9	56	2.2
Total	60	11	13	4	13	101	3.9
Other	7	19	1		20	47	1.8
Total	2474	30	24	4	35	2567	
Percent	96.4	1.2	0.9	0.2	1.4		

Wild Bird Populations

North America has a large and growing waterfowl population (USFWS, 2012). An estimated 62.3 million migratory waterfowl are present in North America, with the majority being ducks (48.6 million). Geese and swans account for an estimated 13.7 million individuals. Currently the waterfowl population in North America is 43 percent above the long-term average and increased 7 percent between 2011 and 2012 (USFWS, 2012). The majority of these species breed at mid-to northern-latitudes in North America with a substantial number annually migrating to the far northern margins of the continent. During the summer breeding season, this northern margin receives species from six continents: North America, South America, Asia, Africa, Australia, and Antarctica (Winker and Gibson, 2010). This results in North America, and in particular the northern margins (i.e. Alaska and Northern Atlantic region), having direct annual connections with wild birds from most of the world. Eurasian birds are common in in the northern region of North America (specifically Alaska) during the summer breeding period (Conant and Groves, 2005; Eldridge et al., 2005; Winker et al., 2007; Winker and Gibson, 2010).

Specific numbers on the estimated populations moving between Asia and North America are limited and only a few studies have attempted to quantify the totality of these populations. An estimated 33 species of waterfowl (*Anatidae*), 46 species of shorebirds (*Charadriidae* and *Scolopacidae*), and 15 species of gulls and terns (*Laridae*) move between Asia and North America (primarily Alaska) (Winker and Gibson, 2010). Of these 94 species, 11 species of waterfowl, 32 species of shorebirds, and 4 species of gulls and terns are Asian taxa that occur in relatively small numbers in North America (Winker and Gibson, 2010). The remaining species are shared between Asia and North America and represent the largest number of individuals moving intercontinentally. It is estimated that 195,000 to 593,000 waterfowl (*Anatidae*), 1.0 to 1.8 million shorebirds (*Charadriidae* and *Scolopacidae*), and 277,000 to 481,000 gulls and terns (*Laridae*) move between Asia and North America annually (Winker and Gibson, 2010). In aggregate, waterfowl, shorebirds, gulls, and terns represent 1.48 to 2.91 million birds annually moving between Asia and North America (**Table 3**). Furthermore, two analyses have estimated that 1,898 to 3,732 of these individuals are infected with Asian origin AI annually (Winker et al., 2007; Winker and Gibson, 2010).

Table 3. Estimated population sizes for families of waterfowl, shorebirds, gulls, and terns that move between Alaska and Asia (Winker and Gibson, 2010).

	Alaska		Asia	
Family	**Min**	**Max**	**Min**	**Max**
Anatidae	5,286,970	5,356,770	195,496	593,015
Charadriidae	85,680	100,670	2180	18,170
Scolopacidae	6,378,125	7,640,125	1,003,475	1,814,955
Laridae	3,521,901	3,645,175	277,151	481,175
Total	15,272,676	16,742,740	1,478,302	2,907,315

Susceptibility of Wild Birds to Avian Influenza

Experimental studies have investigated the susceptibility of various species of aquatic birds to infection and the potential for them to disseminate virus. Previous to the A(H5N1) outbreak that began in Asia and spread to Europe and Africa (1997-2006; circulation is still ongoing), most studies of waterfowl susceptibility to AIV were performed in mallards (*Anas platyrhynchos*) and Pekin ducks (*A. platyrhynchos domesticus*). With few exceptions, these studies produced asymptomatic infections in ducks and variable levels of viral shedding (Brown et al., 2011). There are few published studies of experimental AIV infections in shorebirds (Hall et al., 2011; Reperant et al., 2011; Hall et al., 2013). A few experimental trials have been conducted in gulls and terns using HP H5N2 (USA), HP H5N3 (South Africa) and various LPAIV. Further information on these studies is nicely summarized in Brown et al. (2011) and is not repeated here. In the past ten years, experimental studies of species susceptibility to AIV has mostly focused on HPAI H5N1 Eurasian strain that spread to multiple continents in the 2000's. These studies provided evidence that susceptibility to infection and the potential to spread avian influenza virus varies not only by virus subtype, but also on the species exposed.

Studies evaluating the potential for aquatic birds to become infected with poultry adapted strains of AIV are inconsistent. Research has shown that LPAIV that have adapted to chickens or other gallinaceous poultry do not necessarily replicate as efficiently in ducks (Spackman et al., 2010; Brown et al., 2011). The inference is that once an AIV has switched host preferences and adapted to gallinaceous poultry, it loses its adaptation for wild birds (Brown et al., 2011). However this may depend on the species and the specific AIV. Experimental infections of wild bird species with some HPAIV (i.e. A(H5N1) and HP H5N2) have shown that they survive infection and shed the virus without apparent disease (Stallknecht and Shane, 1988; Sturm-Ramirez et al., 2004; Hulse-Post et al., 2005). Furthermore, studies suggest that HPAIV may become less pathogenic to ducks, while retaining high pathogenicity to chickens (Hulse-Post et al., 2005; Sturm-Ramirez et al., 2005; Chen et al., 2006; Olsen et al., 2006). Despite these studies unidirectional adaptation appears generally likely for HPAIV, with the exception of A(H5N1), which has been widely documented in wild aquatic birds (Liu et al., 2005; Suarez, 2010).

There is less evidence for unidirectional adaptation of LPAIV to gallinaceous poultry. Sá e Silva et al. (2011), for example, demonstrated that cinnamon teal (*Anas cyanoptera*) and Chiloe wigeons (*Anas sibilatrix*) were susceptible to infection with poultry strains of Chilean H7N3 LPAIV and HPAIV. They suggested that cinnamon teal could be reservoirs for these AIV based on contact transmission between birds and evidence of a genetically similar virus isolated from a wild cinnamon teal. Canada geese (*Branta canadensis*) were not susceptible to experimental infection with A/turkey/Wisconsin/l966 but this virus was re-isolated in mallards which developed low levels of HI antibody following infection (Homme & Easterday, 1970). Similarly, experimental infection of mallards with A/turkey/Ontario/7732/66 resulted in minimal antibody titer (Stallknecht and Shane, 1988). In a challenge experiment with Franklin's Gulls (*Leucophaeus pipixcan*), Bahl and Pomeroy (1977) detected shedding of a LP A(H6N2) virus, originally isolated from turkeys, in tracheal swabs from naïve gulls co-housed with the inoculated gulls, indicating that the virus was able to transmit to contact birds.

Experimental infection studies may not reflect natural conditions or other factors that contribute to increased mortality of infected individuals. Within species, factors such as age, body condition, AIV exposure/infection history, and variation in immune responses may also influence a bird’s capacity to transport and shed influenza virus (Tolf et al., 2013). Most experimental infection studies have used immunologically naive aquatic birds which may overestimate the susceptibility (Tolf et al., 2013). At least three studies have reported high variation in LPAIV apparent prevalence among species in North America and proposed that differences in susceptibility to HP H5N1 (Eurasian) might be due to differential reproductive capabilities of AIV between host species (Perdue and Swayne, 2005; Garamszegi and Møller, 2007; Farnsworth et al., 2012). Mallards, likely the most investigated species in North America, have some of the highest levels of LP and HP AIV prevalence among wild waterfowl species (Munster et al., 2007; Ip et al., 2008; Kou et al., 2009). Studies on the effectiveness of mallards as long-distance virus carriers, however, are incongruent. One experimental infection study suggested that mallards are more likely to act as long-distance disseminators for AIV than other common duck species (Keawcharoen et al., 2008). However, field studies of wild mallards at staging areas suggested that AIV shedding times are too brief to make mallards an effective vector across continental and intercontinental scales (Latorre-Margalef et al., 2009). AIV infections in mallards appear to be largely influenced by previous exposure to the virus, although there are significant differences between individuals in terms of immune responses (Tolf et al., 2013). Seropositive individuals mostly showed no clinical disease and reduced cloacal excretion and shedding time post infection, providing arguments both for and against a potentially healthy carrier of AIV in mallards (Latorre-Margalef et al., 2009).

Several studies have observed the ability of AIV infected birds to migrate long distances. A recent study used feather stable isotopes and phylogenetic analysis to assess the influence of AIV infection on mallard migration and found no detectable difference in AIV prevalence between migrants and resident birds. Furthermore, the investigation did not detect changes in body condition which might be linked with AIV infection status of migrating birds (Hill et al., 2012a). In Africa a study documented the long-distance migration (at least 655 km) of an AIV infected white-faced whistling duck (*Dendrocygna viduata*) (Gaidet et al., 2008). Notably, one modeling study found that delayed migration of AIV-infected individuals led to a predicted lower total number of cases of infection each year. The reduced rate of AIV infection at staging sites caused the epidemic to proceed more slowly (Galsworthy et al., 2011). Such an effect would, of course, depend on species susceptibility to the strain of AIV.

The diversity of data, often incongruent, regarding the susceptibility of aquatic birds to AIV infection and the ability of AIV infected birds to migrate—particularly long distances—indicates that there is likely a high degree of inter- and intra-species heterogeneity regarding the affect AIV infection may have on migration or long-distance movements.

Environmental Persistence of AIV

An issue related to the infection and shedding of AIV by aquatic birds is the role of environmental persistence in the ability of aquatic bird populations to move these viruses. In poultry infections, it is known that AIV can be transmitted through contaminated water and other materials. Protection of the virus by organic material (such as feces), and cold, moist conditions allow virus to persist longer. HP H5N1 and H5N2 remained viable in liquid manure for 105 days in the winter during freezing conditions (Swayne, 2008). Several studies have detected AIV in surface water and chicken feces (summarized by Stallknecht et al. (2010)) and LP H3, H8, H11 and H12 subtypes have been found in the sediment of a frozen pond. HP H5N1 Eurasian strain RNA was detected during a local outbreak in samples collected from households, not only in poultry feces and swabs from the feathers of recently dead birds, but also in dry soil, water plants in ponds, and mud using real-time RT-PCR (Vong et al., 2008). However, no live virus was isolated, so it was unclear whether all of these substrates could actually infect a bird (wild or domestic) upon contact or ingestion.

Numerous experimental studies have demonstrated the ability of avian influenza viruses to persist in water. These include LP H5N2, H5N3, H5N8, H7N3 and H7N4 strains and HP A(H5N1) Eurasian strain (Brown et al., 2007b). The estimated persistence varied by subtype and strain, as well as with water temperature and salinity. Under some conditions, certain subtypes were estimated to persist over 365 days. This is consistent with other subtypes of avian influenza, where duration of persistence in water of LP strains of H1N1, H4N6, H5N1, H5N2, H6N2, H6N4, H6N8, H8N4, H9N2, H10N7, H11N6 and H12N5 was affected by water temperature, salinity, and pH (Stallknecht et al., 1990; Swayne and Halvorson, 2008; Nazir et al., 2010). Depending on the water conditions, these viruses could persist from a few days to several months.

A recent study by Farnsworth et al. (2012) in North America evaluated the evidence for environmental persistence at the landscape scale using data collected as part of the USDA A(H5N1) surveillance program (Deliberto et al., 2009; Pedersen et al., 2010). Furthermore, they evaluated if the presence of AIV in a watershed during the over wintering period increased the probability of AIV presence during the following breeding season within the watershed. This study found strong support over wintering of the virus. Specifically, for every seven days the minimum temperature fell below zero, the chance a bird would test positive for AIV increased by 5.9 percent. In addition the study found a 12.0 percent increase in the chance an individual would test positive during the breeding season for every 10.0 percent increase in the interval apparent prevalence during the prior overwintering season. These observations suggest that viral deposition in water and sub-freezing temperatures during the overwintering season may act as determinants of waterfowl infection risk during the subsequent breeding season.

All these studies demonstrate that AIV can persist in the environment, with survival depending on a number of factors. Most isolates of AIV from aquatic bird habitats are associated with feces and water. These findings indicate that environmental processes play an important role in the ecology and persistence of AIV, however much is not understood. Virus has been detected in sediment and in surface water, but no evaluation has been done to determine the distribution of virus in the whole ecosystem. Virus may not be concentrated evenly throughout the water column, and if diluted sufficiently may not infect birds that feed at the water sediment interface; conversely, it is possible that virus is present from association with feces or other organics within or at the sediment surface where it could infect birds such as dabbling ducks and geese that feed at this interface.

Intercontinental Movement of Avian Influenza

Analyses of LPAIV viral ribonucleic acid (RNA) segments from Eurasia and North America have described two distinct groupings of lineages that correspond to hemispheric origins (Gorman et al., 1990a; Gorman et al., 1990b; Ito et al., 1991). Several lines of evidence from recent surveys of LPAIV in aquatic birds have suggested that intercontinental transfer of AIV from Asia to North America via aquatic birds is rare (Kilpatrick et al., 2006; Krauss et al., 2007; Winker et al., 2007). These findings include lack of detection of Asian HPAIV H5N1 in North America, phylogenetic divergence between Asian and North American lineages of LPAIV (Ito et al., 1995; Widjaja et al., 2004) and low levels of reassortment between Asian and North American lineages of LPAIV (Makarova et al., 1999; Wallensten et al., 2005; Krauss et al., 2007; Dugan et al., 2008). However, most of these studies examined viruses obtained from species that are not transcontinental migrants or from mid-latitude locales of North America, which are far removed from sources of Asian lineages of avian influenza. Genetic characterization of LPAIV viruses obtained from a large sample of known intercontinental migrants, and from an area close to the Asian continent, would provide a better test of whether migratory birds can transfer Asian lineages of LPAIV into North America (Koehler et al., 2008).

More recent phylogenetic analyses have documented evidence of genetic exchange between North American and Eurasian strains of LPAIV via reassortment in long-tailed ducks (*Clangula hyemalis*) and white-winged scoter (*Melanitta deglandi*) in the Great Lakes region (Fries et al., 2013), northern pintails (*Anas acuta*) and dunlin (*Calidris alpina*) in Alaska (Koehler et al., 2008; Wahlgren et al., 2008), ruddy turnstones (*Arenaria interpres*) and herring gulls (*Larus argentatus*) along the Atlantic Coast of North America (Makarova et al., 1999), in mallards (*Anas platyrhynchos*) in Minnesota (Jackwood and Stallknecht, 2007), and waterfowl in Alberta, Canada (Krauss et al., 2007) (Appendix A). Intercontinental genetic exchange appears to be bi-directional. RNA segments from North American AIV have been observed in guillemots (*Uria aalge*) in Europe (Wallensten et al., 2005) and waterfowl in Asia (Bean et al., 1992; Liu et al., 2004). North American RNA segments were found in a South American influenza virus isolated from cinnamon teal (*Anas cyanoptera*) (Spackman et al., 2007) and some Asian lineages of the N8 RNA segment were more similar to North American virus isolates from northern pintails (*Anas acuta*) than to other Asian reference samples (Koehler et al., 2008). At least one study has documented the persistence of Eurasian AIV genetic components following introduction into North America (Bahl et al., 2009).

Phylogenetic analyses have also documented evidence of genetic exchange of AIV between Eastern Asia and Europe. Recent studies by Gao et al. (2013), Liu et al. (2013) and Kageyama et al. (2013) have indicated that A(H7N9) most likely resulted from a reassortment of avian influenza viruses of at least four origins—East Asian duck origin for HA (likely wild), European wild duck origin for NA, and at least two H9N2 chicken viruses for the internal genes. Based on their data they extrapolated potential intermediate hosts that facilitated reassortment. Analysis indicated that the HA genes were circulating in the East Asian flyway in a wild passerine bird (*Fringilla montifringilla*) and in wild ducks and domestic ducks. The NA genes were introduced from European lineages via migratory ducks and then transferred to ducks (wild or domestic) in China along the East Asian flyway. The H9N2 AIV circulating in chicken and duck populations in eastern China possibly reassorted with the H7 and introduced N9 avian influenza viruses in ducks, resulting in the emergence of the new H7N9 lineage.

The observed frequency of AIV reassortment events varies greatly between studies (Appendix A) ranging from 0.25 to 45.0 percent (17 studies). Krauss et al. (2007) excluded closely related AIV replicates and reported the frequency of intercontinental exchange between Asian and North American virus lineages at the gene segment level as 0.64 percent. Koehler et al. (2008) observed a frequency of reassortment events of 45 percent for Asian and North American virus lineages. However, when closely related AIV replicates were excluded using the method reported by Krauss et al. (2007), the frequency of reassortments was reduced to 3.1 percent. In a global study by Dugan et al. (2008) a 6 percent hemispheric reassortment was found, and when corrected for closely related AIV replicates was reduced to 0.64 percent. Even with correction, Koehler et al. (2008) and Pearce et al. (2011) found a considerably higher frequency of AIV reassortment events that contain Asian lineages in North America than previously reported studies. Because the likelihood of detecting intercontinental reassortment events is directly related to the degree of contact among host populations, Koehler et al. (2008) proposed that the higher frequency of intercontinental reassortment may be associated with Alaska northern pintails that are highly migratory and use breeding grounds on both sides of the Bering Strait, closer to the source of Asian lineages. The lower frequency of LPAIV strains found with Asian lineages by Krauss et al. (2007) and Dugan et al. (2008) may be due to dilution (i.e., further reassortment) related to the increased distance, temporal separation, and ecological barriers from areas where Asian lineages commonly circulate.

In North America, a greater number of AI genes of Eurasian origin have been documented along continent margins in regions such as western Alaska (Pearce et al., 2010; Ramey et al., 2010b) where North American flyways overlap with Eurasian migratory flyways (Ramey et al., 2010a). In contrast, birds sampled in areas further from continental margins appear to have viruses with fewer lineages of Eurasian origin (Krauss et al., 2007; Pearce et al., 2009). This phenomenon was supported in a recent study using stable isotope assays for quantifying migration status of wild mallards within the Pacific flyway (Hill et al., 2012a). The study found that AIV prevalence and diversity did not differ in wintering mallard ducks with different migration strategies, and while migrant mallards did introduce AIV, the viruses did not circulate as the predominant viruses in resident birds. Rather, resident mallards from more temperate latitudes appeared to act as reservoirs, possibly contributing to the unseasonal circulation and extended transmission period of AIV (Hill et al., 2012a).

Lam et al. (2012) indicated that some of these differences may be explained by strongly spatially structured waterfowl populations in North America, with relatively infrequent gene flow among localities and especially between those that are spatially distant or belong to different flyways. Doherty et al. (2009) assessed the connectivity of North America flyways for 18 waterfowl species using an annealing algorithm and bird banding data and found a high degree of spatial segregation for some species at the flyway scale. They also suggested that the strongly spatially dependent pattern of AIV gene flow may serve as a useful estimator of disease spread when a novel AIV lineage enters aquatic bird populations of North America. More specifically, the new colonizing AIV may be similarly structured by flyway and distance, following the same route of dispersal as endemic AIV in North America. This may indicate ecological and bio-geographical barriers for movement of AIV into central North American flyways (Lam et al., 2012). However, there are incongruent studies. Another study reported a recent transfer of Southeast Asian lineage AIV N6 subtype into the Great Lakes region of North America (Fries et al., 2013). The study determined that an N8 reassortment event supports a point source introduction of an H14 subtype and subsequent reassortment with co-circulating waterfowl AIV in the region.

LIMITATIONS OF EXISTING DATA AND STUDIES

Aquatic Bird Populations, Movements, and Flyways

North America has the largest and most comprehensive system in the world for monitoring populations and movements of migratory birds (USFWS, 2012). However, significant uncertainties in population estimates of aquatic birds are well recognized (Smith, 1995). In addition, for populations that are shared between continents there is greater uncertainty (Winker et al., 2007; Winker and Gibson, 2010). These issues may impact, by orders of magnitude, assessments of the intercontinental movement of aquatic birds between North America and Eurasia and existing and future assessments for AIV introduction into North America (Kilpatrick et al., 2006; Winker and Gibson, 2010). Beyond estimations of population size, the specific regions aquatic birds move between North America and Eurasia are vague and require further investigation, particularly as they relate to movement of pathogens such as AIV (Koehler et al., 2008; Lam et al., 2012).

In Asia there is no detailed description of the ecological and bio-geographic factors which may result in spatial structuring of Asian flyways. There are a limited number of telemetry studies for species such as northern pintail that are thought to be important for AIV movement (Prosser et al., 2009; Gaidet et al., 2010; Takekawa et al., 2010; Yamaguchi et al., 2010; Iverson et al., 2011).

Thus it has been difficult to draw broad conclusions from these studies due to generally small sample sizes, limited number of species marked, and a focus on movement of A(H5N1) by aquatic birds in Asia. Broader studies that investigate the ecology and life history of Asian aquatic birds are needed to further understand continental movement, maintenance, and ecology of AIV in aquatic birds. Several studies have noted that biased estimates of bird movement—or in some instances complete lack of information regarding movement—have led to erroneous conclusions about AIV movement in aquatic birds (Winker and Gibson, 2010). These are significant limitations to existing studies on intercontinental movements of aquatic birds and the spread of AIV that will need to be addressed to fully assess pathways for intercontinental movement of AIV.

Movement of AIV by Aquatic Birds

Koehler et al. (2008) postulated that studies estimating the frequency of intercontinental transfer of AIV segments may be biased low. This is because studies of hemispheric reassortment among LPAIV viruses in North American birds have made comparisons to Asian gene sequences available on the National Center for Biotechnology Information (NCBI) database. Because many Asian AIV strains in the database were not classified to species, studies likely compared isolates from species that do not interact with ducks and that have different movement patterns and breeding/wintering distributions. Koehler et al. (2008) suspected that a whole-genome comparison of LPAI viruses sampled from northern pintails in Asia and Alaska would show greater evidence of exchange than they found. They predicted that such a comparison would reveal individuals with a mixture of both Asian and North American lineages in Asia. Indeed, similar observations were made in a single green-winged teal (*Anas crecca*) wintering in Japan (Kida et al., 1987; Bean et al., 1992).

Some studies have also questioned whether LPAIV, in which all eight gene segments are of Asian descent (i.e. completely Asian-origin viruses), persist in substantial frequency in some North American waterfowl species (Koehler et al., 2008). Several studies (Krauss et al., 2007; Dugan et al., 2008), observed no completely Asian viruses, but this may be related to the fact that the species evaluated (e.g. northern pintails) tend to show high rates of LPAIV exposure (Ito et al., 1991; Ip et al., 2008) combined with 'extremely frequent' reassortment (Dugan et al., 2008). Reassortment can only occur when individuals are co-infected by multiple LPAIV strains (Sharp et al., 1997) and the probability of co-infection appears positively related to overall virus prevalence. Wang et al. (2008) found co-infection in 16 percent of samples where the overall prevalence of influenza viruses was 26 percent (i.e. 61 percent of the positive samples were co-infections). Novel virus types may be more likely to result in co-infections (Sharp et al., 1997). However, direct long-distance movement of individual birds may not be necessary to facilitate virus movement. Viruses may also spread via sequential contact among aquatic birds along a wide range of migratory pathways and through environmental reservoirs, although the exact conditions necessary for host-to-host transfer, such as species assemblages, animal densities, and environmental characteristics remain largely unknown (Smith, 1995; Uchida et al., 2008a).

It remains unclear whether the level of gene reassortment observed in LPAIV of intercontinental migrants, such as northern pintails, is common to all avian hosts that breed in Alaska or only those with strong migratory connections to Eurasia. While there are numerous birds that migrate from Australasian and Eurasian wintering areas to Alaska each year, there are also many species and populations that remain in Alaska for breeding and within North America for wintering (Winker and Gibson, 2010). Determining the degree of migratory and population connectivity of avian taxa between Asia and North America (e.g. Alaska) is important for the prioritization of species to target for AIV surveillance and for understanding what factors influence the introduction of novel pathogens to North America. It has not been determined which bird species that do not regularly migrate between hemispheres can be effective sentinels for detecting foreign-origin viruses in North America. At least one study (Pearce et al., 2011) determined that mallards, which are ubiquitous in North America and share habitat with a diversity of waterfowl, including highly migratory northern pintails, would be a good sentinel species even though their intercontinental movements are rare. Moreover, mallards may be an effective bridge species, distributing AIV from their cosmopolitan relatives to new locations during their annual migrations (Pearce et al., 2011).

Transmission of AIV from Poultry to Aquatic Birds

Studies evaluating the potential for aquatic birds to become infected with poultry adapted strains of AIV have shown variable results and may reflect differences in susceptibility among host species, viral characteristics, and experimental design (Stallknecht and Shane, 1988). Differences in response among species have been demonstrated for specific AIV isolates following experimental infection of both wild and domestic birds. Despite significant experimental work studies—specifically exploring the potential of poultry adapted strains to infect aquatic birds and identifying factors that may limit transmission—are somewhat limited and are often restricted to a limited number of species. Unidirectional adaptation appears in question for some LP and HP AIV and should not be assumed for all viruses without experimental evidence (Stallknecht and Shane, 1988; Hulse-Post et al., 2005; Sá e Silva et al., 2011).

PATHWAY ASSESSMENT

We identified four factors (location, viral characteristics and pathogenicity, host species affected, and unforeseen stochastic events) that—based on the available scientific literature—may affect the likelihood of a successful introduction of AIV, including influenza A(H7N9), into aquatic birds in North America. We use this framework to assess three issues, a) the overall likelihood of an unreassorted AIV to be introduced by aquatic birds into North America from Eurasia, b) the potential for A(H7N9) to be translocated intercontinentally into North America via aquatic birds and, c) the potential emergence of new AIV strains in North America through the aquatic bird pathway. We provide an overall assessment of the likelihood for these three issues using standard terms described in Appendix C.

Assessment of Translocation of Avian Influenza into North America

Location and timing—First among these factors is the location and timing of the virus in relationship to North American migratory bird breeding grounds and flyways. Eastern Russia and Western Alaska represent an area of overlap between Asian and North American flyways. Some AIV host species of aquatic birds (notably northern pintails) are known to nest – and migrate – on opposite sides of the Bering Strait (Flint et al., 2009; Hupp et al., 2011). Sympatry among breeding ducks, and possibly other breeding aquatic birds in these areas, provides opportunities for the spread of divergent AIVs between Eurasia and North America during seasonal migrations (Flint et al., 2009).

Multiple lines of evidence, including published studies, bird banding data, and known locations of flyways suggest that the continental margins of North America are the most likely areas for the introduction and spread of Eurasian origin AIV by aquatic birds. A larger number of avian influenza genes of Eurasian origin have been documented in areas such as Western Alaska (Pearce et al., 2010; Ramey et al., 2010b) where North American and Eurasian flyways overlap. However, there appears to be relatively infrequent AIV gene flow between locations that are spatially distant or belong to different flyways (Krauss et al., 2007; Pearce et al., 2009; Lam et al., 2012). This has been observed not only between flyways but also along a north-south gradient within North American flyways where birds sampled in areas farther south (i.e. mid-continent) and more distant from continental margins appear to have viruses with fewer lineages of Eurasian origin (Krauss et al., 2007; Pearce et al., 2009; Hill et al., 2012a; Hill et al., 2012b; Lam et al., 2012).

These observations are consistent with strongly spatially structured waterfowl populations that are influenced by ecological and bio-geographic barriers, which influence migratory movements of birds – and their viruses (Doherty et al., 2009; Lam et al., 2012). Combined with other factors (described below), these ecological barriers may reduce the probability of whole-genome transfer of Asian lineage AIV into North America. To date, no such occurrences of whole-genome Eurasian AIV have been documented among migratory birds in North America (Krauss et al., 2007; Dugan et al., 2008). However, AIV with genetic segments of Eurasian origin have been found in North American aquatic birds, particularly in Alaska and along the Atlantic coast. These viruses are the result of reassortment between Eurasian and North American phylogenetic lineages of AIV that co-infect birds in these regions.

Timing of AIV infection in aquatic birds may contribute to probability of long distance movement and persistence of the virus (Krauss et al., 2007; Pearce et al., 2009; Hill et al., 2012a; Lam et al., 2012). Several studies observed a pattern of dilution by time and distance, indicating that the likelihood of AIV reassortment (or extinction) increases with distance from the source where the virus was found (Krauss et al., 2007; Pearce et al., 2009; Lam et al., 2012). Contrasting with these observations is the rapid, long-distance dispersal of A(H5N1) out of Southeast Asia across Central Asia into parts of Europe and Africa (Kilpatrick et al., 2006; Salzberg et al., 2007). Several studies have indicated that concentrated breeding of migratory aquatic birds from different regions of Asia contributed to the spread of A(H5N1) across Central Asia (Liang et al., 2010; Newman et al., 2012). A cold weather anomaly is thought to have played a pivotal role in the subsequent movement of this virus out of Central Asia and into Europe (Ottaviani et al., 2010).

Viral characteristics and pathogenicity—Viral pathogenicity and the susceptibility of host species to particular strains of AIV are important factors in potential transport of the viruses to new areas. Experimental challenge studies focusing on HP A(H5N1) have shown that different bird species vary greatly in their resistance to infection, clinical signs, and quantity and duration of viral shedding (Perdue and Swayne, 2005; Brown et al., 2006; Feare, 2007; Garamszegi and Møller, 2007; Kalthoff et al., 2008; Keawcharoen et al., 2008). Within species, factors such as age, body condition, AIV exposure/infection history, and variation in immune responses may also influence a bird's capacity to transport and shed influenza virus (Tolf et al., 2013). Gaidet et al. (2010) demonstrated that migratory aquatic birds have the potential to disperse HP A(H5N1) over hundreds of kilometers. They reached this conclusion by analyzing movements of birds monitored by satellite telemetry in relation to experimental infection studies. Other characteristics of the virus may influence viral survival or reassortment once introduced to a new area or population. The fitness of the virus (i.e., its ability to 'compete') has been hypothesized to play a role in the persistence of some LPAIV in North America and other regions (Bahl et al., 2009; Hill et al., 2012a). Similarly, in some parts of the world Asian HPAIV H5N1 became the dominant virus, all but replacing endemically circulating AIV (Kayali et al., 2011). These factors would likely contribute to the ability for a novel AIV from Eurasia to become established in North America or move intercontinentally.

Host species affected—Host species ecology, particularly with regard to breeding and migratory behavior, is also important to the spread of AIV between continents (Ito et al., 1991; Runstadler et al., 2007; Ip et al., 2008).Species life history traits, such as social factors, , gregariousness, vagility, site fidelity, dispersal characteristics, and habitat preferences, all influence viral exposure (Ely et al., 2013). As a result, different species of aquatic bird play varying roles in the geographic dispersal of AIV (Lam et al., 2012). In order for affected species in Eurasia to translocate AIV over long distances, particularly intercontinentally, they must frequently move between Eurasia and North America or have contact with North American species. They must also be able to successfully move while infected and shedding virus. These host characteristics and host ecology are important factors. Species, such as northern pintails, which move long distances intercontinentally among divergent AIV gene pools and maintain relatively high prevalence of AIV, may have a greater chance for long-distance translocation of AIV or co-infection with Eurasian and North American lineages (Runstadler et al., 2007; Ip et al., 2008). These birds can act as bridge species with waterfowl such as mallards, which then carry virus to other locations during their annual migrations (Pearce et al., 2011).

Unforeseen stochastic events—Unforeseen stochastic events have contributed to the long distance movement of AIV virus across continental barriers. An example is the extreme cold weather in Eastern Europe that caused large numbers of waterfowl to move from Asia into

Europe and Africa in 2005 and 2006. This event is believed to have contributed to the spread of HP A(H5N1) in the eastern hemisphere (Kilpatrick et al., 2006; Bragstad et al., 2007; Salzberg et al., 2007; Ottaviani et al., 2010). Furthermore, there remains the potential for unique, unanticipated, long-distance movement of a single infected bird (Uchida et al., 2008b; Gaidet et al., 2010). While these types of events are impossible to predict they do play a role in the potential for intercontinental movement of AIV from Eurasia to North America.

Aggregate assessment of potential—Together the four factors described above provide a framework for evaluating the likelihood of a whole-genome introduction of a specific strain of AIV, such as influenza A(H7N9), from Eurasia into North America through migratory aquatic birds. For such an event to occur, a series of conditions must be met, including:

1) Sufficient proximity of the emergent virus to North America, or North American wild bird flyways, that it doesn't reassort before reaching the continent.
2) Exposure of a susceptible species to a sufficient viral dose that the bird becomes infected, carries the unreassorted virus while the bird is still healthy – without the virus reassorting – across the Pacific Ocean (most likely the Bering Strait);
3) If the bird does not reach North America, it must shed the virus in a location where it persists in the environment long enough for another susceptible bird to be infected and then transport the virus – unreassorted – intercontinentally;
4) Once the virus reaches North America, it must survive, remain intact (i.e., unreassorted), infect another susceptible species, and be carried, unreassorted from northern breeding grounds to other parts of the flyway, or to other flyways; and
5) The virus must be robust enough to compete with other AIV strains infecting aquatic birds in North America.

Certainly, there could be multiple viral exposures and thus multiple opportunities for such events to occur through bird migrations. However, to meet all these conditions, even for multiple exposures, may be a limiting circumstance for intercontinental translocation of unreassorted AIV from Eurasia into North American aquatic birds.

Currently there are few data describing species assemblages, flyways, and migratory movements of aquatic water birds in Asia. These limitations hinder our ability to make definitive statements about the probability of an unreassorted (whole genome) AIV from a mid- to southern-latitude location in China entering North America through aquatic bird populations. Given the available information, we judge the potential for introduction of a complete AIV (whole genome) into North America as possible with a likelihood of such an event ranging from low to extremely low.

Assessment of Translocation of A(H7N9) into North America

Location and timing—Assuming that Asia and North America have a similar spatially structured pattern with regard to bird migration, flyways, and AIV spread, then there may be similar ecological and bio-geographic barriers that limit the potential for A(H7N9) to move –

unreassorted – from its current location into North America. The putative origin of the A(H7N9) outbreak (in poultry and humans) is at a latitude of 31.2° (i.e., similar to San Diego California which is at latitude 32.7°), which is approximately 8,800 km south of waterfowl breeding areas in northern Asia and Alaska (**Figure 1**). Nevertheless, based on a recent analysis, A(H7N9) virus is thought to have resulted from reassortment of AIV from wild birds origination in Europe and East Asia as well as domestic chickens and ducks in eastern China (Liu et al., 2013). Studies by Gao et al. (2013), Liu et al. (2013), and Kageyama et al. (2013) have indicated that A(H7N9) most likely resulted from a reassortment of avian influenza viruses of at least four origins—east Asian duck origin for HA (likely wild), European wild duck origin for NA, and at least two H9N2 chicken viruses for the internal genes. Furthermore, they proposed that the reassortment events most likely took place in Shanghai, or provinces Zhejiang and Anhui.

Thus, it is possible that components of A(H7N9) exist closer to North America than the current location of the outbreak in China. There is additional uncertainty regarding details of migratory bird flyways in Asia. Currently, information is lacking to confirm that these flyways are structured similarly to North American flyways. Furthermore, it is worth noting that European, African, and Asian waterfowl flyways are also spatially structured, yet A(H5N1) was rapidly translocated to distant locales via migratory birds (Kilpatrick et al., 2006; Salzberg et al., 2007) so the potential does exist for long-distance movement of an unreassorted AIV from Asia. Spring migration is currently taking place in China and if A(H7N9) is present in wild aquatic birds there may be some potential for northward movement to the breeding grounds in northern Asia and Alaska.

Viral characteristics and pathogenicity—Recently completed pathogenicity studies for A(H7N9) suggest that gallinaceous poultry are highly susceptible to this new A(H7N9) strain. These studies have found that A(H7N9) appears to be poultry adapted, replicating to high titers in upper respiratory tract of gallinaceous species, specifically chickens and quail (ARS, 2013) Previous studies have shown that some LPAIV that replicate well in poultry do not necessarily replicate well in aquatic birds, specifically ducks (Spackman et al., 2010; Brown et al., 2011). It is thought that the viral mutations required to adapt to poultry result in the virus being less adapted to ducks (Brown et al., 2011). However, there are currently no experimental infections with A(H7N9) in duck or goose species and unidirectional adaptation appears in question for some AIV, both HP and LP, and should not be assumed for all viruses without experimental evidence (Stallknecht and Shane, 1988; Hulse-Post et al., 2005; Sá e Silva et al., 2011). If waterfowl are susceptible to the virus and mortality is high, the potential for A(H7N9) to move long distances via aquatic birds would be inhibited. There are no data to aid in determining how A(H7N9) may interact with endemic North American AIV circulating in aquatic birds.

Host species affected—To date no migratory species have yet been reported to be infected by influenza A(H7N9) virus. However, domestic and feral bird species have been identified in Guangdong province. Five of the affected provinces, including Guangdong, are located in an important ecological zone in China where large potential AIV reservoir populations are present, including migratory waterfowl and traditional farming systems using ducks and chickens (**Figure 2**) (Martin et al., 2011). In addition, an important aquatic bird congregation site (Poyang Lake) is in Jiangxi province. This region has been suspected of playing an important role in A(H5N1) epidemiology where the internal segments of the 1996 geese HPAIV H5N1 virus may have originated (Mukhtar et al., 2007; Martin et al., 2011). One recent risk analysis of A(H7N9) found increasing risks of spread in Northern China as the migratory season continued this year (Shi et al., 2013a). Different species of aquatic birds play varying roles in the geographic dispersal of AIV so the potential for A(H7N9) to move via aquatic birds would depend on the biology of the affected species biology (Lam et al., 2012). Recent phylogenetic analysis indicates that wild birds played some role in the emergence of the virus (Liu et al., 2013). Until wild aquatic birds are identified as potential carriers of A(H7N9), and sufficient surveillance is conducted to determine if the virus is present in aquatic birds, there will remain uncertainty concerning how affected species biology will play a role in the spread of this virus.

Unforeseen stochastic events—There is always potential for unforeseen stochastic events that may contribute to the rapid long-distance movement of A(H7N9) potentially across continental barriers. While rare, there are several examples of events contributing to the spread of AIV by aquatic birds. These include a rare cold weather event in Eastern Europe that influenced the intercontinental movement of A(H5N1), and the occasional long-distance movement of an infected bird (Uchida et al., 2008b; Gaidet et al., 2010). However, it is impossible to determine the likelihood of these events and how they contribute to the uncertainty of this assessment.

Aggregate assessment of potential—Currently, influenza A(H7N9) is affecting provinces in Eastern China. This region has been identified by two studies as high risk for A(H5N1) (Fang et al., 2008; Martin et al., 2011). Both studies found that human population density and poultry density were driving factors in characterizing risk in this region. Moreover, five of the affected provinces, including Guangdong where bird species have tested positive, are located in an important ecological zone in China where key epidemiological drivers for AIV emergence, persistence, and spread are present (Martin et al., 2011). These include a large potential AIV reservoir population of migratory waterfowl, coupled with a traditional farming system (i.e. typical duck pond system) and high animal and human population densities (Mukhtar et al., 2007; Martin et al., 2011). Recent phylogenetic analysis of A(H7N9) indicates that migratory birds from two distinct and distant flyways (Mediterranean-Black Sea and East Asian) may have contributed to the emergence of the virus (Liu et al., 2013).

The available data indicate that the potential for introduction of A(H7N9) into North America through aquatic migratory birds is possible, but the likelihood ranges from extremely low to low due to:

1) The distance and potential bio-geographical barriers between Asian and North American flyways;

2) Preliminary pathogenicity studies, which suggest gallinaceous poultry are highly susceptible to A(H7N9) - there is evidence that some poultry adapted strains of AIV are less efficiently transmitted in aquatic birds.
3) Lack of evidence (to date, on the basis of limited surveillance) that migratory aquatic bird species have yet been affected by influenza A(H7N9).

There is a large degree of uncertainty in our assessment. Currently there are few data available describing which species are affected, the extent of surveillance in aquatic bird species, and the susceptibility of waterfowl to A(H7N9). These limitations hinder our ability to make definitive statements about the likelihood of A(H7N9) spreading into and through aquatic bird populations to North America. As new information emerges the potential for introduction of A(H7N9) could change rapidly and stochastic events that may influence the movement of the virus are possible.

Potential for Emergence of New AIV in North America

There is strong scientific evidence that aquatic birds introduce Eurasian origin AIV genetic material into North America annually, that AIV prevalence in populations shared between Eurasia and North America can be high (<0.1 to 32.2%), and two studies have estimated that between 1,898 and 3,732 birds in Alaska are infected with Asian origin AIV annually (Winker and Gibson, 2010). Furthermore, co-infection with multiple AIV strains is common (i.e. 61% of positive birds are co-infected) suggesting that some small proportion (albeit an extremely small proportion) of the aquatic bird population along the margins of North America may be infected with unreassorted Eurasian AI viruses. In the case of A(H5N1) several studies have indicated that while South China and Southeast Asia may constitute the virus pool, Northern Asia may be the source of the A(H5N1) resulting from concentrated breeding migratory aquatic birds from different regions of Asia which increased the possibility of gene mutation (Liang et al., 2010; Newman et al., 2012). Recent phylogenetic analysis of A(H7N9) indicates that migratory birds from two distinct and distant flyways (Mediterranean-Black Sea and East Asian) may have contributed to the emergence of this virus (**Figure 3**) (Liu et al., 2013).

There is a demonstrated and continual emergence of new AIV resulting from reassorted Eurasian and North America AIV strains among aquatic birds, especially in Alaska (Pearce et al., 2010; Ramey et al., 2010a; Ramey et al., 2010b; Pearce et al., 2011; Lam et al., 2012). These new strains of AIV are generally well tolerated by their natural avian hosts, but have unknown pathogenic potential for poultry and humans. Several studies have suggested that surveillance of AIV using genetic markers (changes in the viral genome) of adaptation could help identify which new strains of AIV may be of concern (Pepin et al., 2010; Tejeda and Capua, 2011; Flanagan et al., 2012).

Figure 3. Distribution of important aquatic bird wetlands, including Poyang Lake, in comparison to provinces currently affected by avian influenza A(H7N9) (ECDPC, 2013). Inset map depicts affected provinces in comparison to three aquatic bird flyways. The Americas flyway (blue polygon), and two flyways, East Asian/Australia flyway (red polygon) and Mediterranean-Black Sea (orange polygon), thought to have migratory birds which contributed genetic material to A(H7N9) (Liu et al., 2013).

Conclusion of Pathway Assessment

Research over the past decade resulting from significant investment in A(H5N1) surveillance has dramatically expanded our understanding of AIV in North America and the evolutionary and ecological forces that lead to the spread and persistence of AIV within and between continents. Yet, there remain gaps in our knowledge of these complex systems. The framework and factors we identified may affect the likelihood of intercontinental movement of AIV and aid in addressing these scientific gaps.

AIV have been circulating among wild aquatic birds for millennia, frequently reassorting into novel genetic forms. In light of these characteristics, and of the uncertainties and obstacles to the transmission of AIV among multiple hosts and across bio-geographic barriers, it is extremely difficult to predict where, when, and what particular strain of influenza virus might emerge and to which hosts it might cause harm. The assessment of the likelihood that a specific virus will be carried within or between continents through aquatic birds – or by some other pathway – is currently limited by available data. It is well established that aquatic birds have moved strains of AIV, largely unaltered, over long distances. However, this phenomenon appears to be rare. Of greater likelihood is the movement of segments of the AIV genome which continually appear in new regions, including intercontinentally, as a result of reassortment events that take place along the migratory routes of aquatic birds.

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APPENDIX A. SUMMARY OF EXPERIMENTAL SUSCEPTIBILITY STUDIES OF AVIAN INFLUENZA VIRUSES IN WATERFOWL SPECIES WITH SPECIFIC FOCUS ON H5 AND H7 SUBTYPES.

Species Susceptible (common name[a])	AIV subtype	Route of Infection	Age Inoculated	Morbidity (observed clinical signs)	Mortality (death or euthanasia)	Shedding (route[b], duration)	Citation
Mallard (MA) Northern pintail (NP) Blue-winged teal (BWT) Redhead (RH) Wood duck (WD) Laughing gull (LG)	A(H5N1) (strain A known to affect wild birds, strain B not)	Intranasal	10-16 week	WD (strain A: 2/3 , strain B: 1/3) LG (strain A & B: 2/3 each) Others: none	WD (strain A: 2/3 , strain B: 1/3) LG (strain A & B: 2/3 each) Others: none	MA: OP (B only), CL (A only) NP: OP (A&B), CL (B only) BWT: OP (A&B), CL (A only) RH: OP (A&B), CL (A only) WD, LG: OP & CL (A&B)	(Brown et al., 2006)
Wood ducks	A(H5N1)	Intranasal	12-16 week	Low dose: 4/5 High dose: 5/5	Low dose: 4/5 High dose: 5/5	Primarily OP, 4-7 days	(Brown et al., 2007a)
Whooper swan (WS) Black swan (BS) Trumpeter swan (TS) Mute swan (MS) Bar-headed goose (BHG) Cackling goose (CG)	A(H5N1)	Intranasal	12-16 week	WS (4/4) BS (5/5) TS (5/5) MS (5/5) BHG (2/5) CG (3/4)	WS (4/4) BS (5/5) TS (5/5) MS (5/5) BHG (2/5) CG (3/4)	OP and CL: 1-3 dpi until death (up to 8 days)	(Brown et al., 2008)
Mute Swan (MS)	A(H5N1)	Oculo-oronasal	1-4 year		5/5 (high dose) 6/7 (low dose)	High-dose: OP, CL (2-6 dpi) Low dose: OP, CL (3-11 dpi)	(Kalthoff et al., 2008)
Tufted duck (TD) Eurasian pochard (EP) Mallard (MA) Common teal (CT) Eurasian wigeon (EW) Gadwall (GA)	A(H5N1)	Intratracheal and Intra-esophageal	8-11 month	TD (7/8) EP (4/8) Others: None	TD (3/8) EP (1/8)	TD: OP EP: OP, CL MA: OP CT: OP, CL EW: OP GA: OP	(Keawcharoen et al., 2008)
Mallard (MA) Redhead (RH) Wood duck (WD) Laughing gull (LG)	LP: H5N2, H7N3, H3N8	Intranasal	10-16 week	None	None	MA: CL (expt H7N3: OP) – up to 21 days RH: Primarily OP – up to 12 days WD: Primarily OP – up to 12 days LG: Primarily OP – up to 16 days	(Costa et al., 2011)
Mallard (MA)	LP: H7N7, H5N2	Intra-esophageal	3 month	None	None	H7N7: feces, OP, CL – up to 12 days (plus intermittent, up to 3.7 days) H5N2: unclear	(Jourdain et al., 2010)

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[a] Bar-head goose (*Anser indicus*); black swan (*Cy. atratus*); blue-wing/common teal (*Ana. crecca*); cackling goose (*Branta hutchinsii*); Eurasian pochard (*Ay. ferina*); Eurasian wigeon (*Ana. penelope*); gadwall (*Ana. strepera*); laughing gull (*Larus atricilla*); mallard (*Anarhynchos platyrhynchos*); mute swan (*Cygnus olor*); northern pintail (*Ana. acuta*); redhead (*Ay. americana*); trumpeter swan (*Cy. buccinator*); tufted duck(*Aythya fuligula*); whooper swan (*Cy. cygnus*); wood duck (*Aix sponsa*).

[b] Shedding infectious virus route: OP = oropharyngeal, CL = cloacal; duration of shedding provided, if available in publication

APPENDIX B. OBSERVED FREQUENCY OF REASSORTMENT AMONG ASIAN AND NORTH AMERICAN VIRUS LINEAGES FROM WILD BIRDS (*ANSERIFORMES* AND *CHARADRIIFORMES*).

Study	Year	Reassortment Frequency	AIV Type	Region/Species Assessed	Conclusion	Comments
Fries et al. Evidence for the Circulation and Inter-Hemispheric Movement of the H14 Subtype Influenza A Virus. PloS ONE. 2013.	2013	Not analyzed.	LPAIV	Great Lakes – USA Long-tailed ducks and white-winged scoter	Circulation of novel AIV in sea ducks; previously found only in Asia; point source movement or undetected reservoir?	Three H14 isolates sequenced.
Hill et al. Migration strategy affects avian influenza dynamics in mallards (Anas platyrhynchos). Molecular Ecology. 2012.	2012	Not analyzed.	LPAIV	AK, CA, OR, WA – USA Mallard	Migrants imported virus that did not readily circulate. Residents were AIV reservoirs, facilitating early AIV circulation at lower latitudes.	34 AIV isolated for stable isotope analysis. Migrants and residents had similar AIV prevalence and diversity.
Lam et al. Migratory flyway and geographical distance are barriers to the gene flow of influenza virus among North American birds. Ecology Letters. 2012.	2012	Pacific – 16 transferred lineages from East Asia Atlantic – 20 transferred lineages from East Atlantic	LPAIV	All USA Flyways 5 spp. of Dabbling ducks, 1 Shorebird (ruddy turnstone)	Intra-continental spread of AIV by migratory birds is subject to major ecological barriers, including spatial distance and avian flyway.	Approximately 1,000 AIV sequences studied.
Van Borm et al. Phylogeographic analysis of avian influenza viruses isolated from Charadriiformes in Belgium confirms intercontinental reassortment in gulls. Archives of Virology. 2012.	2012	Not calculated but stated as rare.	LPAIV	Northern Europe Gulls, ruddy turnstone	American virus components existed in some European Charadriiformes	Whole genome sequencing of 9 LPAIV
Pearce et al. Interspecific exchange of avian influenza virus genes in Alaska: the influence of trans-hemispheric migratory tendency and breeding ground sympatry. Molecular Ecology. 2011.	2011	Mallard 14.5% (0.65% by Krauss et al. 2007 method) Pintail 35% (3.5% by Krauss et al. 2007 method)	LPAIV	AK – USA Summer breeding grounds Mallard, pintail	Mallards infected with Asian origin viruses through transfer from sympatric highly migratory species.	Intercontinental movements and occurrence of Asian AIV reassortment events within North American waterfowl are correlated.

Study	Year	Reassortment Frequency	AIV Type	Region/Species Assessed	Conclusion	Comments
Wille et al. Reassortment of American and Eurasian genes in an influenza A virus isolated from a great black-backed gull (Larus marinus), a species demonstrated to move between these regions. Archives of Virology. 2011.	2011	1 of 1 isolated virus (out of 38 birds)	LPAIV	Newfoundland Great black-backed gull	Long-distance gull migration helps to move of AIV genes between Eurasia and America.	AIV prevalence low in gulls (<0.1–13%) and seabirds (<0.1–4.26%) but many have serology of past infection.
Bahl et al. Gene flow and competitive exclusion of avian influenza A virus in natural reservoir hosts. Virology. 2009.	2009	No percentage given.	LPAIV	North America Ducks and shorebirds	Viral gene flow from Eurasia led to exclusion of some endemic AIVs in North America; no whole genome replacement.	H6 AIV have changed (both external and internal genes), H4 AIV have been stable.
Pearce et al. Avian influenza at both ends of a migratory flyway: characterizing viral genomic diversity to optimize surveillance plans for North America. Evolutionary Applications. 2009.	2009	6.6% of California AIV segments were in mixed lineage clades w/ sequences from Asia and North America	LPAIV	AK, CA – USA Pintail	LPAIV lineages observed in Alaska and the nucleotide composition of LPAIV lineages is not maintained through fall migration.	3,045 samples, 30 AIV isolates were compared to 38 Alaska AIV isolates from Koehler et al. 2008.
Dugan et al. The Evolutionary Genetics and Emergence of Avian Influenza Viruses in Wild Birds. PloS Pathogens. 2008.	2008	6% (<1% by Krauss et al. 2007 method)	LPAIV	AK, MD, MO, OH – USA Ducks, gulls, shorebirds	AIV in wild birds forms transient "genome constellations," continually reshuffled by reassortment.	167 complete viral genomes – 26% mixed subtypes; Large geographic separation from Asia
Koehler et al. Genetic evidence of intercontinental movement of avian influenza in a migratory bird: the northern pintail (Anas acuta). Molecular Ecology. 2008.	2008	45% (3.1% by Krauss et al. 2007 method)	LPAIV	AK – USA Pintail	Wild birds move AIV between continents with higher degree of transfer in Alaska than elsewhere in North America.	Whole genome analysis; underestimates viral exchange due to inappropriate Asian reference samples.
Wahlgren et al Gene Segment Reassortment Between American and Asian Lineages of Avian Influenza Virus from Waterfowl in the Beringia Area. Vector-Borne and Zoonotic Diseases. 2008.	2008	1 of 3 isolated AIV samples had 1 Asian lineage segment.	LPAIV	Siberia – RUS AK – USA Dabbling ducks, geese, gulls, waders	AIV isolate from Delaware was closest relative; H6 from Asia is established in North America.	Fecal swabs and feces collected. 4 of 676 samples AIV-positive

Study	Year	Reassortment Frequency	AIV Type	Region/Species Assessed	Conclusion	Comments
Jackwood and Stallknecht. Molecular Epidemiologic Studies on North American H9 Avian Influenza Virus Isolates from Waterfowl and Shorebirds. Avian Diseases. 2007	2007	No percentage given.	LPAIV	MN – USA Mallard DE/NJ – USA Ruddy turnstone	Waterfowl and shorebird H9 viruses no longer segregate into clear North American and Eurasian lineages.	Examined the HA gene from six H9 AIV compared to 44 known H9N2 viruses.
Krauss et al. Influenza in Migratory Birds and Evidence of Limited Intercontinental Virus Exchange. PloS Pathogens. 2007.	2007	No whole genome transfers; gene segment transfers 0.25-1.77% outsider events	LPAIV	AB – CAN DE/NJ – USA Ducks, gulls, shorebirds	Exchange of whole AIV between the Eurasian and American lineages occurs infrequently.	6,767 segments and 248 whole AIV analyzed; closely related LPAIV replicates excluded as not representing independent events.
Wallenstein et al. Multiple gene segment reassortment between Eurasian and American lineages of influenza A virus (H6N2) in Guillemot (Uria aalge). Archives of Virology. 2005.	2005	3 of 26 (11.5%)	LPAIV H6N2	Baltic Sea – EUR Guillemot	Isolated AIV is chimera of North American and Eurasian strains.	All 3 positive birds were nestlings. 10 adults negative.
Krauss et al. Influenza A Viruses of Migrating Wild Aquatic Birds in North America. Vector-Borne and Zoonotic Diseases. 2004.	2004	No information provided on reassortment.	LPAIV	Alberta – CAN Ducks DE/NJ – USA Gulls, shorebirds	AIV prevalence in ducks = 22.2% – high rate due to nestlings. AIV prevalence in shorebirds = 6.1%.	Cyclic 2-year periodicity of AIV infection in ducks and shorebirds
Liu et al. Interregional Transmission of the Internal Protein Genes of H2 Influenza Virus. Virus Genes. 2004.	2004	2 of 4 isolated viruses had one gene segment each of North American lineage.	LPAIV	Hokkaido – JAPAN Ducks	Interregional transmission of AIV occurred between the North American and Eurasian ducks.	Compared HA sequences of four isolated H2 viruses with two H2 viruses from Japan database.
Marakova et al. Transmission of Eurasian avian H2 influenza virus to shorebirds in North America. Journal of General Virology. 1999.	1999	No percentage given.	LPAIV	Alberta – CAN Northern USA Dabbling ducks DE/NJ – USA Gulls, shorebirds	Divergence of H2 HA into American and Eurasian lineages. Transfer of Eurasian H2 gene into one group of shorebirds.	Group of recent American AIV isolates from gulls and shorebirds in Delaware Bay were Eurasian lineage AIV.

APPENDIX C. QUALITATIVE SCALES OF LIKELIHOOD

This appendix defines the qualitative likelihood scale used to describe the probability of events in this pathways assessment. Qualitative scales attach a specific narrative phrase which conveys a meaning to terms used to describe the likelihood of an event occurring. Generally, it is best to choose an expression where there is some evidence for a high degree of consensus for its interpreted meaning (Theil, 2002). For example, use of the narrative phrase *"there is a high likelihood that the event will occur"* has been interpreted as a probability that ranges from 0.60 to 0.97 (60 to 97 % chance of occurrence); and the expression *likely* has been interpreted to range from 0.63 to 0.77 % (Behn and Vaupel, 1982; Reynolds et al., 1987). To date, there is not one universally accepted or utilized likelihood scale, and the scales are customized as appropriate for specific assessments. The OIE handbook on qualitative risk analysis does not prescribe a specific likelihood scale, although it provides examples for terms which might be used in likelihood scales such as *low, negligible, high* etc. (World Organization for Animal Health (OIE), 2006). Error! Reference source not found. **Table A-1** lists adjectives to describe likelihoods used in this assessment.

Table A-1. The modified likelihood scale used in this assessment adapted from Standards Australia for qualitative risk assessment in fisheries management (Fletcher, 2005).

Category	Probability Range
Likely	It is expected to occur
Occasional	May occur sometimes
Possible	Some evidence to suggest this is possible here
Unlikely	Uncommon, but has been known to occur elsewhere
Rare	May occur in exceptional circumstances
Low	It is very unlikely that the event will occur
Extremely Low	It is very unlikely that the event will occur but is not insignificant
Negligible	The likelihood that the event will occur is insignificant

Drake Northern Pintail in flight.

Photo Credit: Mimi Drake