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Predicting the global spread of H5N1 avian influenza

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The spread of highly pathogenic H5N1 avian influenza into Asia, Europe, and Africa has resulted in enormous impacts on the poultry industry and presents an important threat to human health. The pathways by which the virus has and will spread between countries have been debated extensively, but have yet to be analyzed comprehensively and quantitatively. We integrated data on phylogenetic relationships of virus isolates, migratory bird movements, and trade in poultry and wild birds to determine the pathway for 52 individual introduction events into countries and predict future spread. We show that 9 of 21 of H5N1 introductions to countries in Asia were most likely through poultry, and 3 of 21 were most likely through migrating birds. In contrast, spread to most (20/23) countries in Europe was most likely through migratory birds. Spread in Africa was likely partly by poultry (2/8 introductions) and partly by migrating birds (3/8). Our analyses predict that H5N1 is more likely to be introduced into the Western Hemisphere through infected poultry and into the mainland United States by subsequent movement of migrating birds from neighboring countries, rather than from eastern Siberia. These results highlight the potential synergism between trade and wild animal movement in the emergence and pandemic spread of pathogens and demonstrate the value of predictive models for disease control.

emerging | introduced species | model | trade | zoonotic disease

Highly pathogenic H5N1 avian influenza emerged in Hong Kong in 1996–1997 (1) and by early October 2006 had subsequently caused outbreaks in poultry or wild birds in 53 countries and 256 human cases, including 151 deaths (www.who.int/csr/disease/avian_influenza/en/). Hundreds of millions of chickens, ducks, turkeys, and geese have died or have been culled to prevent the spread of the virus. Coupled with export bans on affected countries the disease has had an economic impact of >$10 billion (2). Despite efforts to eradicate H5N1 in southeast Asia it has spread to central Asia, Europe, and Africa. Migratory birds, the transport of poultry and poultry products, and the trade in wild birds all have been hypothesized as pathways of introduction. However, their role in individual H5N1 introduction events and in future spread is not well understood and has been debated extensively (3–9).

Determining the pathways by which H5N1 is spread has critical implications for predicting and preventing the future spread of this virus (10). If the risk of H5N1 spread to a country is highest through the movement of migratory birds, then surveillance at migratory stopovers such as Alaska will likely yield the first evidence of introduction, a strategy that matches current U.S. Department of Interior and Agriculture policy (11). In this scenario, the timing of the highest risk of introduction would coincide with periods of peak bird movement and pathways of migration (12). Prevention of future outbreaks would be facilitated by eliminating contact between farmed poultry and migrating birds, as was attempted by several European countries in 2005–2006. Alternatively, if the risk of H5N1 introduction is higher via the trade in poultry and poultry products, then monitoring poultry imports and eliminating imports from high-risk countries should be a higher priority for reducing the probability of H5N1 introduction. Finally, H5N1 has been found in wild birds imported into Europe and other countries as part of commercial trade in wild birds (4), making this another potentially important pathway unless all imported birds are quarantined, tested for avian influenza, and culled where necessary.

We determined the most likely pathways for the introduction of H5N1 into each of 52 countries by using global data on country-to-country imports and exports of live poultry, trade in wild birds, and the migratory and cold weather movements of wild ducks, geese, and swans, which are considered to be the main reservoirs for highly pathogenic H5 and H7 subtypes of avian influenza, including H5N1 (6, 13, 14). For each of these three pathways we estimated risk as the number of H5N1-infectious bird days for an introduction by multiplying the number of birds entering or passing through a country by an estimate of the prevalence of H5N1 and by an estimate of the number of days that each bird would shed virus. We then used data on the trade in poultry and wild birds and the migration patterns of wild birds to predict the future risk of spread of H5N1 to new countries. Because of the variability in trade restrictions and the delays of several days to over a month between the start of an H5N1 outbreak and the implementation of trade bans, we predicted future spread under two scenarios. First, we estimated the number of infectious bird days caused by the poultry trade assuming no restrictions on the poultry trade were imposed on H5N1-infected countries. Second, we predicted the risk of introduction assuming that no country would import poultry from another country that had reported H5N1 in poultry (unless that country was considered H5N1-free), but that countries reporting H5N1 in wild birds could export poultry freely, and that these exports might contain infected birds.

Results and Discussion

Past Spread of H5N1. We found that estimated numbers of H5N1-infectious bird days associated with poultry trade was >100-fold higher than for the two other pathways (wild bird trade and migratory birds) for introductions of H5N1 into Indonesia, Vietnam, Cambodia, Laos, Malaysia, Kazakhstan, Azerbaijan, Iraq, and Cote D'Ivoire and 57-fold higher for Sudan [Fig. 1*a* and supporting information (SI) Data Set]. In contrast, the number of infectious bird days was >58-fold higher for migrating birds passing first through regions with H5N1 and then to Thailand, Croatia, Ukraine, Niger, Bosnia and Herzegovina, Slovakia, Switzerland, Serbia, Burkina Faso, Poland, Denmark, Israel, the United Kingdom, and Djibouti at the time of H5N1 outbreaks than for imports of poultry and captive wild birds (Fig. 1*a* and SI Data Set). In addition, the number of infectious bird days associated with movements of wild birds after a cold weather event in Eastern Europe in January 2006

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The authors declare no conflict of interest.

Abbreviation: IBA, important bird area.

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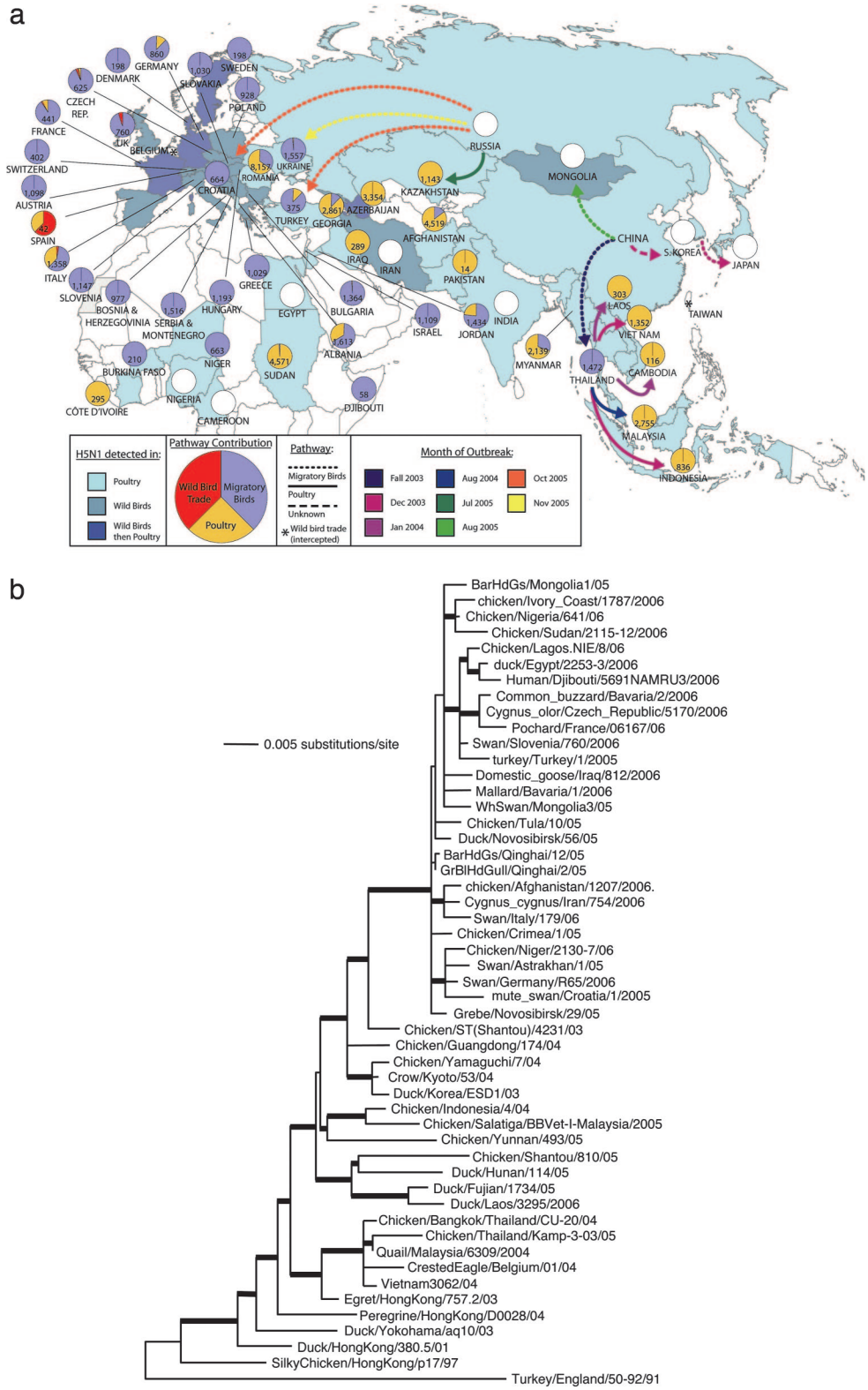

Fig. 1. Spread of H5N1 avian influenza and phylogenetic relationship of viral isolates. (*a*) Spread of H5N1 in Asia, Europe, and Africa. Pie charts show the total number of infectious bird days (number of infected birds × days shedding virus) and fraction from each pathway for birds moving between previous H5N1 outbreak countries and the focal country. Arrows give the month of the outbreak and hypothesized direction of spread for 2003–2005 introductions. The introductions of H5N1 into some countries (white pie charts) were inconsistent with reported wild bird and poultry trade (no imports from an H5N1-infected country were reported) and the direction of migratory birds in the months of the outbreaks (outbreaks occurred outside periods of bird movement; see *Methods*). Introductions into Belgium and Taiwan through the trade in wild birds were intercepted and did not lead to outbreaks in poultry or wild birds. (*b*) Maximum-likelihood phylogram showing the genetic relationship between samples of strains of H5N1 avian influenza isolated between 1997 and 2006 (with England 1991 as an outgroup) for the hemagglutinin gene. Nodes with thick, gray lines have bootstrap support >70%, based on 100 replicates.

ECOLOGY

were >100-fold greater than the trade in poultry or wild birds for H5N1 introductions into other European countries (Greece, Bulgaria, Hungary, Slovenia, Sweden, Austria, Bosnia and Herzegovina, Slovakia, Switzerland, and Serbia and Montenegro) (Fig. 1*a* and SI Data Set). The fact that poultry were not subsequently found infected in most of the countries in these latter two groups (all but Denmark and Sweden) after H5N1 was detected in migratory birds also argues against the involvement of the trade in live poultry, in which the virus usually causes substantial mortality. Migratory birds, the trade in wild birds, and the trade in poultry were all possible pathways for H5N1 introductions to Turkey, Romania (which imports poultry from Turkey), Albania, Italy, France, Germany, Georgia, Afghanistan, Myanmar, Jordan, and Spain (Fig. 1*a* and SI Data Set). However, genetic analyses (see below) and the details of the outbreaks for Italy, France, and Germany suggest that introduction by cold weather-induced movement of wild birds was more likely (www.oie.int/downld/AVIAN%20INFLUENZA/A_AI-Asia.htm). In summary, the synergistic spread of H5N1 first by poultry in Southeast Asia and then by migratory birds to Europe facilitated rapid dissemination and introductions into many countries that would likely have remained free of the virus without this synergism.

H5N1 outbreaks in South Korea, Japan, Russia, Mongolia, Nigeria, India, Pakistan, and Cameroon were inconsistent with both reported poultry trade (no poultry imports were reported from H5N1-infected countries) and the timing and direction of migratory bird travel in the month of the outbreaks. This finding suggests that unreported or illegal trade of poultry or poultry products [e.g., chicken feces for fertilizer and aquaculture (7)], the trade in wild birds, movement of free-grazing domestic ducks (15), or irregular movements of wild birds led to these introductions and may have contributed to others. Alternatively, H5N1 may have been introduced earlier by migratory birds, but not detected until later (e.g., when it spread to poultry). This sequence of events is highly likely for introductions into Russia, Mongolia, Nigeria (9), and Spain, which occurred 1–2 months after periods of peak migration and involved isolates that were genetically closest relatives to isolates along migratory bird routes (see below).

The phylogenetic relationships of the H5N1 isolates currently available strongly support the spreading pattern outlined in Fig. 1*a* and provide additional insight into the introduction of H5N1 into Japan, Russia, Mongolia, Turkey, Italy, and France, and thus clarify three introductions that could not be resolved based on trade data. The recent isolates from South Korea and Japan (eastern clade) and Qinghai (China), Russia, Mongolia, Europe, and Africa (western clade) formed well supported clades, suggesting a common ancestor for each of these groups of isolates (Fig. 1*b*). Thus, the introduction into Japan was most likely from migratory birds passing through South Korea (16) and not from China (neither of which reported poultry exports to Japan in 2003), whereas the introductions into Russia and Mongolia likely originated from China. The France, Italy, and Turkey isolates formed a well supported clade with Russian (Novosibirsk, Tula) and Ukraine (Crimea) isolates (the source for migrating birds) that was distinct from south Asian isolates where poultry imports into France and Italy (India) and Turkey (Thailand) originate.

Birds imported into the United Kingdom, Belgium, and Taiwan from Southeast Asia (Fig. 1*a*) as part of the wild bird trade also tested positive for H5N1, although none of these introductions resulted in outbreaks in poultry or wild birds (4). The trade in wild birds may also have played a part in the introductions into Japan, Indonesia, and Malaysia, where imports number in the thousands per year.

These results represent an important advance over earlier general assertions that the spread of H5N1 involves both poultry and wild birds in Asia and Europe, respectively (5, 6, 12, 17, 18). Most importantly, they identify the most likely individual pathway for 36 of 52 H5N1 introduction events, which is not possible based solely on phylogenetic relationships of viral isolates (17). Our results demonstrate that the spread of H5N1 through Asia and Africa involved both migratory birds and poultry, whereas wild bird movements were the most important pathway for the spread into and throughout Europe.

Predicting Future Spread. In the absence of trade bans, H5N1 may be introduced through poultry to the remaining countries in Europe, throughout much of Africa, and to the Americas in the near future (Fig. 2*a*). However, even if countries with current outbreaks of H5N1 in poultry cease exports, the risk of H5N1 spread continuing through Europe, Africa, and into the Americas is possible through poultry exports (Fig. 2*b*) from countries with H5N1 in wild birds (which has repeatedly spilled over into poultry; Fig. 1*a*). In addition, because few birds regularly migrate between the Americas and areas of the Old World where H5N1 has been reported (Fig. 2*d*; H5N1 has not been reported in eastern Siberia or Ireland) both poultry and the trade in wild birds currently represent a larger risk than migratory birds for the spread of H5N1 to the Americas (Fig. 2 *b* and *c*) unless all birds are quarantined and tested for influenza on import (as they are in the United States). However, if H5N1 spreads into northeastern Siberia (including Wrangel Island), then the risk of introduction into the mainland United States by migratory birds will increase substantially, because several species of ducks, geese, and swans regularly cross the Bering Sea between their breeding and wintering grounds (3, 19) (Fig. 2*d*).

Our analyses demonstrate an important consequence of the synergistic spread of H5N1 by both poultry trade and wild birds (Fig. 1*a*): Although the risk of H5N1 introduction into the mainland United States by any single pathway is relatively low (Fig. 2), the risk of introduction by poultry to other countries in the Americas, including Canada, Mexico, and Brazil, is substantial unless all imported poultry is tested for H5N1 or trade restrictions on imports from the old world are imposed (Fig. 2 *a* and *b*). Subsequent spread by >4 million migratory ducks, geese, and swans (representing ≈2,600 H5N1 infectious bird days) from the south would then make introduction into the United States likely (Fig. 2*d*). Thus, current American surveillance plans (11) that focus primarily on the Alaskan migratory bird pathway may fail to detect the introduction of H5N1 into the United States in time to prevent its spread into domestic poultry.

Examination of previous outbreaks and surveillance efforts also provides insight into the most effective means of surveillance. Of the 23 H5N1 primary outbreaks detected first in wild birds (Fig. 1*a*), 17 were in dead or sick swans (*Cygnus* spp.) and the other 6 were in dead or sick geese or ducks (other species, including a gull and several birds of prey were also occasionally found infected with H5N1) (www.oie.int/downld/AVIAN%20INFLUENZA/A_AI-Asia.htm). Surveillance of live birds, even in areas where H5N1 is known to be circulating, has shown that the prevalence of H5N1 is quite low (0.0013 ± 0.00052) (17). These findings suggest that surveillance measures should focus on sick or dead birds such as swans and other waterfowl (family Anatidae) for the early detection of H5N1.

In this analysis, we used data on the numbers of migratory birds and the magnitude of the trade in poultry and wild birds to understand previous spreading events and predict the risk of introduction of H5N1 to currently uninfected countries. Although we were not able to account for illegal and unreported poultry trade, it is unlikely that doing so would alter the following four conclusions: (*i*) the spread of H5N1 in Asia and Africa included introductions both by poultry and wild birds, whereas the spread to European countries was more consistent with the movements of wild birds (Fig. 1); (*ii*) currently, the highest risk of H5N1 introduction to the Americas is through the trade in poultry, not from migratory birds (Fig. 2*b*); (*iii*) because of

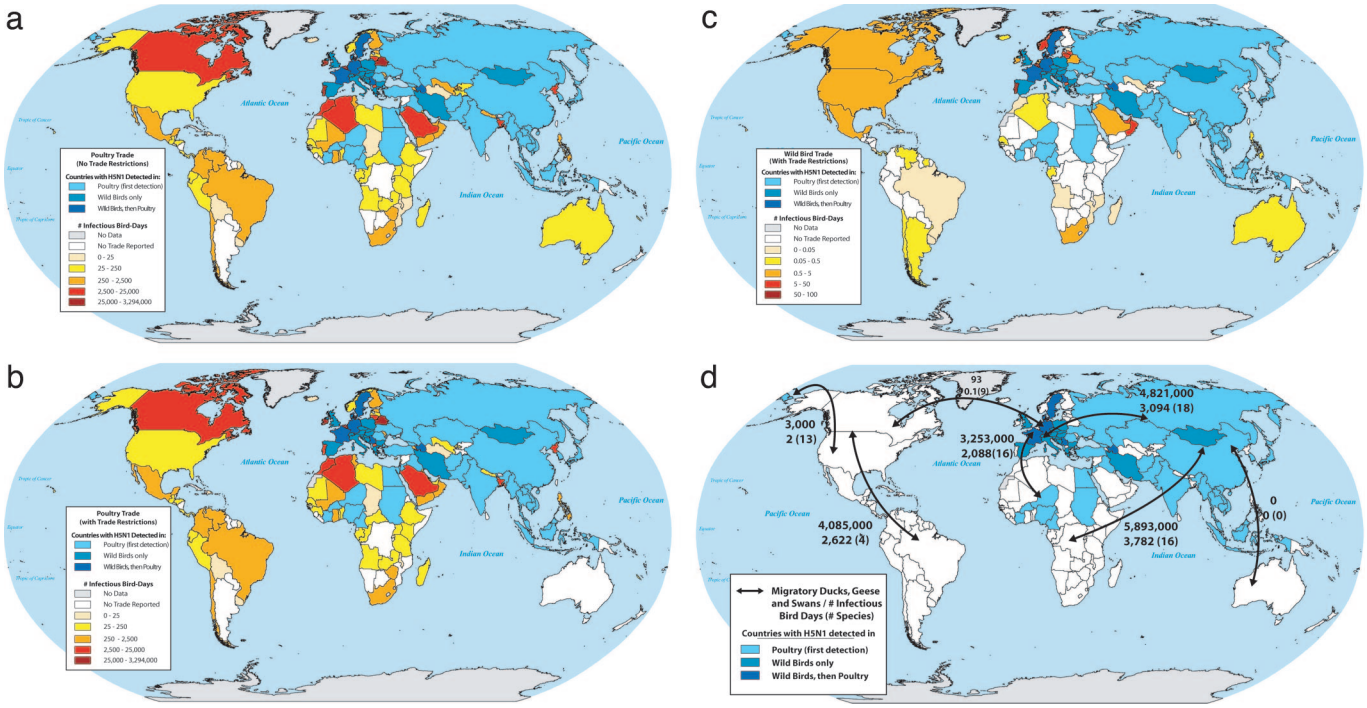

Fig. 2. Predicted risk of H5N1 avian influenza introduction from countries that have had H5N1 outbreaks (in blue). (*a–c*) Risk was estimated as the number of infectious bird days (number of infected birds × days shedding virus) caused by trade (presented as yearly totals/12 months) in: live poultry with no trade restrictions (*a*), live poultry with no exports from countries reporting H5N1 in poultry (France, Denmark, Sweden, and Germany are considered H5N1-free) (*b*), and captive wild birds with no exports from countries reporting H5N1 in poultry (*c*) as in *b*. (*d*) Estimated number of ducks, geese, and swans migrating between mainland continents, number of infectious bird days, and number of species (in parentheses). Numbers given between Asia and North America include only those that breed on mainland Asia and winter in North America south of Alaska; an additional 200,000–400,000 ducks breed in Siberia and molt or winter in or off the coast of Alaska. In addition, ≈20,000 geese migrate between Ireland and North America.

synergy between poultry and migratory bird pathways, countries adjacent to poultry importers, including the United States, are at higher risk for H5N1 introduction (Fig. 2*c*); and (*iv*) surveillance for H5N1 introduction in wild birds should focus on searching for and testing sick and dead (rather than live) birds arriving from the south and the north. We conclude that the most effective strategy to prevent H5N1 from being introduced into the western hemisphere would be strict controls or a ban on the importation of poultry and wild birds into the Americas and stronger enforcement to curb illegal trade. More broadly, our results highlight an important consequence of trade and globalization and show how predictive modeling can be used as a valuable tool for controlling the spread of pathogens.

Methods

To examine the past and future spread of H5N1 avian influenza, we determined the number of infectious bird days for each pathway as the simple product of three quantities: (*i*) the number of birds entering a country, (*ii*) the prevalence of infection, and (*iii*) the number of days that infected birds would be likely to shed virus. We obtained data on the country-to-country trade in poultry and wild birds from the Food and Agricultural Organization of the United Nations (http://unstats.un.org/unsd/comtrade/) and the U.S. Census Bureau, Foreign Trade Division. For poultry we used commodity code H1-0105 (live poultry, domestic fowls, ducks, geese, etc.). For wild bird trade we summed the totals from three commodity codes: H2-010632 [live birds (order Psittaciformes), including parrots, parakeets, macaws, and cockatoos], H2-010631 (live birds of prey), and H2–010639 [live birds (excluding H2-010631 and H2-010632)]. These numbers include all reported trade in wild birds, but do not include illegal or unreported trade.

Trade data were reported in dollar values and either kilograms or numbers of birds. We converted all of the data to numbers of live birds by using the median number of birds per kilogram (obtained from trade data where both were reported), 10.61 for poultry (most traded poultry are domestic fowl <185 g) and 1.92 for wild birds. To minimize errors in trade data caused by underreporting we used the maximum of the two quantities: (*i*) imports reported by country A from country B; and (*ii*) exports reported by country B to country A. To provide an additional check on the Food and Agriculture Organization of the United Nations (FAO) wild bird trade data, we also compared the 2003 FAO data to the 2003 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) bird trade data (http://sea.unep-wcmc.org/citestrade/trade.cfm) (the most recent complete data). We found that, on average, wild bird trade reported to FAO was 41.7 times higher than CITES trade (which only includes species of conservation concern), with only two countries reporting trade >100 birds per year having CITES numbers >15% higher than those reported to FAO (Bahrain and Portugal, with 1,000 per year and 7,520 per year, respectively).

We quantified migratory birds as a possible mechanism for H5N1 outbreaks reported in the months of peak migration [spring: March-May in Europe and Asia and February-April in Africa; fall: September-November (20)]. To obtain the most robust estimate, we used up to three different methods to calculate the number of ducks, geese, and swans (Anseriformes: Anatidae) migrating between H5N1-affected countries (SI Data Set). We included both diving ducks and dabbling ducks in the analysis, but dabblers were >75% of the total number of individuals for all countries considered. We multiplied estimates of the population size for each subpopulation (using midpoints if a range was given) (21) of waterfowl by the approximate

ECOLOGY

fraction of each subpopulation whose migration pathway from their breeding grounds to their wintering grounds would result in them passing both over an area with a reported H5N1 outbreak and then over the outbreak area (within a ≈50-mile radius) in the focal country. For many species where migration pathways are unknown we estimated this fraction by drawing parallel lines along the migration pathways connecting the breeding and wintering areas. We used the same method to determine the number of waterfowl migrating between continents. Given the uncertainty in the population estimates (21) and migration routes for each species our estimates for the total number of migrating waterfowl along each pathway have an estimated coefficient of variation of 50% (i.e., estimates are accurate within a 3- to 5-fold range).

We used two additional methods to estimate the number of migratory birds that may have introduced H5N1 into European countries in 2005. First, we summed the average of the minimum and maximum numbers of duck, goose, and swan species recorded as nonbreeding (excluding summer nonbreeders) or passage birds in important bird areas (IBAs) (22) within 50 miles of an outbreak in a focal country for species whose migratory pathway (19, 23) is likely to have passed through an H5N1 outbreak area. Approximately one-quarter of the IBAs near the H5N1 outbreak in Turkey had no data, so we divided the IBA estimate for this country by 0.75. Second, we obtained estimates of the mean wintering populations in H5N1 outbreak countries for the same species considered in the first two methods (24).

The first of our three methods estimated the fraction of each species that passed through an infected area and then into or through the focal country based on the size of breeding and wintering areas and approximate migratory routes. The second technique was based on actual counts at sites near focal country outbreaks and is likely to be the most accurate for species that congregate in large numbers on IBAs and are easily counted (geese and swans). Although some of these counts may be overestimates caused by double-counting of the same individuals at different sites, for most species they are likely to underestimate the number of individuals of species that migrate through, rather than winter in, IBAs, because single or repeated counts cannot account for turnover of individuals. In addition, several species migrate through the affected areas in small groups and are not concentrated into IBAs, and thus are unlikely to be accurately counted (e.g., Garganey, *Anas querquedula*). The third technique estimates the total population for each species in the entire country, rather than just within 50 miles of the outbreak area. It is likely to be more accurate for species that winter in substantial numbers near the H5N1 outbreak, but outside of IBAs, but may overestimate the number of individuals of a species that may have introduced H5N1 to a country if that species winters in areas far from the outbreak area (e.g., eastern Turkey). Our qualitative conclusions for determining the most likely pathway for European H5N1 introductions were identical with all three methods. For the 2003–2005 H5N1 introductions in Fig. 1*a* we used the mean of the estimates from the first and second methods for the number of migratory birds that may have introduced H5N1 into a country.

In January 2006, a period of cold weather in eastern Europe resulted in the movement of large numbers of waterfowl to the north and west and these birds may have introduced H5N1 in their movements. We estimated the number of birds that may have been responsible for these introductions by calculating the numbers of ducks, geese, and swans that overwinter in countries that were previously affected by H5N1 in the late fall of 2005 (Turkey, Romania, Croatia, and Ukraine). We assumed that 5–25% of the birds wintering in these countries would have flown west to other European countries, with the fraction decreasing with the distance between these countries (e.g., 5% of the waterfowl wintering in Turkey was estimated to reach France). Although these estimates involve substantial uncertainty, our qualitative conclusions were unaffected by a 5-fold increase or decrease in these fractions.

We did not include migratory shorebirds (Charadriidae) in our calculations because, although they can become infected with H5N1 (12), they appear to shed low quantities of virus (25) and as a group are generally thought to carry different types of influenza (14). Including shorebirds in our calculations would have increased the predicted future risk of H5N1 introduction (Fig. 2*d*) to two areas, Australia and Southern Africa. Neither of these regions are wintering grounds for migratory ducks, geese, or swans summering in Europe or Asia, but both are used by large numbers (>1 million) of shorebirds that breed in Asia and Europe (21).

To analyze the previous spread of H5N1 we compared migratory bird numbers to trade data from 2003 if H5N1 was first detected in 2003 or January 2004, and 2004 trade data if the first detection of H5N1 occurred between February 2004 and December 2004, and 2005 trade data for introductions that occurred in 2005 through July 2006. However, several countries had not yet reported trade data for 2005, and Vietnam did not report in 2004 or 2005. We used 2003 data for Vietnam and an average of 2003–2004 data for the others. To examine past spread, we assumed that a country's poultry exports were infected only if it reported H5N1 in poultry. In addition, we assumed that poultry exports from France, Germany, Denmark, and Sweden were not infected, because these countries had at most two infected farms with no subsequent outbreaks.

To compare the risk for H5N1 introduction of poultry and wild bird trade to migratory birds we divided the yearly import/export numbers by 12 months, and the migratory bird numbers by 2 because the peak period of migration for individual migratory species is ≈2 months (20). To compare the number of migratory birds and poultry that might be shedding H5N1 we multiplied the numbers generated above by estimates of periods of viral shedding, $2 \pm \mathrm{SD} = 1.0$ d for poultry (26) and 6.0 ± 0.95 d for migratory ducks (17), and 3 ± 3 d for the trade in wild birds (this was unknown so a wide range was assumed); and prevalence, 6/4,674 ($0.0012 \pm$ SE 0.00052) for highly pathogenic H5N1 in asymptomatic migrating ducks, 6/13,115 (0.00046 ± 0.00020) for highly pathogenic H5N1 in all wild birds tested (which may underestimate prevalence in traded wild birds because of crowded shipping conditions), and 512/51,121 (0.010 ± 0.00044) for H5N1 in apparently healthy poultry, based on large-scale surveillance efforts in China (17). Another smaller-scale study in Russia found 22 of 466 wild birds were positive for H5 by PCR, including 4 that were positive for H5N1 (pathogenicity was not reported) in the Republic of Kalmikya, a region that had not yet reported H5N1 in poultry (27). These data confirm the presence of H5N1 in wild birds away from outbreak areas. We estimated confidence bounds on the contribution of each pathway to each introduction (Fig. 1*a*) and categorized introductions as being more likely the result of one of the pathways if the lower limit of the 95% confidence interval for the pathway with the greatest number of infectious bird days was at least 5-fold higher than the mean value for the next most important pathway (SI Data Set).

DNA sequences for the hemagglutinin gene (and a subset of neuraminidase sequences including isolates from India) of H5N1 influenza A viruses were downloaded from GenBank. Initially, all sequences that could be easily aligned were examined (>475 sequences) and used in a simple analysis of phylogenetic relationships (these trees are available from R.C.F.). We then pared down the data set to 50 sequences that represented the variation within particular clades or geographic regions of interest, with a goal of at least two isolates per country. We used the sequence from a 1991 isolate from a turkey in the United Kingdom as an outgroup. Maximum-parsimony (MP), maximum-likelihood (ML), and neighbor-joining (NJ) analyses were used to recon-

struct relationships among the sequences (28). For ML and NJ analyses, we used a Kimura two-parameter model of sequence evolution. All analyses resulted in very similar trees, with strong support by bootstrap for a number of the possible clades. Bootstraps of 1,000 replicates were conducted for the MP and NJ analyses and of 100 replicates for the ML tree.

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