excerpts from
Reducing Pandemic Risk, Promoting Global Health

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INFLUENZA
When called upon by host governments, PREDICT provided assistance with influenza outbreak response and preparedness by providing technical guidance and transfer of technologies. PREDICT also worked to address critical research gaps as needed to inform on global surveillance and testing strategies by examining the factors influencing the emergence and spread of influenza A viruses, especially from or by potential wildlife reservoirs.

While PREDICT surveillance activities were not initially designed to specifically target influenza A viruses, our diagnostic strategy did include protocols to detect the viruses. Thus PREDICT contributed protocols for testing to laboratories as needed and also facilitated influenza A screening of wildlife and selected human samples in collaborating laboratories.

INFLUENZA A H7N9 VIRUS OUTBREAK INVESTIGATION

In 2013, an H7N9 influenza A virus strain caused a deadly outbreak in people in the provinces of southeastern China. This outbreak came as a surprise, as this strain had never before been diagnosed as the cause of disease in people. PREDICT researchers investigated potential source populations and the conditions for the genesis of the H7N9 virus outbreak using active surveillance, screening of virus archives, and evolutionary analyses. This research revealed that the H7N9 outbreak lineage originated from reassortment of H7 viruses and enzootic H9N2 viruses and that the H7 viruses likely were transmitted from domestic ducks to chickens in China during two separate events (Lam et al. 2013). The researchers discovered a related H7N7 influenza virus in chickens that has the ability to infect mammals experimentally, which provides evidence that H7 viruses may pose a greater public health threat than previously recognized (Lam et al. 2013).

Also, in response to the knowledge gaps surrounding the source of infection for human cases of the avian H7N9 subtype in China, PREDICT estimated the historical prevalence and distribution of H7N9 viruses in wild bird populations. Because the prevalence of the H7N9 subtype was found to be historically low in wild birds, the researchers recommended that future work should focus on identifying H7N9 sequences
that are linked to increased human pathogenicity and transmissibility and to conduct risk-based surveillance to detect these viruses in domestic and wild birds (Olson et al. 2013).

Genetic analysis has provided evidence that the human H7N9 viruses in this outbreak were of avian origin through novel reassortment of the influenza A virus subtypes H9N2 and H7N3 (Gao et al. 2013). PREDICT was the first to report H9N2 viruses in wild birds and suggested that wild birds were natural reservoirs and may carry the virus along migratory routes allowing for transmission to other host species (Zhu et al. 2013). Fifteen H9N2 viruses were isolated from two species of wild ducks (spot billed ducks and mallard ducks) in Poyang Lake of southeast China in 2011. Eleven representative viruses were further characterized by complete sequencing of the eight gene segments. One isolate replicated efficiently in laboratory mice tissues and led to mortality in 20–40% of experimentally infected mice cohorts (Zhu et al. 2013) revealing the virus’s ability to cause fatal infections in a mammalian species and therefore, the potential to pose a threat to human health.

SURVEILLANCE STRATEGIES FOR INFLUENZA A VIRUSES

In an effort to improve surveillance and detection of new influenza subtypes and outbreaks, scientists are looking to better understand and monitor the diversity, ability to change genetically, and distribution of all influenza A viruses – not just those known to cause disease. By completing the first global inventory of influenza A strains in birds and mammals, PREDICT provided a key step in building that understanding and providing new insight into competent bird and mammal hosts and drivers of viral diversity and the emergence of influenza in domestic animals and people.

In order to understand the role wild birds play in the emergence of zoonotic influenza viruses, PREDICT scientists examined 11,870 sequences from the GenBank database to provide a baseline inventory and insight into patterns of global influenza A subtype diversity and richness in wild birds. Further, they conducted an extensive literature review and communicated directly with scientists to gather data from 50 studies and over 250,000 birds to assess the historic sampling effort to better understand the current knowledge. Virus subtype richness was examined in order to estimate the diversity of influenza subtypes in a particular location using sample-based accumulation curves that examine the presence and absence of a subtype in each location.

This research identified over 116 influenza A strains in wild birds globally, which is approximately twice the number found in domestic birds. The majority of all known sequenced subtypes were found in wild ducks, geese, and swans (Anseriformes; 90%) or gulls and shorebirds (Charadriiformes; 63%); mallard ducks were found to carry the highest number of viral strains (Olson et al. 2014). The more a strain was shared across wild bird types, the more likely it was to be found in domestic birds, and thus thought to be a risk factor for spillover events. Geographically, the North American Atlantic flyway, Europe, and Asia were global hotspots for subtype richness (Olson et al. 2014). Results from the analysis showed that sampling plans for surveillance needed to include a minimum of 10,000 samples to detect an estimated 75% of circulating virus subtypes from a targeted bird population, and thus should help to guide future surveillance to understand the influence of host and virus biodiversity on emergence and transmission.
In an effort to understand factors driving the evolution and diversity of all high-risk influenza A virus subtypes and more accurately identify hotspot areas of emergence to better design diagnostic strategies, PREDICT investigators also evaluated mutation rates of high priority influenza A subtypes detected globally as well as socio-economic, biodiversity, and agricultural drivers that may be associated with subtype diversity and reassortment. Results indicated that potentially pathogenic influenza A strains may be more likely to evolve in East Asia, reinforced by the fact that the majority of subtypes that have caused disease and mortality in humans in recent years, such as H5N1, H5N6, H6N1, H7N9, H9N2 and H10N8, were first detected in China and Hong Kong. Other factors that were associated with detected subtype diversity were sampling effort, measured by the number of strains reported, and healthcare spending, as a measure of the ability to test and detect multiple influenza subtypes. An important recommendation for diagnostic testing was to revise current strategies of targeted surveillance for specific influenza subtypes – instead performing broader testing to detect all subtypes in order to better understand the total diversity globally and to facilitate the early detection of emerging subtypes and strains.

**FACTORS DRIVING EMERGENCE AND PERSISTENCE OF H5N1 INFLUENZA A VIRUSES**

PREDICT also examined drivers for highly pathogenic H5N1 influenza A persistence. Hosseini et al. (2013) used mathematical models of H5N1 virus dynamics in different-sized poultry farms to understand the virus’ ability to persist in different types of poultry operations and to investigate the effects of culling and cleaning as control measures. Results indicated that moderately-sized poultry farms can sustain H5N1 virus for over two years without wild bird involvement. In addition, a mixture of intensive and backyard farming within a country sustained H5N1 infection and circulation indefinitely (Hosseini et al. 2013). In countries with a need for intensive poultry production, larger scale commercial poultry operations with more intensive H5N1 virus monitoring and increased biosecurity may be the best strategy for reducing risk of human infection with H5N1 virus and persistence in farmed poultry (Hosseini et al. 2013).

In addition, Murray and Morse (2011) assessed whether human H5N1 cases occurred seasonally in Indonesia and Egypt in association with changes in temperature, precipitation, and humidity. The incidence of human H5N1 in Egypt, but not Indonesia, was strongly associated with meteorological variables. In addition, incidence of infection was highest in Egypt when precipitation was low, and temperature, along with absolute and relative humidity, were moderate compared to the average daily conditions in Egypt; suggesting that human infection may be occurring primarily via droplet transmission from close contact with infected poultry (Murray and Morse 2011).
CONTRIBUTIONS TO INFLUENZA RESEARCH IN OTHER WILDLIFE SPECIES OF INTEREST: EMERGENCE OF INFLUENZA A STRAINS IN MARINE MAMMALS

Transmission between wild avian reservoirs and mammalian hosts is an important factor in the dynamics and evolution of influenza A viruses. Marine mammals come into contact with aquatic birds presenting opportunities for interspecies transmission and the emergence of new strains of influenza viruses that may pose a risk to public health. Previous studies have documented interspecies transmission events between marine mammals and birds and marine mammals and people (Webster et al. 1981; Hinshaw et al. 1984; Mandler et al. 1990).

In 2009, a new strain of H1N1 emerged in people and resulted in a global pandemic. The following year, PREDICT investigators detected pandemic H1N1 influenza in free-ranging northern elephant seals in 2010 off the central California coast upon their return from their migration. Virus isolation, whole genome sequencing, and hemagglutination inhibition assay confirmed exposure to pandemic H1N1 influenza virus in the seals. In vitro characterizations showed that replication of the virus was similar to that of reference strains of pandemic H1N1 in canine kidney cells. However, the virus did not replicate well in human epithelial respiratory cells, demonstrating that the virus isolates may be elephant seal adapted (Goldstein et al. 2013). This was the first isolation of H1N1 in a marine mammal. These findings provided evidence for cross species transmission of influenza viruses among free-ranging wildlife and between wildlife and people and provided evidence that oceanic transmission and movement of pathogens should not be eliminated from consideration of amplification and spread (Figure 1; Goldstein et al. 2013).

Figure 1. A visual representation of the geographical and interspecies spread of influenza A. In 2010, exposure to H1N1 was documented in free-ranging northern elephant seals after returning to the California coast from their short migration in the northeast Pacific. Given that direct contact between people and elephant seals is unlikely while at sea, exposure may have occurred through contact with aquatic birds, thus expanding the virus’s host and geographical range by circulating among marine mammals, birds, and people on land and at sea. From Mazet et al. (in press).
The following year, in 2011, 162 harbor seals died off the New England coast of the US in an outbreak of pneumonia. A PREDICT investigator detected an influenza A virus H3N8 subtype in the harbor seals that died, a subtype typically associated with infection of avian, equine, and canine hosts. Sequence analysis revealed that the H3N8 influenza A subtype was most similar to avian H3N8 strains previously detected in North American waterfowl, but with mutations consistent with adaptation to mammalian hosts (Anthony et al. 2012).

Both of these examples documented the emergence of new strains of influenza viruses in new mammalian hosts and are of public health concern because of the potential for viral persistence and cross-species transmission. In both cases, the viruses had naturally acquired mutations that may increase transmissibility and virulence in mammals. Monitoring the spillover and adaptation of avian viruses in mammalian species is critically important if we are to understand the factors that lead to both epizootic and zoonotic emergence.

REFERENCES


