MINIREVIEW

H7N9 Avian Influenza A Virus and the Perpetual Challenge of Potential Human Pandemicity

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ABSTRACT The ongoing H7N9 influenza epizootic in China once again presents us questions about the origin of pandemics and how to recognize them in early stages of development. Over the past ~135 years, H7 influenza viruses have neither caused pandemics nor been recognized as having undergone human adaptation. Yet several unusual properties of these viruses, including their poultry epizootic potential, mammalian adaptation, and atypical clinical syndromes in rarely infected humans, suggest that they may be different from other avian influenza viruses, thus questioning any assurance that the likelihood of human adaptation is low. At the same time, the H7N9 epizootic provides an opportunity to learn more about the mammalian/human adaptational capabilities of avian influenza viruses and challenges us to integrate virologic and public health research and surveillance at the animal-human interface.

The ongoing epizootic of H7N9 influenza in Eastern China (1), associated with 132 confirmed human infections and 39 related deaths (as of 18 June 2013), once again presents us questions about the origins of influenza pandemics, the risk to humans of avian influenza viruses (AIVs), and their potential to infect large numbers of humans via poultry epizootics (2–4). The new virus in question has a subtype 7 hemagglutinin (HA), which heightens concern because other HA subtype 7 (H7) viruses not only have caused large-scale poultry epizootics but also have become enzootically established in or have otherwise infected mammals, including humans. In attempting to assess the possible risk of an H7N9 pandemic, it is important to consider not only AIVs in general but also specific H7 influenza outbreaks that may give us clues about what to expect from H7N9. In this regard, the evidence as a whole is complex and the implications of past outbreaks for predicting the future course of the current H7N9 epizootic are uncertain.

All influenza A viruses are derived from a large global pool of AIVs circulating in, and moving geographically between, hundreds of species of migratory and nonmigratory wild birds (5, 6). AIVs appear to be stably adapted to these wild avian hosts, and their HA and neuraminidase (NA) genes seem to be under little or no immune selection pressure within each subtype (7). However, our concept of the stability of AIVs in wild birds may be erroneous. Wild birds are often infected simultaneously with multiple influenza viruses (7); this leads to constant/ongoing genetic reassortment that creates endless variations of new gene constellations. Such gene constellations may not exist as stable viruses until such time as they leave the wild-bird avian host to infect such "nonnative" hosts as galliform poultry or mammals.

Influenza host-switching events that result may follow either of two different viral evolutionary pathways. One such pathway begins with AIV host switching and adaptation to galliform poultry, such as chickens, turkeys, or quail. Another entirely different pathway begins with host switching and adaptation to a mammal, such as a horse, a pig, or a human. Because AIV host switches are associated with viral mutations that reflect adaptation to the new host, they seem generally to lead the virus away from an ability to back-adapt to wild birds. Evidence also suggests that poultry and mammalian adaptation may independently increase the evolu-

tionary distance between diverging viruses (i.e., between viruses adapting in galliform poultry and viruses adapting in mammals). If this were the case, the distinctive genetic specializations resulting from viral replication and accumulating mutations that occur during host adaptation may arguably make poultry-adapted viruses less likely to cross-adapt to mammalian species (8, 9). This possibility has important implications for the emergence of influenza pandemics.

Were it not for several highly unusual aspects of H7 viruses, such information about AIV evolution, coupled with the fact that since at least 1918, no poultry-adapted influenza virus has ever stably infected humans, let alone caused a pandemic (10, 11), might be reassuring. However, such aspects of H7 viruses challenge any sense of assurance, including (i) the repeated involvement of H7 avian viruses in large-scale poultry epizootics, (ii) their capacity to spontaneously develop mutations leading to high pathogenicity in poultry, (iii) the association of poultry-adapted viruses with human spillover infections and epidemiological features at times reminiscent of highly pathogenic avian influenza (HPAI) H5N1 (4, 10), (iv) a propensity for ocular replication and transmission, and (v) the ability of H7 viruses to infect several other mammals (e.g., seals and swine), including an H7N7 lineage that achieved long-term stable mammalian adaptation in horses, challenge any sense of assurance.

EPIZOOTIC BEHAVIOR OF H7 VIRUSES

The current H7N9 epizootic is not the first time that H7 viruses have exhibited unexpected and unusual epizootic and epidemiologic behaviors. Over recent decades, both HPAI and low-pathogenicity avian influenza (LPAI) H7 viruses have caused nu-

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merous explosive poultry outbreaks, notably massive 1999 H7N1 HPAI and 2003 H7N3 LPAI outbreaks in Italy (12, 13), a 2003 H7N7 HPAI outbreak in the Netherlands (14, 15), 2004 and 2007 HPAI H7N3 outbreaks in Western Canada (16), a 2012 HPAI H7N3 outbreak in Mexico (17), and ongoing enzootic circulation of H7N2 viruses in bird markets in New York (18). Each of these epizootics has been associated with the independent emergence and adaptation to galliform poultry of a novel virus from the wild-bird AIV pool. Moreover, with the exception of the 1999 H7N1 outbreak, each has caused spillover human cases; one of them, the 2003 Netherlands outbreak, led to an estimated 450 human cases, one death, and three secondary cases that may have arisen from person-to-person transmission (14, 15). Human infections have also resulted from other poultry epizootics caused by various H7N2, H7N3, and H7N7 viruses (19–22). Another unusual epizootic feature is that the Chinese H7N9 virus contains a cassette of internal genes acquired from an avian H9N2 virus (1). In recent decades, H9N2 viruses have spread panzootically in poultry and have also caused swine infections and widespread but mostly asymptomatic human infections (23, 24).

POULTRY PATHOGENICITY

Similar to H5 viruses, but unlike viruses with any other HA, H7 viruses sometimes acquire polybasic HA cleavage site insertional mutations that render them highly pathogenic to poultry. Indeed, in some of the H7 poultry epizootics noted above, mutations from LPAI to HPAI were documented during the epizootics (12, 16). The Chinese H7N9 virus has remained a low-pathogenicity virus to date; however, it is conceivable that if it spreads further in poultry, it may undergo a similar spontaneous mutational change to HPAI. Although HPAI viruses do not as a rule infect humans or cause severe human disease (10), large-scale culling of diseased poultry during HPAI outbreaks can create intense exposure situations for large numbers of people, potentially leading to human infections and even deaths in persons who may have specific but uncommon and as-yet-uncharacterized susceptibilities (4, 10, 14, 25).

THE H7N9 EPIZOOTIC SHARES EPIDEMIOLOGICAL SIMILARITIES WITH THE HPAI H5N1 EPIZOOTIC

Unlike the H5N1 virus, which has caused widespread fatal poultry outbreaks, the current LPAI H7N9 virus spreads silently in poultry. Moreover, to date, H7N9 seems to have been confined to live-bird markets and circumscribed poultry foci, compared to H5N1, which has spread panzootically in poultry and wild-bird populations. Nevertheless, the two avian viruses share a number of epidemiological features, as follows (4): both viruses have produced a characteristic clinical picture in humans that includes bilateral pneumonia, acute respiratory distress syndrome, and multiorgan failure (1, 26); human cases have been rare but unusually severe and often fatal; large numbers of humans have apparently been exposed to both viruses without immunologically detectable or clinically apparent infection; person-to-person transmission has rarely if ever occurred; and although uncommon, case clusters seem to indicate common source exposures in persons who are genetically related. As appears to be the case for H5N1 (10, 25), H7N9 may be exhibiting features of a poorly adapted avian influenza virus that is now and may remain unable to infect humans easily but which is at the same time capable of

causing severe disease in rare persons with as-yet-uncharacterized genetic susceptibilities (4, 10, 26).

TROPISM OF H7 VIRUSES FOR CONJUNCTIVAL CELLS

Another unique aspect of some H7 viruses is a predilection for infecting human conjunctival cells, a property of potential importance for prevention and clinical management. Although most influenza viruses can infect multiple epithelial cell types of the nasopharyngeal and respiratory tracts (including conjunctival cells), human infections caused by some but not all H7 viruses have featured unusually prominent conjunctival signs and symptoms—often without traditional respiratory signs and symptoms—as well as possible conjunctival-associated person-to-person spread. In the 2003 Netherlands H7N7 outbreak, the majority of infected humans had conjunctivitis alone (91 percent of 86 virologically confirmed primary cases) (14, 15). Recent work has linked conjunctival tropism to different H7 viral lineages, to host responses, and to host receptor binding affinity (27–29), although the underlying mechanisms of enhanced conjunctival cell tropism with certain H7 viruses remain unclear. The clinical implications of a human influenza virus that causes high-viral-titer conjunctival infection and is associated with conjunctival spread are speculative. Various other viruses (e.g., some adenoviruses and some enteroviruses) have been associated with conjunctivitis and conjunctival spread (30), and two unrelated enteroviruses, EV70 and coxsackievirus A24 variant, emerged in 1969 and 1970, respectively, to cause explosive pandemic conjunctivitis (31). At the very least, analogous human conjunctival adaptation of an influenza virus would have important implications for patient treatment and public health prevention given the possibility of conjunctival-associated transmission.

H7 VIRUSES HAVE INFECTED OTHER MAMMALS

From the 1940s, if not earlier (32), until the 1970s, a well-adapted H7N7 virus was widely enzootic in horses and seems also to have caused uncommon and mild spillover infections in humans (33). The origins of this virus lineage are incompletely understood, making it difficult to predict the length of time it had been adapted to horses, but it was generally believed at the time of its initial isolation in 1956 to have circulated for decades beforehand (32, 34). Equine influenza-like epizootics had been exceedingly common for centuries but began to disappear as horses were replaced by automobiles and farm machinery, around the time of World War I (35). Since the 1970s, the equine H7N7 virus has become extinct or at least virtually undetectable by surveillance. Nevertheless, the ability of an H7 virus to adapt stably to a mammal over several decades raises the possibility that another H7 virus, such as H7N9, may adapt to a mammal in an analogous manner or even to humans. LPAI H7N7 viruses have also caused fatal outbreaks in seals without stable adaptation and have been associated with spillover seal-to-human infections (36). In addition, H7 viruses have infected pigs (37), a species that seems to be permissive for many influenza viruses and which is considered to be a "mixing vessel" that allows dynamic reassortments of the kind that led to the 2009 H1N1 pandemic. The novel H7N9 virus infects pigs experimentally (38) but has not so far been detected in swine populations.

What does the H7 viral track record reviewed here tell us about the potential for the current H7N9 epizootic to evolve into a human pandemic and about the consequences if such a pandemic

occurs? On the one hand, in 94 years of virologic surveillance, we have never seen a poultry-adapted influenza virus cause widespread human transmission (10, 35), and archaeserologic evidence from the 1950s and later, including subjects born as early as the 1880s, makes it doubtful that H7 viruses have circulated in humans at any time since then (39). On the other hand, H7N9 is only the latest of a series of H7 subtype viruses that have exhibited unusual behaviors not only in poultry but also in mammals, including adapting to epizootic/enzootic transmission in at least one mammalian species, the horse. H7 viruses have also repeatedly infected humans, sometimes causing atypical clinical features, such as conjunctivitis. It is concerning that the recent H7N9 virus contains an internal gene cassette from an H9N2 virus, representing a lineage that has caused widespread human infection, although there is no evidence that the specific subtype strains of internal genes of the Chinese H7N9 virus have themselves infected humans as components of an H9N2 virus. Nevertheless, given the ubiquity of H9N2 viruses and their capacity to evolve dynamically and to infect and move rapidly between numerous avian and mammalian hosts (24), H7N9 might arguably be more likely than other avian viruses to become human adapted (4).

The history of H7 viruses constitutes a set of complex observations that may or may not be relevant to the emergence of an H7N9 pandemic, and we thus find it challenging to fit them into calculations of potential human risk. If it was not obvious before, the emergence of H7N9 makes a strong argument supporting the need for much more basic and applied research on the mechanisms of evolution of human and particularly avian and mammalian influenza viruses as well as better integration of influenza virology within human and veterinary public health. Threats like those posed by H7N9 also reflect natural experiments from which we can gain important fundamental knowledge about the characteristics and behavior of influenza viruses. Looked at from the combined points of view of epidemiology, epizootiology, and virology, there may be no more complex infectious disease problem than that posed by influenza. Regardless of whether the current H7N9 outbreak dies out or proceeds to pandemic spread, we have a unique opportunity to learn more of influenza's many secrets and thereby enhance our ability to prevent and control an important disease that seems destined to appear again and again, in multiple guises, far into the foreseeable future.

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