H7N9 Influenza: What We Learned from H5N1

The H7N9 flu that emerged in 2013 is 30% lethal in humans but, unlike H5N1, causes no apparent illness in birds

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During the past year, a second bird flu emerged in Asia—an avian influenza virus that is sporadically transmitted to humans. This virus of the H7N9 subtype proved lethal in 33% of the 135 cases reported as of October 2013, yet it is termed a “low-pathogenic” virus because of the mild to inapparent respiratory disease it causes in domestic poultry. In contrast, the H5N1 influenza virus that emerged in Southern China in 1997 is highly pathogenic to poultry, killing as many as 100% of the birds it infects. It, too, is sporadically transmitted to humans, and is lethal in about 60% of reported cases.

Both the newer H7N9 and H5N1 viruses were triple reassortants, having obtained their eight gene segments from three different sources. Their hemagglutinin (HA) and neuraminidase (NA) genes, which encode their surface glycoproteins, come from the avian influenza gene pool in wild aquatic birds, while the six genes of their replication complex, their “internal” genes, also originate from wild birds or poultry birds—in both cases, from H9N2 influenza viruses (Fig. 1). Here we briefly review the genesis of the H7N9 influenza viruses, compare their features to those of the H5N1 viruses, and consider what lessons learned in managing H5N1 may be useful in controlling H7N9.

Emergence of the H7N9 Influenza Virus and Its Relative Transmissibility

The H7N9 bird flu virus emerged in humans in China early in 2013. Although the virus is more than 30% lethal in human cases, it has no apparent effect on domestic poultry or wild birds. Therefore, there are no sentinel disease outbreaks in poultry to warn of potential danger to humans. The first three human cases in Shanghai and Anhui provinces involved severe pulmonary infection.

After H7N9 avian influenza virus was isolated from these patients, the viruses were rapidly characterized. The initial details, including complete genome sequences, were quickly made available on the Global Initiative on Sharing Avian Influenza Data (GISAID) site for immediate worldwide use. The Chinese authorities acted generously and admirably in rapidly sharing this information, which is important to human and veterinary public health and represents a major contribution to global health efforts.

The H7N9 virus isolated from humans is a triple reassortant (Fig. 2). Its HA gene is most similar to that of an H7N3 influenza virus that infects ducks (A/duck/Zhejiang/12/2011-like), while its NA gene resembles NA genes of H11N9 and H2N9 viruses from wild waterfowl. Meanwhile, its six internal gene segments come from an H9N2 virus isolated from small finch-like birds (A/Brambling/Beijing/16/2012).

The H7N9 human isolates carry a number of molecular characteristics that facilitate the transmission of avian influenza viruses to mammals, including an HA mutation (Gln226Leu) that enhances the binding of HA to mammalian α2–6 sialic acid receptors. This mutation promotes aerosol transmission of the H5N1 viruses.

SUMMARY

➤ The H7N9 influenza virus that emerged in Asia in 2013 is, like H5N1, a triple reassortant and appears to cause severe human disease, but causes no apparent illness in birds.

➤ The H7N9 influenza viruses are more readily transmissible to humans than are the H5N1 viruses.

➤ Although the avian reservoir of the H7N9 virus is not yet known, more than 60% of the 135 humans it infected had been to markets selling live poultry.

➤ H9N2 influenza viruses provided all six internal gene segments to the H5N1 and H7N9 viruses and may be the key influenza subtype that opens the way for forming multiple genotypes.
to mammals, according to gain-of-function studies. The H7N9 isolates also are deleted of residues 69–73 in the stalk of the NA glycoprotein, a mutation that is associated with adaptation to domestic poultry and with replication in the respiratory tract.

A characteristic of even greater concern is this strain’s acquisition of the Glu627Lys substitution in PB2, which is also associated with adaptation to mammals. All of the H7N9 viruses isolated from birds in live poultry markets possess characteristic avian residues at this PB2 site, suggesting that the Glu627Lys mutants are rapidly selected during virus replication in humans.

The H7N9 influenza viruses are more readily transmissible to humans than are the H5N1 viruses. The emergent H5N1 virus took 3 years to infect 100 people, while the H7N9 virus required only 57 days. In ferrets, which respond to influenza much as humans do, H7N9 viruses are transmitted from one animal to others by direct physical contact and, to some extent, by contact with respiratory droplets. In similar studies, H5N1 virus transmission by contact was rare. Together, these findings suggest that H7N9 viruses are much more easily transmitted from poultry to humans than are the H5N1 viruses. Despite a few cases of human-to-human transmission of H7N9 virus, sustained transmission does not occur.

### Epidemiology of the H7N9 Influenza Virus and Efforts to Contain It

The initial H7N9 outbreak was in the Yangtze River delta of eastern China, with its epicenter in Shanghai municipality. Additional human cases of H7N9 are being detected in nearby provinces and in Beijing and Shanghai (Fig. 3). A single case in Taiwan involved an individual who had recently visited Shanghai. The incidence of human H7N9 cases peaked early in 2013 and declined after April.

Although the avian reservoir of the H7N9 vi-
rus is not yet known, more than 60% of the 135 humans it infected had visited wet-markets selling live poultry. Surveillance indicates a low incidence of infection in chickens, pigeons, and ducks in China, but they are highly susceptible to H7N9, shedding this virus for as long as 10 days without overt disease. Quail shed the highest titters of virus, followed by chickens, ducks, and pigeons. Swine are also susceptible to H7N9, but show no signs of disease and do not transmit the virus to other swine.

Although H7N9 viruses infected more humans in a shorter timespan than did H5N1, H7N9 infection progressed more slowly. The symptoms and complications of infection with these two viruses are similar, including fever and dyspnea, acute respiratory distress syndrome, and multiple organ failure.

Sex and age of infected humans differ markedly between the two viruses. Most cases of H7N9 occurred in males, and the median age was 62 years; in H5N1 infection, there was a trend toward female predominance, and the median age was 26 years. Unlike cases of H5N1, many H7N9 infections were associated with underlying illness, and patients on respiratory support were vulnerable to secondary bacterial pneumonia. Like H5N1 viruses, most H7N9 viruses are sen-
sitive to the neuraminidase inhibitors but resistant to the adamantane inhibitors. Patients in whom neuraminidase inhibitor-resistant mutants (Arg292Lys) arose had poor clinical outcomes.

According to serology studies, 6.3% of poultry workers in China were exposed to H7N9 virus, whereas the general population was not. Clinical studies suggest that multiple doses of vaccine with a high antigen content or vaccines with adjuvants will be needed. H7N9 vaccine seed stocks so far are low antigen producers.

Closing live poultry markets dramatically reduced human H7N9 cases, which dropped to zero in the initial epicenter by early May. Subsequently, two additional human cases were reported, one in Hebei province and the other in Guangdong province. Summer warmth may have helped to reduce virus circulation in poultry and transmissions to humans, as was documented with H5N1 viruses. It remains to be seen whether interventions in live poultry markets will provide a practical method to control H7N9 if it reemerges.

**H9N2 Virus: the Enabler?**

The H9N2 influenza virus, which is enzootic in Eurasian domestic poultry, is not found in the Americas. Although it provided all six internal gene segments to the H5N1 and H7N9 viruses, the H9N2 virus by itself causes no apparent illness in domestic poultry. While subsequent reassortants of H5N1 viruses obtained internal
gene segments from other avian influenza viruses, we speculate that the H9N2 influenza viruses opened the door for the genesis of multiple genotypes.

What properties H9N2 viruses contribute to other influenza viruses is unknown. For example, although H5 influenza viruses caused no known human infections before 1997, H7 influenza viruses caused low numbers of human conjunctivitis cases, while 89 persons in the Netherlands showed serological evidence of H7N7 infection with one death. After reassortment with Asian H9N2 influenza virus, both H5 and H7 influenza viruses show a greater propensity to infect humans. The ubiquitous presence of H9N2 influenza viruses in Asian domestic poultry and their predisposition to reassortment may help to explain the role of H9N2 viruses as donors of internal genes for both H5N1 and H7N9 viruses that infected humans.

Another troubling property of H9N2 viruses is their acquisition of mutations that enable them to infect mammals, including the capacity to bind to the predominant α2-6 sialic acid receptors in the mammalian upper respiratory tract. Multiple sublineages of H9N2 viruses have a high potential to reassort with other influenza subtypes and have been transmitted to humans and swine, but they have not established lineages in mammals.

Will H9N2 viruses soon acquire the ability to cause disease in mammals and be transmitted human-to-human? Will they reassort with other low-pathogenicity influenza viruses that have spread to domestic chickens in Asia? H9N2 may well be facilitating the emergence of influenza viruses, such as H5N1, and H7N9, that can establish themselves in domestic poultry.

Conclusions
As human populations increase in density, they tend to consume more animal proteins, increasing the demand for large-scale poultry farms and live markets near and in metropolitan areas. This trend leads to more opportunities for emergence and spread of novel influenza virus reasortants. One lesson from both the H5N1 and H7N9 out-

Experience with H5N1 Influenza Virus
Some lessons learned from dealing with H5N1 viruses provide guidance for controlling H7N9.

Poultry market interventions. Live poultry markets support the reassortment, evolution, and spread of novel influenza viruses, increasing the risk of zoonotic infection and facilitating the spread of virus to uninfected farms via contaminated cages. Closing live poultry markets interrupts transmission of the virus to humans. Other interventions, such as rest days or rules against overnight stay of live poultry, can also help.

Seasonality of virus prevalence. The H5N1 influenza viruses are more prevalent during the cooler months in Asia and Egypt, and human infection is more frequent. The seasonality of H7N9 influenza viruses is not known.

Without intervention, spread will continue. Circulating H5N1 viruses generated more than 32 distinguishable clades; clade 1 continues to circulate, but the predominance of clade 2 and the emergence of multiple subclades illustrate the continued evolution of these viruses. During the past 16 years, these viruses spread throughout Eurasia but not to the Americas.

Gain-of-function changes may confer mammalian transmissibility. Experiments by three research groups formally showed that three to five mutations or mutations "plus" reassortments with human epidemic strains can generate H5N1 viruses that are transmissible among mammals. Despite controversy about the potential malicious use of this information, these studies indicate how highly pathogenic H5N1 viruses can become transmissible among humans.

Control in domestic poultry. Locales that opted to stamp out H5N1 by eradicating commercial poultry flocks succeeded at least temporarily, notwithstanding the potential for its reintroduction. In countries such as China, Vietnam, Indonesia, and Egypt where the virus was widespread, vaccinating rather than destroying poultry substantially reduced outbreaks in both humans and poultry, but at the cost of enzootic infection of the latter.

Role of wild birds. Wild birds contribute to the spread of H5N1 viruses, but there is no convincing evidence that birds serve as a reservoir (Microbe, December 2006, p. 559). Domestic poultry species maintain the virus in countries where it is enzootic, but wild birds may be a vector for at least the H5N1 clade 2.2- and 2.3.2.1-like viruses.

Inapparent disease in reservoir species. Ducks maintain and spread H5N1 viruses. While some variants of H5N1 can kill all breeds of ducks, others cause little or no disease in breeds such as the Pekin white duck, which is farmed in China, and migratory mallards.
breaking is that closing such markets can dramatically reduce transmission of these viruses to humans. Whether H7N9 viruses will reemerge and, if so, whether closing live poultry markets will prevent their extended spread remains to be seen.

Because infections of poultry with H7N9 virus are not easily detectable, reinstituting timely preventive measures could prove challenging. Meanwhile, health officials should consider banning overnight stays of birds in live markets, introducing market rest days, and improving hygiene. In the longer term, phasing out live poultry markets may be the most effective way to reduce human health risks, although it will likely depend on infrastructure changes such as improved availability of refrigeration.

While H7N9 and H5N1 influenza viruses continue to circulate in poultry in Asia, they pose a continuing threat to public health worldwide. Therefore, we must promote strategies to improve biosecurity, reduce circulation of these viruses within poultry, and diminish opportunities for zoonotic transfer of viruses from domestic animals to humans. Because avian influenza viruses continue to emerge and evolve, pandemic preparedness requires updating of conventional vaccine seed stocks along with development of universal vaccines and new antiviral agents.

Four additional human cases of H7N9 influenza have occurred since the beginning of October 2013, with serious to critical disease signs in three of the four cases, while the younger person recovered and was discharged from the hospital. The human cases occurred in provinces as far apart as Zhejiang and Guangdong, making it likely that the H7N9 is widely dispersed in poul-

**AUTHOR PROFILE**

Webster: from Influenza in Sea Birds along the Beach to Storms off the Florida Coast

In 1958, Robert Webster arrived at the Australian National University in Canberra to pursue his Ph.D., hoping to work on poxviruses with Frank Fenner, who became a leader of the team that eradicated smallpox. “The day I arrived Frank said: ‘Rob, you are going to work on influenza,’ and I said, ‘Oh, no,’” Webster recalls. “And here, at the end of my life, I am still working on influenza.”

Webster, 81, devotes considerable energy to influenza research, even while contemplating retirement. “I keep thinking about it,” he says. “But influenza keeps making changes. And so long as it’s exciting, I will continue to study it.” He holds the Rose Marie Thomas Chair at St. Jude Children’s Research Hospital in Memphis, where he has worked since 1968. He also advises the World Health Organization, the federal Biomedical Advanced Research and Development Authority, and other public health organizations.

When Webster began studying influenza in the 1960s, he and his colleagues were among the first to propose that pandemic strains could arise from antigenic shifts when such viruses mixed genes in wild birds or other animals. He and the late Graeme Laver, a longtime collaborator from Australian National University in Canberra, grew curious when they saw dead birds along a southeastern Australian beach where the two of them walked during a break from their fishing trip. These observations evolved into studies on the Great Barrier Reef and detection of flu antibodies in migratory birds. Eventually, they isolated flu viruses, and those efforts contributed to the first antiflu drug, “all from a simple walk on the beach,” Webster says.

Webster grew up in southern New Zealand on a dairy farm, “plumb in the middle” of 13 children. “We used to get up at daybreak to milk the cows before catching the bus to school,” he says. “We didn’t have any electricity. I went straight through high school on candle power and kerosene lamps.”

Although Webster set out to become a chemist, he changed course after hearing a lecture by microbiologist Molly Marples during his first year at Otago University in New Zealand. “She talked about diseases caused by bacteria, viruses, and fungi, and the interface between infection in animals and humans,” he says. “The next year, I took microbiology.” Webster received his B.Sc. in 1955 and his M.Sc. in 1957, both in microbiology from Otago. He then spent three years as the first agricultural virologist in New Zealand in the Department of Agriculture. His Ph.D. is from the Australian National University in 1962, and that year he traveled as a Fulbright postdoctoral scholar to the University of Michigan, Ann Arbor.

Webster, who became an American citizen 20 years ago, holds dual citizenship from New Zealand. He and his wife Marjorie have two sons, a daughter, and eight grandchildren. He has two main hobbies, deep-sea fishing and gardening. “I grow sweet corn and feed the whole department,” he says. He recently returned from a fishing trip off the coast of Florida where he caught a 23-pound red snapper while also contending with a tropical storm. “It was very wet and extremely rough,” he says.

Marlene Cimons

Marlene Cimons lives and writes in Bethesda, Md.

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try. The newly emerged H7N9 influenza virus did not disappear over the summer months, and it is likely that we will see a significant re-emergence of H7N9 human cases in the winter months.

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