# Keeping an eye on H7N9, and learning from the past

## Ann Griswold, PhD

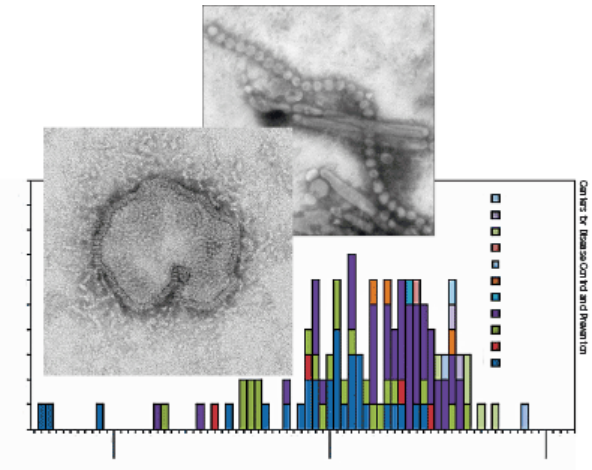
June 2013—What began as a trickle of reports in China earlier this year swelled into a flood of patients with grave flu-like symptoms. Each time, PCR assays returned the same result: unsubtypable influenza A. Amid a rising mortality rate, viral samples were sent to China's national laboratories for sequencing analyses. On March 31, Chinese officials posted the results to an open-access database and alerted the World Health Organization to a public health emergency of international concern: The H7N9 epidemic had begun.

Working quickly, the CDC developed an H7-specific real-time RT-PCR assay using the 19 viral sequences posted by the Chinese researchers to the Global Initiative on Sharing All Influenza Data (GISAID) database. In a rare move, the FDA approved test kits, each capable of testing about 1,000 specimens, under an emergency use authorization. This allowed distribution of the test kits to health departments in 50 states and the District of Columbia, as well as U.S. Department of Defense labs, private diagnostic test manufacturers, and WHO-recognized National Influenza Centers worldwide. Public health labs that have been certified by the CDC can order the H7N9 reagents from the Influenza Reagent Resource, and test protocols can be downloaded from the CDC's Laboratory Support of Influenza Surveillance Web site.

With testing capabilities in place, clinicians have been advised to obtain samples from any patient with acute respiratory symptoms and a recent history of travel to an affected region. Hospital laboratories are encouraged to send samples that yield "unsubtypable" results on the seasonal flu test to their state health departments, where the CDC's seasonal Flu rRT-PCR Dx panel will rule out other forms of influenza before the new test is used to confirm H7N9.

Though private manufacturers are modifying existing assays to detect H7N9, those assays will have to embark on a lengthy FDA approval process. Meanwhile, the test kit empowers state labs to detect H7N9, preventing a backlog of samples at the CDC. Until the FDA approves those tests, all H7N9 cases in the U.S. must be confirmed by public health laboratories following CDC-approved protocols.

In recent weeks, the lull in H7N9 cases has many wondering whether the storm has passed or whether China is merely rotating in the storm's eye. "It's really hard to forecast flu," says Daniel Jernigan, MD, MPH, deputy director of the Influenza Division in the CDC's National Center for Immunization and Respiratory Diseases. The trouble, he says, is that occurrences of human infection with avian influenza viruses normally decline in number during the spring and summer months, so the lull in H7N9 cases is to be expected.



"If you look at the epidemiology of H5 infections, in China and elsewhere, almost all of the bird and human cases occur between January and March. It's possible we'll see the human cases drop off. But the virus is likely to keep moving through bird markets throughout China and some of the adjacent countries, and may start to take off again when the cooler temperatures come back this fall and winter."

And that's if the viral sequence remains the same. If H7N9 evolves, all bets are off. "If in the course of its circulation, this new H7N9 virus has the opportunity to move back and forth between mammals and poultry, then there's a chance it will gain adaptations that would make it more likely to spread from person to person. But

there's a fair amount of work that will need to happen before that occurs," Dr. Jernigan says.

Soon after the H7N9 epidemic was announced, the number of cases swept into the hundreds and public health officials realized the H7N9 virus was targeting a peculiar population: older men. "The people who are getting sick are an interesting lot," says Dr. Jernigan. By mid-May more than 130 people had been infected and about a quarter of the patients had died, many from multiorgan failure or respiratory failure. Of these, 71 percent were men and most were older than age 50. "That's a fascinating epidemiologic finding," Dr. Jernigan says. "It's not what we would expect."

Explaining the link between H7N9 and older men has proved challenging. For one thing, epidemiologists aren't sure whether the numbers reflect exposures that are unique to older men, biological differences related to sex or age, or simply a surveillance bias. It's possible that younger people and women are infected at similar rates as older men but less likely to develop severe illness. Indeed, one asymptomatic child in China has tested positive for H7N9. Amid the possible mechanisms, one theory has emerged as particularly plausible: "We've learned that in older men the prevalence of smoking is very high, and in older women it's not very high at all," Dr. Jernigan says. In fact, 76 percent of the cases so far have had at least one underlying condition such as lung disease.

Of these common threads in the patient medical histories, the most predictable is recent contact with live poultry. The first wave of patients included a butcher, a farmer, and a number of people who had visited live bird markets. "For a city as large as Shanghai, live bird markets require quite a lot of chickens coming in and out every day," Dr. Jernigan says. The constant flux of feathers and fowl offers ample opportunity for avian influenza viruses to evolve. In 2006, steam spewing from the centrifuge-like defeathering machines used at live bird markets was found to transmit H5N1 influenza to bystanders, but a definitive link has not yet been established for H7N9. Epidemiologists have, however, noted a marked reduction in cases since officials closed live bird markets in China and Taiwan in early April.

China's government has been praised for its efficient efforts to curb the epidemic. As of May 31, the H7N9 outbreak has been restricted to eight provinces and two municipalities, with just one confirmed case in a Taiwanese businessman who had traveled recently to mainland China. Still, it remains unclear whether additional infections are going undetected. "There are probably a lot of animal infections still occurring in the rural areas, and probably some human infections there too, but the surveillance systems are not in place to pick up those cases," Dr. Jernigan notes.

In parsing the pandemic potential of H7N9, Dr. Jernigan explains, two factors are particularly worrisome. The first is the severity of the reported infections. "About 25 percent of the identified cases have died and about 30 percent have been discharged from the hospital," he says. "So it's still pretty dynamic. We don't know what the outcomes will be, but we think many of the patients will probably die or have significant illness for a while."

The second concern is the virus' ability to move silently within the poultry population. "If birds are dying, you know where the virus is," Dr. Jernigan points out. "You can be careful about how you handle them, and you can test people who have had contact with the infected birds." But less than one percent of the chickens, ducks, and pigeons tested have been infected with H7N9 and in the absence of symptoms it's impossible to know which birds are putting humans at risk.

"Together, these things indicate that H7N9 is a virus we need to watch closely," Dr. Jernigan says. The CDC has ranked H7N9 fairly high on the Influenza Risk Assessment Tool's "potential for emergence" and "severity of disease" scales, placing the pandemic potential of H7N9 between that of H1N1 and H5N1.

At this point, investigators have found no evidence of efficient and sustained H7N9 transmission between humans. But that could change. The H7N9 virus seems uniquely positioned to infect humans, possessing features in the hemagglutinin gene that may enhance the virus' ability to attach to cell-surface receptors in the respiratory tracts of humans and other mammals. "That means the virus is one step closer to becoming capable of transmitting from human to human," Dr. Jernigan warns. "Has it taken off? No, it hasn't. Does it have the potential? Yes. But it will take some additional adaptations before that can occur."

The H7N9 influenza virus is the latest in a string of potentially pandemic viruses that have shaped the relationship between public and private laboratories in the United States. “The molecular testing community has come an enormous way since the SARS outbreak in Hong Kong in 2002,” says David Hillyard, MD, medical director for molecular infectious disease testing at ARUP Laboratories. During the SARS outbreak, three labs quickly produced and shared genomic sequences, paving the way for laboratories to develop their own tests and distribute inactivated calibration proficiency materials. “We learned that people could discover efficiently, that they could get complete viral genomic sequences quickly, and that testing could be brought to bear very rapidly,” Dr. Hillyard recalls.

Things became more complicated with the avian threat of 2006, he says, when China’s nonrelease of sequences crystalized the importance of open-access information. With limited sequence data, the laboratory community found it much more challenging to mount a widespread response of the scale used to monitor and diagnose SARS.

In 2009, the H1N1 pandemic changed the landscape again. Within a short time—as the virus moved from Mexico to North America and beyond—viral sequences were released from several labs, and the CDC began distributing diagnostic reagents to state laboratories.

“So we find ourselves now with an influenza strain that has a very high case mortality rate, and we’re just learning about its source and how it is transmitted to humans,” Dr. Hillyard says. “There’s nothing to indicate we’ll have a widespread human pandemic, but fortunately there is a much improved infrastructure in place compared to previous threats. And the most important thing is how government, reference, and local laboratories have learned to better work together.”

The collaborative spirit lives on: In May, Nancy Cox, PhD, director of the CDC’s Influenza Division, spoke by teleconference to attendees at the Pan American Society for Clinical Virology annual meeting in Daytona, Fla. She provided a clinical and epidemiologic update on the state of the H7N9 outbreak and monitoring and containment efforts, as well as a description of the CDC’s current testing capabilities. Beyond meetings and updates, the CDC and WHO maintain excellent H7N9 Web pages that include information for laboratory testing, Dr. Hillyard notes. “Communication and cooperation between state laboratories and hospital, reference, and other non-government testing entities also seems to have improved,” he says.

“Our plan, based on early access to H7N9 sequences, and probably the plan of many other laboratories like ours, is to have H7N9 test reagents on hand now, not knowing what’s going to happen with this current threat,” Dr. Hillyard says. “We’ll perform initial preliminary validation testing in order to have an independent test’s capability in the eventuality of a need for spillover testing.”

One thing that hasn’t changed much since the early 2000s is the technology used to detect novel viruses like SARS or H7N9. Real-time PCR has remained the tool of choice, with a few modifications and enhancements over the years. “What has progressed are the databases we have for the design of real-time PCR tests,” Dr. Hillyard explains. “And what’s really progressed are the logistic relationships and algorithms for determining who to test, when to test, and when to forward specimens.”

In 2009, the first H1N1 assay to complete the full regulatory approval process was the Simplexa test, developed by Focus Diagnostics for the 3M Integrated Cycler system. Since the news broke in spring about H7N9, Focus, a business of Quest Diagnostics, has been building the capability to unveil an H7-specific test should the need arise.

“Our flu A detection is very robust, but right now we don’t have a multiplex test to differentiate H7,” explains Michelle Tabb, PhD, vice president of research and development at Focus Diagnostics. “Basically, it would involve dropping [H7] into a different channel on an existing test.” The new test would detect influenza A in one channel and H7 in another channel, and combine those results to generate a positive result for H7N9. In May, Quest received viral stock from the CDC and began producing contrived samples to confirm that its new test can detect H7 in human patients.

“We want to make sure we have the detection system worked out and that we have a predetermined sensitivity,

so we can launch this right away if needed," says Rick Pesano, MD, PhD, medical director for infectious disease at Quest Diagnostics. "Because in the case of H7N9 and similar viruses, it's imperative that the physician has the information as rapidly as possible. If you're standing in front of someone with a communicable disease that has the ability to spread, it's important to get sensitive, rapid answers in order to intervene and provide supportive care."

Numerous laboratories, public and private, have also begun to reverse-engineer the viral genes and produce seed viruses for use in vaccine development. Several vaccines have been designed, providing the U.S. with a reasonably strong arsenal to ward off H7N9 in the event it crosses the Pacific.

Meanwhile, clinicians remain watchful. "You want reasoned and thoughtful preparedness," says Gary Procop, MD, chairman of the Department of Molecular Pathology and medical director of the molecular microbiology, mycology, and parasitology laboratories at the Cleveland Clinic. "The medical community is always trying to prepare for the next outbreak. We never know how bad next year's flu will be, so we tend to plan for the worst without becoming too frantic over it."

Novel influenza viruses receive a lot of press, notes Dr. Procop. "But it's also important to circle back and recall that there are many preventable deaths every year from the seasonal influenza." The H7N9 virus is definitely worth watching, he says, but routine preparedness and vaccination for the regular influenza virus will save lives in the here and now. And for the time being, that's what America needs most.

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