Nowhere to hide. Lightweight transmitters enable satellite tracking of migratory birds and the flu viruses they carry.

AVIAN INFLUENZA

Flu Virus Research Yields Results But No Magic Bullet for Pandemic

As concerns wane that the bird flu strain H5N1 will spark a global pandemic, scientists are warning that the virus, perhaps less of a threat, is here to stay

BANGKOK—Just a couple of years ago, scientists, public health officials, and journalists were nervously tracking every move of the deadly H5N1 avian influenza virus, fearing that a few simple mutations might give it the ability to spread readily among humans, sparking a global pandemic that could kill tens of millions. But since alarms were sounded when the virus started spreading in earnest among birds in late 2003, the dreaded pandemic hasn't come. "I'm less worried about this virus than I was 5 years ago," says virologist Robert Webster of St. Jude Children's Research Hospital in Memphis, Tennessee.

But H5N1 hasn't gone away—and increasingly, say scientists, the virus appears to be here to stay. "H5N1 is going to be with us for a long time," says Les Sims, a veterinary consultant based in Palm Cove, Australia, continuing to devastate poultry flocks and posing an ongoing threat to human health.

In 2007, the virus surfaced in poultry flocks in eight new countries as widely separated as Bangladesh, Poland, and Ghana. Outbreaks returned in 23 countries stretching from Japan to the United Kingdom; in Indonesia and Nigeria, in particular, they are now more or less continuous. Although the number of human cases and deaths declined by 25% compared with 2006, Nigeria, Laos, and Pakistan had their first human cases last year, and Indonesia, the hardest-hit country, reported 42 cases and 32 deaths. As long as the virus is circulating in birds, experts warn, there will continue to be sporadic human cases, and most of them will be fatal.

Research is providing insights into how the virus spreads and the viral mutations that might be needed for H5N1 to infect humans more easily, as was evident at a recent meeting here.* "The spinoff is a better understanding of flu viruses in general," says microbiologist Peter Palese of Mount Sinai School of Medicine in New York City.

But David Fedson, a vaccine expert and former executive at Aventis Pasteur now based in Sergy Haut, France, worries that these advances, although valuable, are not doing much to help prepare for an influenza pandemic. He and others believe a pandemic is inevitable, whether it is caused by H5N1 or another flu strain that has yet to emerge. "Nobody has a clue [how] to take some of these findings from the lab and turn them into something that addresses public health," laments Fedson.

* "Bangkok International Conference on Avian Influenza 2008," 23–25 January, Bangkok, Thailand.

Out of the wild

One continuing uncertainty is whether wild birds are "victims or vectors" of H5N1, says wildlife health specialist Scott Newman of the Food and Agriculture Organization (FAO) of the United Nations in New York City. Poultry trading is the primary means of spreading the virus. But the role wild birds play in long-distance spread is still unclear, says Newman. Several groups are studying the question both in the lab and in nature, taking advantage of new lightweight transmitters that enable satellite tracking of migratory species.

Nicolas Gaidet of the French Agricultural Research Centre for International Development in Montpellier, France, described one of the most ambitious efforts. The group, which includes researchers from FAO, the U.S. Geological Survey, Italy's Istituto Zooprofilattico Sperimentale delle Venezie, and others, collected cloacal, tracheal, and fecal samples from more than 11,000 birds in 19 countries in eastern Europe, the Middle East, and Africa in 2006 and 2007. The researchers fitted some of the migratory birds with transmitters. Overall, 2% of the birds were carrying influenza viruses, says Gaidet, and that number rose to 14% in certain species.

The group did not find any living wild birds infected with H5N1, which is in line with other surveys. That suggests that H5N1, which is lethal to many types of wild birds, may kill its victims before they travel far. The team did, however, find four birds in Nigeria carrying an H5N2 virus that genetic analysis indicates would be highly pathogenic to chickens. One, a white-faced whistling duck, subsequently flew 650 kilometers and is still apparently healthy. "This is the first time anyone has found a bird carrying a highly pathogenic virus over a great distance," Gaidet says.

At the meeting, researchers also described progress in understanding how avian influenza viruses mutate into human pandemic strains. Previous work had shown that the viruses' hemagglutinin protein, a surface protein that comes in 16 subtypes, preferentially binds to a host cell receptor known as alpha 2,3; human viruses prefer alpha 2,6. Evidence suggests that a mutation affecting hemagglutinin binding is necessary for an avian influenza virus to switch to a human virus. Whether additional mutations are needed is not known.

Mikhail Matrosovich of the Institute of Virology at Philipps University in Marburg,

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Germany, is trying to answer that question by working with the 1968 H3N2 pandemic virus. The hemagglutinin protein of that virus differs from its putative avian ancestor by seven amino acid substitutions. Two of these had been linked to the receptor-binding preference of the hemagglutinin. To find out the role of the other five substitutions, Matrosovich's group is creating viruses with various combinations of the mutations and testing how well they bind and replicate in cultures of human airway epithelium cells. The team took the pandemic virus and switched the two mutations associated with binding preference back to their avian version. As expected, this engineered virus replicated far less efficiently in human cells than the pandemic virus did.

The biggest surprise, says Matrosovich, is that the virus with the avianlike binding protein grew at all in human cells. "These [findings] do not support the quite-common theory that there are no receptors for avian viruses in the human airway," Matrosovich says. Another virus construct, with the two binding mutations of the pandemic strain left intact but the remaining five substitutions reworked to their avian state, replicated much less efficiently than the pandemic strain as well, suggesting that these substitutions might also be needed for conversion to a pandemic virus, a finding likely to apply to all avian viruses.

Preliminary results from similar studies of changes in the neuraminidase protein common to the 1918, 1958, and 1967 pandemic viruses suggest that mutations in that protein also play a role in giving a virus pandemic capabilities, Matrosovich says.

Evidence that viruses need multiple mutations to adapt to human hosts might seem reassuring. But Prasert Auewarakul, a virologist and physician at Mahidol University in Bangkok, warned that viruses can adapt quickly. His group sequenced viruses retrieved from three fatal human H5N1 cases and found that genomic domains associated with hemagglutinin binding specificity were mutating far more frequently than other areas, indicating evolutionary pressure for the avian virus to adapt to its new host species. Another site with evidence of rapid change was associated with a protein involved in enabling avian influenza viruses, which thrive at the 40°C temperatures found within birds, to replicate efficiently at the lower 33°C temperature of the human body. Auewarakul notes that all three patients died 1 to 3 weeks after the onset of illness. "This tells us that the virus is evolving very quickly inside the human body," he concludes.

An ounce of prevention

Public health authorities hope an effective vaccine will prevent human H5N1 infections altogether. Numerous groups have reported advances in vaccines, including, for instance, novel ways of making one vaccine protect against several different flu strains and using adjuvants to stretch precious vaccine supplies. But Fedson notes that these advances might ameliorate but don't solve the bottleneck of current vaccine production, which requires incubating the virus in an enormous number of chicken eggs, a time-consuming and expensive process that requires biosecure facilities, a highly trained work force, and long lead times. Fedson calculates that with existing vaccine production capacity and the use of an adjuvant, 9 months after a pandemic virus appears there is likely to be only enough vaccine for 700 million people. "Pandemic vaccination is not going to be a realistic possibility in the near future for more than 85% of the world's people who live in countries that don't have vaccine companies," he adds.

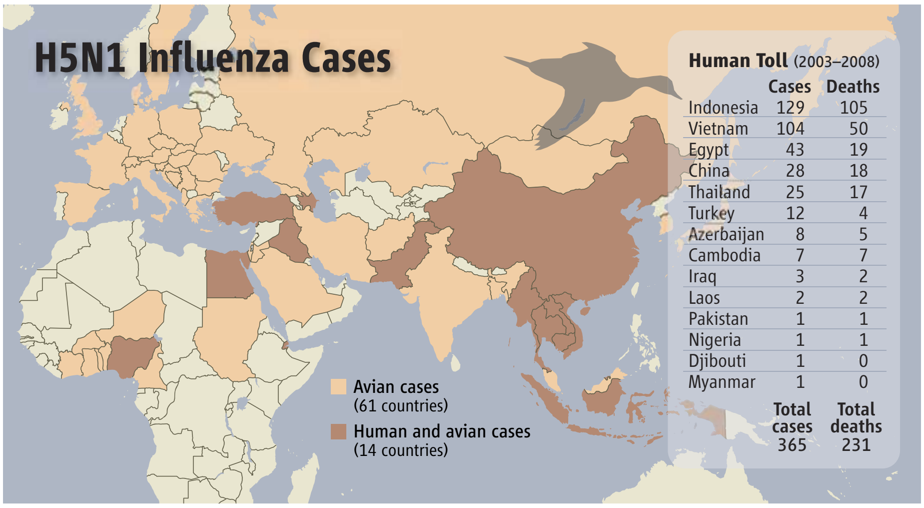
Responding to that challenge, Linda Lua of the Australian Institute for Bioengineering and Nanotechnology at the University of Queensland in Brisbane presented "a radically different vaccine process" that doesn't use eggs. Instead of working with the entire virus, they select a part of a viral structural protein recognized by the human immune system. They then build these protein bits into viruslike particles for use as vaccines. "There is no genetic material," says Lua, which means that the particles are noninfectious; this in turn avoids the need for high-level biosafety production facilities.

"It's a pretty simple process," Lua says. The protein is produced in a bacterial-fermentation process, purified, and then chemically processed into viruslike particles. Lua says they deliberately developed a manufacturing process within the capabilities of some of the more advanced developing countries, such as Thailand and Vietnam. "It is an Asian solution for an Asian problem," Lua says. Anton Middelberg, a chemical engineer at the institute, says that once a pandemic strain appears, whether it is H5N1 or another flu subtype, they could identify target proteins and start production in 1 to 2 weeks. A plant small enough to load into a cargo plane and take to an airport near an outbreak site would be capable of producing about 500,000 doses of vaccine a week. "We can have a rapid response for pandemic influenza using this technology," Lua says. Fedson called the work "extraordinarily exciting."

Middelberg says they "tackled the manufacturing issues first" and are now seeking partners to move into animal testing. He adds that viruslike particle vaccines for hepatitis and human papillomavirus are already on the market and that other groups have gotten promising results with a viruslike particle vaccine against flu in mice. Provided they find a partner and funding, it would take "a few years" to have the process ready to go.

That is cold comfort to Michael Osterholm, a public health specialist at the University of Minnesota, Minneapolis, who warns that every day brings the world closer to the next pandemic. "We don't know if it's going to be H5N1, but there will be another pandemic," he says.

–DENNIS NORMILE



Human Toll (2003–2008)

	Cases	Deaths
Indonesia	129	105
Vietnam	104	50
Egypt	43	19
China	28	18
Thailand	25	17
Turkey	12	4
Azerbaijan	8	5
Cambodia	7	7
Iraq	3	2
Laos	2	2
Pakistan	1	1
Nigeria	1	1
Djibouti	1	0
Myanmar	1	0
	Total cases 365	Total deaths 231

Still on the move. Although not headline news, in 2007 the H5NI virus spread to poultry flocks in eight new countries and returned in 23 others stretching from Japan to the United Kingdom while human cases continued to mount.

SOURCE: WHO AND OIE (WORLD ORGANISATION FOR ANIMAL HEALTH), 21 FEBRUARY 2008