As H5N1 reaches Europe, scientists debate the role of wild birds but agree on the need for greater surveillance

Are Wild Birds to Blame?

Almost as soon as H5N1 avian influenza began its deadly sweep across Asia, people fingered migratory birds as likely culprits in its spread. Migrating birds offer an obvious way to connect the dots of H5N1 outbreaks along the east coast of Asia and, in just the past few months, its unexpected cross-continent jump to Siberia, Kazakhstan, and Turkey. Moreover, researchers have long known that these birds commonly harbor less virulent flu viruses, and many wild birds mingle with Asia's free-ranging domestic poultry, which have been decimated by H5N1.

But avian experts have been almost universally skeptical that wild birds are spreading the virus. One reason is that sampling of tens of thousands of birds has failed to turn up a single healthy wild bird carrying the pathogenic strain of H5N1, which has caused the death of more than 100 million domestic birds—and at least 60 humans—in Asia. Evidence so far suggests that H5N1 kills wild ducks and geese nearly as efficiently as it does chickens. "Dead ducks don't fly" has been the refrain, as avian experts point out that sick and dying birds simply can't spread viruses very far. Instead, epidemiologists investigating the virus's jump, even to geographically far-flung regions, keep turning up evidence suggesting that the poultry trade and other human activities are responsible.

Now, however, evidence implicating wild birds is starting to convince even some of the doubters. "Until about 2 months ago, I was pretty skeptical on whether wild birds were playing a role," says David Suarez, a virologist with the U.S. Department of Agriculture's (USDA's) Southeast Poultry Research Laboratory in Athens, Georgia. "But now I feel that there is much stronger evidence that wild birds are spreading the virus." What changed his mind, he says, was the death of 100 or so ducks, gulls, geese, and swans from H5N1 at a remote lake in Mongolia that he believes can't be explained by human activities. And, he and others add, in an unexpected twist, it's beginning to look as though the culprits might not be the long-suspected migratory waterfowl but another yet-unidentified wild species.

The implications are huge. If wild birds are carrying the disease, says Suarez, "it will be difficult or impossible to control the spread from country to country." Nailing down the answer became even more urgent last week with the confirmation that H5N1 has now entered Europe.

Even before that confirmation, the Netherlands ordered farms along migratory routes to keep poultry inside, and three German states asked farmers to voluntarily take similar precautions. Last month, the European Commission rejected proposals to extend such measures throughout the union, but E.U. officials were reassessing their stance with the news that H5N1 has reached Turkey (see p. 417). Everyone recognizes that if wild birds are involved, new strategies will be needed to halt the virus's spread to domestic flocks—and from them to people. A growing number of scientists and organizations are calling for dramatically increased global surveillance to profile all viruses circulating in wild birds. Says Kennedy Shortridge, a virologist and professor emeritus at the University of Hong Kong, "H5N1 is important, but we still need to be on the lookout for other flu viruses." The costs of surveillance are small, he says, considering the damage that could be done to the poultry industry—or, worse, the potential for a human pandemic.

Heads up. Researchers worry that bar-headed geese might carry the H5N1 virus from the sites of outbreaks in northern China and Mongolia to India and Bangladesh.

From low to high

One reason migratory waterfowl were high on the list of suspects for spreading H5N1 is because they are natural hosts for other bird flu viruses. But Ilaria Capua, a virologist at Italy's National Reference Laboratory for Avian Influenza in Padua, warns that Anatidae, the family that includes ducks and geese, are as genetically distant from gallinaceous birds (chickens, turkeys, and quail) as cats are from dogs. The different families interact with viruses very differently, she says.

Viruses are subtyped by the forms of two of their surface glycoproteins, hemagglutinin (H) and neuraminidase (N). There are 16 forms of hemagglutinin and nine of neuraminidase. Viruses are further classified as being of low or high pathogenicity. Low-pathogenicity viruses are typically carried in a bird's intestinal and respiratory tracts and usually cause mild or no symptoms. Highly pathogenic viruses can infect cells throughout a bird's body and cause systemic disease and, usually, death.

Waterfowl have been shown to carry low-pathogenicity viruses of virtually all possible combinations of H and N, including low-pathogenicity versions of H5N1. So far, however, there is no known natural reservoir for

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highly pathogenic avian influenza viruses. They emerge only after low-pathogenicity viruses jump from water birds into chickens and turkeys. As the virus attempts to adapt to a new host, it somehow acquires the ability to infect cells throughout the bird's entire body. This mutation from low to high pathogenicity, with a resulting bird flu epidemic among poultry, has occurred at least 19 times since 1959. In some cases, researchers have traced the virus from its low-pathogenicity form in water birds to a low-pathogenicity virus that circulated in poultry before becoming highly pathogenic.

No one has yet uncovered the lineage of the highly pathogenic H5N1 strain now endemic in Asia. Presumably, it evolved from a low-pathogenicity H5N1 variant circulating in waterfowl in southern China before the first known outbreak of the disease in chickens in Hong Kong in 1997. By culling all 1.5 million domestic poultry in Hong Kong, authorities stamped out the outbreak. With a few exceptions, the virus was not seen again until December 2003, when a massive outbreak swept chicken farms in Korea. By January, the virus had turned up on farms in Japan and Vietnam; by February it was detected in Indonesia, and it was soon killing chickens in Thailand and China.

When public health experts pointed to migratory birds as a likely source, ornithologists and animal epidemiologists showed that the outbreaks did not neatly fit any known migratory patterns. If migratory birds were carriers, they argued, the virus should have turned up in the Philippines and Taiwan by now, but it hasn't. What's more, since the late 1990s, USDA has sampled more than 10,000 waterfowl crossing the Bering Sea from Asia to Alaska, while University of Hong Kong researchers have tested several thousand entering Hong Kong; neither group has found a single healthy bird carrying the H5N1 virus.

Instead, human movements of infected poultry have spread the virus over seemingly improbable distances. For instance, an outbreak of H5N1 among poultry in Lhasa, Tibet, in January 2004 was traced to a shipment of chickens from Lanzhou in China's Gansu Province, about 1500 kilometers away. An even more bizarre case surfaced in October 2004, when an air traveler was caught at Brussels Airport with two crested hawk eagles, infected with H5N1, in his carry-on bag. The smuggler had bought them at a Bangkok bird market on behalf of a Belgian falconer.

A new paradigm

As the epidemic continues, it's becoming increasingly clear that H5N1 represents a "change in the paradigm" of what is known about avian influenza viruses, says Les Sims, a veterinarian in Manunda, Australia. Before this strain of H5N1 appeared, for instance, waterfowl were thought to be resistant to infection by highly pathogenic viruses. Studies over the last several years have shown that domestic ducks can asymptomatically carry some strains of H5N1 that are lethal to chickens. (Yet other H5N1 strains are lethal to domestic ducks.)

Until last spring, however, there was no sign that H5N1 was infecting any wild birds in a significant way. That changed in April, when an H5N1 outbreak at Lake Qinghai in northwestern China killed an estimated 5000 to 6000 migratory water birds.

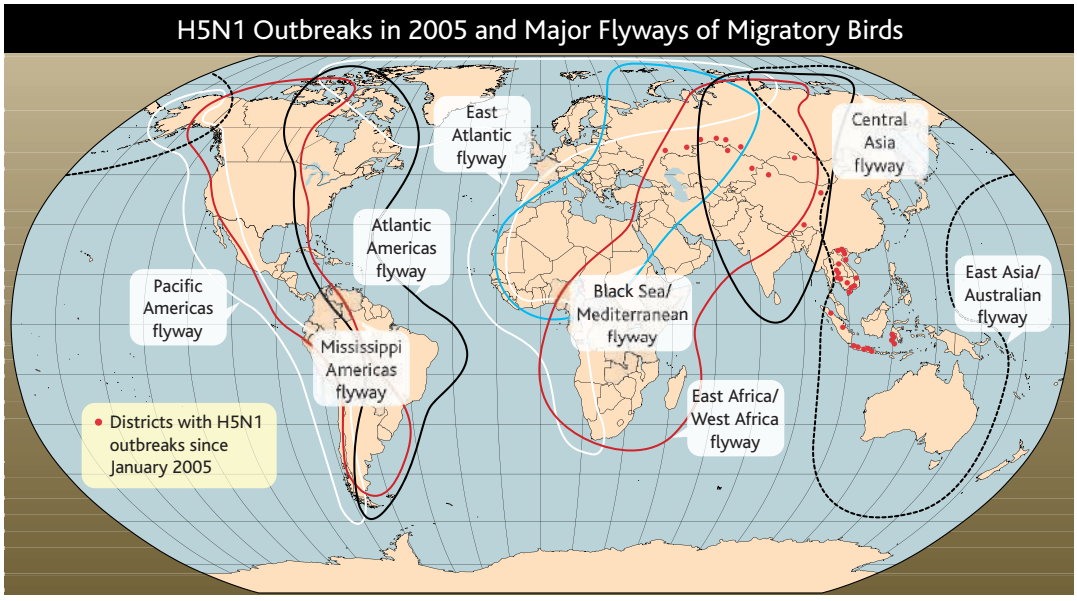
The die-off immediately raised alarms that surviving birds might carry the virus to India and beyond. But, apparently because of infighting between Chinese ministries and institutions, the government barred Chinese and outside scientists from sampling or tracking the travel of surviving birds. "It was a missed opportunity," says ornithologist David Melville from Nelson, New Zealand.

Stowaways. These crested hawk eagles, infected with H5N1, were smuggled from Bangkok to Brussels in an air traveler's carry-on bag.

Researchers are still wondering how the virus got to this remote corner of China. Just after the Lake Qinghai outbreak, the virus turned up on a poultry farm in the same province. This "makes it difficult to tell whether poultry or wild birds brought the virus to the area," says Suarez.

An August outbreak at Erkhel Lake in Mongolia, however, helped persuade Sims that wild birds are to blame, but his change of mind comes not from finding a positive link but from ruling out human movements of poultry, he warns. "All epidemiology is based on probabilities," he adds.

A group of veterinarians from the Wildlife Conservation Society was already in Mongolia in case H5N1 made the 600-kilometer leap when it heard of unusual bird deaths at Erkhel Lake. The group collected 774 samples from both dead and living birds. USDA confirmed highly pathogenic H5N1 in dead birds—but found no evidence of the virus in any samples from the live ducks, gulls, geese, or swans.



On the fly. Flyways might seem to connect the dots of H5N1 outbreaks, but the timings and locations aren't a perfect fit with known migratory patterns.

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Published by AAAS

Keeping Track of Viral Air Traffic

BERKENWOUDE, THE NETHERLANDS—Catching wild ducks, an art that requires skill as well as patience, has a long tradition in this water-rich country. But these days, Dutch duck trappers are helping address a 21st century challenge by taking stock of the dizzying variety of bird flu strains flying overhead—and perhaps providing early warning should the fatal H5N1 strain arrive. At Erasmus Medical Center in Rotterdam, virologist Vincent Munster runs one of the largest surveillance programs for avian influenza in the world, and he relies on dozens of people who catch birds, either for a living or as a hobby, to send him more than 8000 samples a year.

Bert Pellegrom, a forester whose hobby is keeping a 200-year-old duck trap operational, is one of them. At his trap—really a small lake, surrounded by reed screens to hide the trapper from the birds and equipped with elaborate netting structures—Pellegrom catches ducks several times a week, which he kills and sells to the local poulterer. (They fetch about $4 a bird.) On a sunny afternoon last week, conditions weren't favorable—too warm and not enough wind—but Pellegrom caught two mallards. "This may look a bit unpleasant," he cautioned, before wringing their necks. Then he got some sterile cotton swabs from a shed, inserted one in each of the ducks' cloacas, and turned it around once before pulling it out and storing it in a small plastic bag.

Helping hands. Bert Pellegrom (*right*) is one of many people collecting samples for the avian influenza surveillance program run by Vincent Munster.

Between 1% and 20% of all ducks, depending on the species and season, are infected with an influenza strain, usually without symptoms, Munster says. Back at the lab, he and his colleagues culture viruses from the samples, determine the strain, sequence the signature hemagglutinin gene, and check whether they have low or high pathogenicity. Although duck trappers like Pellegrom supply some of the samples, the majority come from ornithologists—in the Netherlands, Sweden, and far-flung places such as Japan, Canada, and South America—who ring wild birds for migration studies and release them. Together, the samples cover hundreds of different bird species, mostly ducks, geese, gulls, and shorebirds. Bit by bit, the Rotterdam group, led by Ron Fouchier, is assembling a detailed picture of which viral strains are out there, which bird species each strain prefers to infect, and how patterns change with the seasons.

When the program started 5 years ago, it was a leisurely academic endeavor, and the researchers analyzed the samples only after the end of each migration season. But after H5N1 started its path of devastation from China to Turkey, the group realized that it offered a possible early warning system as well. Two months ago, they started collecting samples weekly and screening them as soon as they come in. If highly pathogenic H5N1 makes it to northern Europe, Munster hopes he will be the first to know. The group has applied for European Union funds to expand the network across Europe.

Munster rarely goes on field trips himself. But when he accompanied a reporter to Pellegrom's trap, his study produced an unexpected benefit: Rather than selling them, Pellegrom offered the two birds to Munster, who, for the first time in his life, got to carve up, roast, and eat his research subjects at home. **–MARTIN ENSERINK**

Because there are so few poultry in this isolated region, Suarez thinks their involvement is "unlikely." "The most likely scenario," he says, is that wild birds carried the virus to Erkhel Lake and infected the birds that eventually died. "We don't know which species were responsible for spreading the virus," says Sims, who is also involved in the project, although he suspects that those unidentified species could be spreading the virus elsewhere. (The researchers declined to provide further details because they are readying an article for publication.) Figuring out which species might be involved will be tough, others note, as next to nothing is known about avian influenza except in waterfowl.

Searching

Some answers may come from Fu-Min Lei, an ornithologist at the Institute of Zoology in Beijing, part of the Chinese Academy of Sciences (CAS). Since last March, he has collected more than 6000 viral and serological samples from a variety of wild animals throughout China, including 2000 samples from migratory and resident birds, and is searching for H5N1.

Another Chinese team led by George Gao, a virologist at CAS's Institute of Microbiology in Beijing, has collected several dozen serum samples from birds that survived the H5N1 outbreak at Qinghai Lake. If any test positive for antibodies to the H5N1 virus, says Gao, who is preparing to publish a paper, it would suggest that some mildly infected water birds might be carrying the virus long distances.

Even before the virus turned up in Turkey, the incidents at Qinghai and Erkhel and the spread of the H5N1 virus through Siberia and Kazakhstan had sparked new surveillance efforts. In Europe, Albert Osterhaus, a virologist at Erasmus University in Rotterdam, the Netherlands, has proposed a Europe-wide wild bird surveillance program. His group currently gathers cloacal samples from 6000 birds annually, primarily in the Netherlands (see sidebar). Extending such surveillance to critical migratory routes crossing Europe, which he estimates would cost about $2.5 million, would not only serve as an early warning system for a possible pandemic, he says, but also provide data on other viruses that pose a threat to domestic flocks. Osterhaus would like to see similar networks set up to cover flyways in Asia-Pacific and the Americas.

Other nations have not recognized the need, so surveillance is patchy, except in Asia, which has an aggressive program of sampling wild birds and birds brought to live poultry markets.

The United Nations Food and Agriculture Organization (FAO) is helping nascent surveillance efforts in South Asia, and the World Organisation for Animal Health recently sent an expert mission to support surveillance in Russia. "We're very concerned about India and Bangladesh," says FAO's Juan Lubroth, because the bar-headed geese that breed at China's Qinghai Lake winter in South Asia. But Lubroth notes that wild bird surveillance is just one on a long list of veterinary needs that includes strengthening local lab capabilities and improving hygiene on farms and in markets. All these measures are desirable no matter how H5N1 is being spread, he says. FAO has appealed to the international community for $100 million to fight avian influenza in Asia but has so far only raised $30 million—a small sum, Lubroth says, for trying to avert a human pandemic.

–DENNIS NORMILE

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