

By Kathryn Brown

A
Bout
with

Flu

As influenza smashes evolutionary barriers,
scientists wonder: Is this the coming of the
next human pandemic?

PHOTOGRAPH BY STUART RAMSON / AP



A WORKER PROCESSES A CHICKEN AT A PROCESSING PLANT IN SHANGHAI. IN NOVEMBER, CHINA CONFIRMED ITS FIRST CASES OF AVIAN FLU IN HUMANS AND, TO TRY TO STEM THE SPREAD OF THE VIRUS, RUSHED TO VACCINATE BILLIONS OF CHICKENS AND OTHER FOWL.

Last year, a shortage of flu vaccine helped focus public attention on the medical consequences of influenza, to say nothing of its economic impact. This year, flu is again in the news. A lethal strain of avian influenza—bird flu—is spreading. Because this strain can infect humans as well as birds, scientists fear that in a worst-case scenario the avian flu could trigger a human pandemic.

In this context, influenza is a challenge to public health officials. But to scientists, the virus also presents a unique opportunity—a way to study evolution in action.

Since 1933, when scientists first isolated influenza A type viruses from ferrets, they have watched it break evolutionary barriers with alarming ease—infecting not only humans, but also birds, pigs, horses, dogs, and other species.

“Really, it’s a numbers game,” says Robert G. Webster, a virologist at St. Jude Children’s Research Hospital in Memphis. “The more that chickens are infected and the broader the virus’s geographic range, the faster it all adds up. It’s just a matter of time before a new pandemic emerges.”

Flu pandemics in 1918, 1957, and 1968 caused millions of deaths. Both strain H2N2 (the cause of the 1957 pandemic) and strain H3N2 (1968’s pathogen) are believed to have arisen by the exchange of genes between avian and human flu viruses, possibly following dual infection in humans. The deadliest pandemic, in 1918, was different. It was the result of strain H1N1, thought to be derived wholly from an ancestor that originally infected birds.

Concerned about the likelihood that history will repeat itself—and that it will look more like 1918 than 1957 or 1968—Webster has been sounding the avian influenza alarm for years—to the point that some researchers dismiss him as preachy. “I’ve been accused of being a Chicken Little,” he says. “But someone’s got to do it. The H5N1 strain has some very disturbing characteristics.”

Circulating in Southeast Asia since at least 1997, the highly pathogenic H5N1 has killed more than 150 million birds. And the virus is on the move. Wild waterfowl such as geese have been carrying H5N1 across Asia, along migratory routes where they come in contact with domestic poultry, typically near rivers and lakes. In August, officials in Russia and Kazakhstan confirmed the first reported outbreaks of H5N1 influenza among poultry in those countries.

So far, it’s unclear how dangerous H5N1 is to humans. The virus, however, is clearly capable of infecting humans who come in contact with infected poultry—authorities have reported more than 110 confirmed cases, resulting in over 60 deaths, in Vietnam, Thailand, Cambodia, Indonesia, and China.

These numbers are likely to be underestimates—with spotty surveillance data, it’s impossible to reliably gauge the rates of disease incidence or fatality. But, at least for now, H5N1 does not appear to be easily transmitted from human to human—a basic feature of pandemics.

Many scientists think they’re playing a waiting game, however. “H5N1 has been around for 9 years, and I find myself asking, ‘Why *hasn’t* a human pandemic happened?’” says Robert A. Lamb, an HHMI investigator at Northwestern University in Chicago. “The fact is, it wouldn’t necessarily take much. With these H5 viruses, even a single-point mutation can make the difference between the virus’s ability to kill lab mice or not.”

ESSENCE OF EVASION

Influenza’s threat is not limited to this particular avian type. H5N1 belongs to the H5 influenza virus family, just 1 of 16 subtypes. Labeled H1 to H16, each subtype is named for the distinct structural biology of two key influenza surface proteins, hemagglutinin (HA) and neuraminidase (NA). All H5 viruses, for instance, share a similarly shaped HA protein. The influenza viruses within the H5 family as well as in the other families are further distinguished by the shapes of their NA proteins, of which there are nine.

Like a coat of armor, the HA and NA surface proteins stud the tiny influenza virus particle. When the virus mutates, it can essentially “change coats,” altering the shape of its exterior surface and becoming unrecognizable to the human (or animal) immune system. This is the essence of immune evasion, a hallmark of influenza. The virus can under-

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go two types of changes. Small changes in the virus coat's proteins happen continually and result in new strains. This is a main reason why people can get the flu more than once and why they need to get a new flu vaccine every year. The virus coat can also change abruptly into a new subtype that has an HA protein or an HA-NA protein combination that has not been seen in humans, at least not for many years. Most of us would have little or no innate protection against this new virus. And if the virus can spread easily from person to person, a pandemic may occur. If influenza viruses rarely changed shape, immune evasion wouldn't keep researchers up at night. But they constantly evolve, and their physical structure is again the reason. Inside its spherical shell, the virus particle houses eight separate RNA segments—which encode genes for at least 11 proteins—and this kind of segmented genome is ripe for recombination. If two different influenza viruses infect the same cell, for instance, they can easily exchange gene segments—generating, by some estimates, up to 256 different offspring. Scientists call this phenomenon a genetic “reassortment.”

In the case of the H5N1 avian flu strain, waves of genetic reassortment have pushed the virus from geese into chickens, then ducks, and beyond. What are the molecular mechanics behind these interspecies jumps? Scientists are beginning to find out. In the past year, researchers have published several studies of mammals in Asia infected with H5N1—including humans, tigers in a Thai zoo, and mice. Each case appears to harbor the same mutation: a single amino acid substitution, glutamine to lysine, in position 627 of the virus's PB2 protein, a polymerase protein that renders the virus more pathogenic by helping it replicate. The specific cause of the jump from one species to another remains something of a mystery, but many researchers believe it has to do with changes in the HA protein, which is responsible for recognizing receptors on the cells the virus infects.

TRANSMISSIBILITY

Although pathogenicity is a key influenza feature, it's only part of the health equation. Equally important, scientists



CURRY, A 5-YEAR-OLD BICHON FRISÉ, IS ONE OF THE LUCKY ONES. CURRY RECOVERED FROM CANINE FLU, WHICH IS CAUSED BY A VIRUS THAT JUMPED FROM HORSES TO DOGS IN THIS COUNTRY.

The Flu Strikes close to home

FOR A REAL-LIFE EXAMPLE OF INFLUENZA jumping the species barrier, look no further than the family pet. In a study published in the October 21, 2005, issue of *Science*, researchers reported that a decades-old variety of equine (horse) influenza has emerged in dogs.

This discovery began unfolding in 2004, when greyhounds at a Florida racetrack fell ill with an unidentified respiratory disease. Lab studies at the University of Florida failed to turn up the usual pathogens behind “kennel cough” and similar canine conditions, so researchers sent the samples to the veterinary diagnostic lab at Cornell University.

Fearing an influenza virus, Cornell scientists forwarded suspect viral samples to the U.S. Centers for Disease Control and Prevention (CDC). Sure enough, CDC staff recognized the pathogen as H3N8 equine influenza virus. Having occurred for at least 40 years in horses, this virus suddenly made a complete jump into greyhounds. Moreover, this newfound canine influenza, dubbed canine/FL/04, quickly began to spread. Since the winter of 2004 it has been confirmed in outbreaks at racetracks in at least 11 states, affecting thousands of greyhounds. Pet dogs, too, are susceptible, with confirmed cases among many breeds in Florida clinics and 16 other states, although the illness is mild in most dogs.

Nevertheless, “For scientists worried about interspecies transfer of influenza, this is a rare and striking example,” says Ruben O. Donis, a CDC scientist and senior author of the *Science* paper. “Interspecies transmission of influenza happens quite frequently, but what we usually see is the scenario in Asia, where H5N1 avian influenza jumps to a person and then stops. That’s a dead end for the virus, because it can’t be transmitted from person to person. What’s new, in the canine case, is the establishment of a new virus in a new host—the dog—with efficient transmission. Dogs catch this flu from other dogs. In other words, influenza has found a new host, adapted to it, and is thriving.”

How? Donis says that, although the equine and canine influenza strains are at least 96 percent genetically homologous, the canine virus appears to carry 8 to 10 amino acid changes in its hemagglutinin—an important surface protein on influenza particles that is critical for determining host specificity. Changes in other proteins, still under study, may also promote the virus’s interaction with its new canine host.

CDC scientists, in collaboration with scientists at the University of Florida and University of Kentucky, plan to continue comparing recent equine and canine influenza isolates—as well as to survey equine samples that are older. “We have equine influenza virus isolates, taken every year, back to 1963,” Donis says. “So we can look at all of them and ask, ‘Which mutations at what time enabled H3N8 to cross the species barrier into dogs?’”

That may be the top question among scientists, but for pet owners another concern looms. If the flu can jump from horses to dogs, why not from dogs to people? The historical record provides some assurance, as well as uncertainty. “H3N8 has been in horses for more than 40 years,” Donis notes. “In all this time, there has not been a single documented case of human infection. On the other hand, dogs have been living next to horses for the same period of time, and they didn’t catch the equine flu virus until now. The reality is, we just don’t know.”

say, is transmissibility within a species. Peter Palese, a virologist at Mount Sinai School of Medicine, in New York, has lots of questions in that regard: “What makes an influenza virus transmissible from human to human? What are influenza’s rules for this transmission? And how can we study it in the lab, using animal models?” He acknowledges that “we just don’t have good answers right now.”

HHMI investigator Stephen Harrison, a structural biologist at Harvard Medical School, adds that these questions require scientists to blend different approaches. “To understand influenza’s molecular evolution, we must rephrase natural history questions in molecular terms,” Harrison says. “In other words, we have to capture that moment when a virus jumps to a new species, and learn the detailed dynamics of viral infection.”

If H5N1 did trigger a human flu pan-

Pandemic Protection: Build Higher Levees, Now

INFLUENZA RESEARCHERS ARE KNOWN to disagree on the finer points of avian flu, including just how great a threat it may pose to human beings. But the scientists are virtually unanimous on one point: Should this flu cause a pandemic, the world is not prepared for it. “The science is way ahead of the political will to solve these problems,” says Robert G. Webster of St. Jude Children’s Research Hospital in Memphis.

At a September influenza briefing on Capitol Hill sponsored by HHMI and the Center for Strategic and International Studies, Webster and several other researchers highlighted the importance of stockpiling influenza drugs, modernizing vaccine production, and planning for a worldwide disaster.

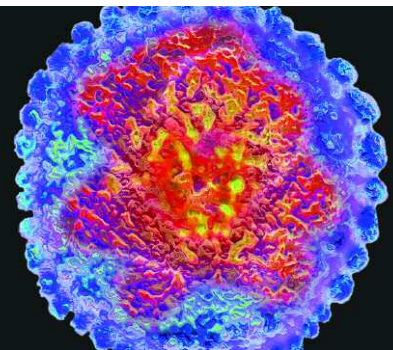
For many, it was a familiar refrain. “Ten years ago, I attended an influenza-pandemic preparedness meeting with some of the same people in this room,” says Dominick Iacuzio, medical director at the pharmaceutical company Hoffman–La Roche, at the briefing. “Here we are again a decade later, still talking about being prepared.”

Along the way, the scientists have actually made significant progress—in science. Hoffman–La Roche has released the antiviral drug Tamiflu (oseltamivir phosphate), designed to alleviate flu symptoms if taken early in the illness. Webster has probed the molecular biology and epidemiology of the H5N1 avian flu strain. Briefing participant Peter Palese of Mount

READY OR NOT

Influenza scientists widely agree on one point: The world is not prepared for a flu pandemic. Scientists have expressed concern that public policy lags the need for action. As Peter Palese of Mount Sinai School of Medicine says: “We have markets for F16 fighters, but not vaccines.”

Currently, no vaccine is available to protect humans against the avian virus known as H5N1 (right). According to the Centers for Disease Control and Prevention, research studies to test a vaccine to protect humans against H5N1, which is known to have infected people in southeast Asia, began in April 2005.



demic, the World Health Organization (WHO) estimates it could kill anywhere from 5 million to 150 million people, although WHO says a valid prediction is possible only after a pandemic begins. Even at the lower end of that range, the numbers are astounding; public health officials cannot afford to wait for influenza to reveal its secrets. The National Institute of Allergy and Infectious Diseases (NIAID) has contracted with two companies to develop H5N1 vaccines.

One of the companies, Sanofi Pasteur, has already demonstrated the feasibility of an H5N1-specific vaccine in preliminary clinical trials of a vaccine candidate. The other company, Med-Immune, announced in September that it will collaborate with NIAID scientists to systematically develop a library of vaccines for all 16 influenza virus HA subtypes. And an H5N1 vaccine is on the list. ■

Sinai School of Medicine and collaborators have reconstituted the 1918 flu strain to reveal its molecular secrets. And attendee Robert A. Lamb, an HHMI investigator at Northwestern University, has discovered the function of important elements of the replicating influenza virus. Other scientists have made similarly impressive gains.

Yet science can only go so far. “At the end of the day, public policy and government planning will make the difference,” says Lamb. “European countries and Australia have done a much better job than the United States at stockpiling Tamiflu, for instance. In the U.S., all the available drugs would probably go to Congress and specific primary-care providers. What would happen if 20 percent, or 5 percent, or even 1 percent of Americans got sick?”

In one revealing moment at the September briefing, when an audience member pointedly asked the panel of scientists how the United States would cope with an influenza pandemic, they replied that the U.S. Department of Homeland Security (DHS) would ultimately be responsible for coordinating the day-to-day management of the crisis. To slow the flu virus’s spread, they suggested, DHS might close schools and offices, shut down public transportation, and basically send the country home.

Weeks after the briefing, Webster reflected on this scenario and felt a lot less sanguine. Americans have since lambasted the federal government, including DHS, for its uneven response to the devastating Hurricane Katrina on the Gulf Coast. “There are so many indicators that a pandemic is brewing,” says Webster. “We really can’t be caught short. As in New Orleans, our levees have got to be built higher.”