

Changes on the farm may be fostering the evolution of the swine flu virus—and if a dangerous new strain crosses back into humans, it could have deadly consequences

Chasing the Fickle Swine Flu

One of the first signs of trouble was a barking cough that resounded through a North Carolina farm in August 1998. Every pig in an operation of 2400 animals sickened, with symptoms similar to those caused by the human flu: high fever, poor appetite, and lethargy. Pregnant sows were hit hardest, and almost 10% aborted their litters, says veterinary virologist Gene Erickson of the Rollins Animal Disease Diagnostic Laboratory in Raleigh. Many piglets that survived in utero were later born small and weak, and some 50 sows died.

The culprit, a new strain of swine influenza to which the animals had little immunity, left veterinarians and virologists alike puzzled. Although related flu strains in birds, humans, and pigs outside North America constantly evolve, only one influenza subtype had sickened North American pigs since 1930. That spell was suddenly broken about 4 years ago, and a quick succession of new flu viruses has been sweeping through North America's 100 million pigs ever since. This winter, for example, up to 15% of the 4- to 7-week-old piglets on a large Minnesota farm died, even though their mothers had been vaccinated against swine flu, says veterinary pathologist Kurt Rossow of the University of Minnesota, Twin Cities.

It seems that after years of stability, the North American swine flu virus has jumped onto an evolutionary fast track, churning out variants every year. Changes in animal husbandry, including increased vaccination, may be spurring this evolutionary surge. And researchers say that the resulting slew of dramatically different swine flu viruses could spell danger for humans, too. The evolving swine flu "increases the likelihood that a novel virus will arise that is transmissible among humans," says Richard Webby, a molecular virologist at St. Jude Children's Research Hospital in Memphis, Tennessee.

Because people have no immunity to many viruses from other species, strains that on rare occasions leap the species barrier can have deadly consequences (see sidebar, p. 1504). And pigs are considered "mixing vessels" in which swine, avian,

and human influenza viruses mix and match. Scientists believe, for example, that the last two flu pandemics, or worldwide epidemics, in 1957 and 1968, occurred when avian flu and human flu viruses swapped genes in pigs, creating a new, hybrid virus that then spread to humans. In each case, the new virus appeared first in Southeast Asia, then around the globe. The 1918 "Spanish flu," which claimed upward of 40 million lives, may also have arisen first in pigs. "We used to think that the only important source of genetic change in swine influenza was in Southeast Asia," says Christopher Olsen, a molecular virologist at the University of Wisconsin (UW), Madison. Now "we need to look in our own backyard for where the next pandemic may appear."

Fortunately, the new pig strains that have appeared in North America so far do not appear to readily infect humans. But researchers are sufficiently concerned that they are calling for increased surveillance of both humans and pigs. "Within the swine population, we now have a mammalian-adapted virus that is extremely promiscu-

ous," says Webby, referring to the virus's proclivity to swap genes with avian and human flu influenza viruses. "We could end up with a dangerous virus."

When pigs fly

Most genetic changes in the flu viruses—human, pig, and bird—are small and subtle point mutations in the virus's RNA. Less common but more alarming are sudden, wholesale changes that replace entire genes and are more likely to circumvent the immune system. This process, called genetic shift, is exactly what is now occurring in North American pigs. Thus, the latest swine influenza virus is a curious hybrid: The genes that code for its coat proteins derive from classical swine influenza, but half of its internal genes have been snatched whole from avian and human viruses.

The structure of the influenza virus lends itself to such radical changes. The virus is made of eight single-stranded segments of RNA that together code for 10 proteins (see illustration). If two or more different viruses infect the same host cell, they can swap segments, creating new viral types.



Crowd control. Changes in pig farming, including the rise of large farms, may be spurring the evolution of new swine flu viruses.

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Most commonly, the virus swaps genes that code for its two surface proteins: hemagglutinin (HA) and neuraminidase (NA). Both proteins spike off the virus's outer coat, and HA initiates infection when it binds to receptors on host cells. The immune system of the infected animal targets sites on these molecules. Therefore, a virus with a novel HA can escape preexisting immune defenses—hence the pig deaths.

Influenza viruses are named after their HA and NA components, as in “H1N1” or “H3N2.” Both human and swine influenzas have been historically limited to only a few of these varieties. Birds, on the other hand, can be infected by every combination of the virus's 15 HA genes and nine NA genes, forming a vast global reservoir of virus. And pigs have receptors for both human and bird flu virus, making them crock pots for new viral combinations.

The “classical” swine influenza virus discovered in 1931 is an H1N1 virus, related to the H1N1 that caused the 1918 pandemic. But in the past 5 years, a quick succession of progeny, which now include at least three additional virus subtypes and four genotypes, have all but supplanted that classical swine virus in North American pigs.

The first new virus, the one that struck the North Carolina hogs in 1998, was an H3N2; in this case, genes had crossed from human viruses to pig viruses. By late 1999, the novel viruses could be found wherever there were pigs in North America and so were presumably spread by cross-country transport. Webby and St. Jude colleague Robert Webster, together with Olsen and others at UW Madison, traced these viruses' evolutions. They found both “double reassortant” viruses, with human and swine flu genes, and “triple reassortants,” containing genes from human, swine, and avian influenza viruses.

The avian flu genes may hold clues to the viruses' evolution. They code for two of the virus's three polymerase proteins: PA and PB2. (A third polymerase, dubbed PB1, comes from either human or avian viruses, depending on the swine flu subtype.) All three polymerases are involved in viral replication, and they tend to do a sloppy job, allowing countless errors to slip by.

Scientists suspect that these three imported polymerase genes form a viral platform that Webby calls “unnervingly adept” at triggering change in the influenza genome. In fact, H3N2 has continued to change, acquiring a succession of HA genes derived from the human influenza viruses that circulated several years previously. By 2000, Olsen's lab had identified a new viral subtype, an H1N2 that is a combination of the classical swine virus and the H3N2.

This season's variant is an H1N1 with the internal genes of an H3N2. Its HA gene, derived from the classical swine influenza virus, appears to be rapidly mutating. The amount of sequence divergence among certain 2001 isolates “is as much as the difference between classical H1N1 viruses isolated in the 1960s and those isolated in the early 1990s,” Webby reports. If enough point mutations accumulate, that HA molecule could become unrecognizable to the immune systems of both pigs and humans.

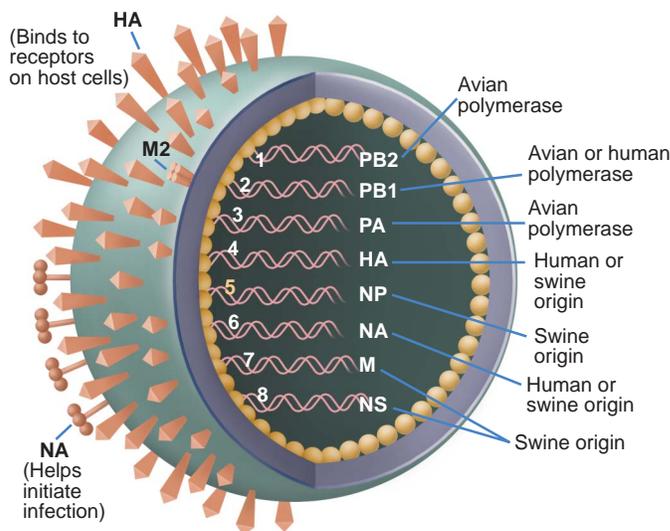
Pig pressures

As they seek to understand what upset the status quo in North American swine, researchers have turned to the environment. For North American pigs, the environment has recently changed dramatically in two ways: herd size and vaccination practices.

In the past decade, big swine producers have gotten bigger, and many small producers have gone out of business. The percentage of farms with 5000 or more animals surged from 18% in 1993 to 53% in 2002, according to Rodger Ott, an agricultural statistician at the National Agricultural Statistics Service in Washington, D.C. Having more pigs under one roof makes it more likely that a rogue virus can take hold. “With a group of 5000 animals, if a novel virus shows up, it will have more opportunity to replicate and potentially spread than in a group of 100 pigs on a small farm,” Rossow says. On the other hand, pigs in outside pens, as is common on small farms, can be exposed to the droppings of migratory waterfowl, which may contain infectious viruses; large-scale confinement agriculture may prevent such exposure, points out Liz Wagstrom, director of veterinary science at the National Pork Board in Clive, Iowa.

Another crucial change has been the recent wide-scale vaccination for swine influenza. In less than a decade, vaccination has become the norm for breeding sows, which in turn pass their maternal antibodies on to their progeny. In 1995, swine flu vaccination was so new that the National Swine Survey conducted by the United States Department of Agriculture didn't bother to assess its extent. In 2000, the

same survey showed that 44.1% of breeding females received a vaccine. Today, more than half of all sows are vaccinated against both H1N1 and H3N2 viruses, says Robyn Fleck, a veterinarian at Schering-Plough, one of the nation's three producers of swine influenza vaccine. But the vaccine is not protecting against all new strains. “We're seeing clinical disease in vaccinated pigs,” says Rossow. Flu is also showing up in piglets thought to be protected by maternal antibodies passed on



New flus. The influenza viruses now found in North American pigs have genes from both human and bird viruses.

from vaccinated sows, such as those on the Minnesota farm.

Widespread vaccination may actually be selecting for new viral types. If vaccination develops populations with uniform immunity to certain virus genotypes, say H1N1 and H3N2, then other viral mutants would be favored. Webby suggests that the combination of avian polymerase genes generating errors in the genetic sequence and immunologic pressure from vaccination may be selecting for unique variants. However, he adds that “the benefits of vaccination far outweigh this side effect.” From a human perspective, reducing the overall viral burden in pigs through vaccination is a plus. “If you can decrease the amount of virus, you can reduce the chances of interspecies transmission,” he says.

Schering-Plough veterinarian Terri Wasmoen acknowledges that vaccines “may be pressuring change.” But she also notes that larger hog confinement operations and more shipping from state to state may play a role. “We need epidemiological

work to understand these issues, and there is no funding now," she says.

Breaking the species barrier

Although scientists know that influenza viruses can jump the species barrier directly, such an event has been seen as a rarity. A review of the literature yields only 18 cases of pure swine influenza directly crossing into people, says Olsen. But his work suggests that many cases of transmission may occur, then fizzle out. Olsen tested 74 swine farm owners, employees, their family members, and veterinarians in rural Wisconsin for antibodies to swine influenza and compared the results to those for 114 city folk in a study published last summer in *Emerging Infectious Disease*. Seventeen of those routinely exposed to pigs tested positive for the antibodies, whereas only one urban dweller did so.

Olsen and his colleagues have also found evidence that a novel H4N6 swine virus isolated in pigs in Ontario—which probably came from local ducks—has already acquired genetic mutations that give it the potential to bind to human cell receptors. Such an event could be catastrophic, as humans have no immunity to H4 viruses. But getting into humans is just the first step. To have pandemic potential, a new influenza virus must also be able to move easily from one person to the next. No new virus from swine or birds, nor any hybrid created in pigs, has been able to accomplish this since the 1968 pandemic.

Even so, experts in both animal and human health are beginning to call for increased surveillance to stop a new pandemic before it starts. The World Health Organization (WHO) constantly scans the globe for new strains of human influenza, which are used to make annual recommendations for next year's vaccines. But "there is no systematic monitoring of [human] populations where there may be interspecies transmission between humans, birds, and pigs," says Carolyn Buxton Bridges, an epidemiologist at the U.S. Centers for Disease Control and Prevention in Atlanta.

In addition, "we don't have any official surveillance system for swine influenza," says Sabrina Swenson, head of bovine and porcine viruses at the National Veterinary Services Laboratories in Ames, Iowa. "We have to bring the human-health people together with the veterinary-health people because of concern that the viruses can



Rapid response. Robert Webster (left) and Richard Webby work to trace the dramatic evolution of swine flu in North America.

move to people. It would be nice to have something better defined, but it's dependent on funding," she says.

Webster, who heads the WHO collaborating laboratory on animal influenza in Memphis, Tennessee, calls for the development of reagents to recognize every possible influenza subtype and for a global

network for monitoring of animal influenzas at the human-animal interface. "If something strange pops up in Georgia or Washington state, do we have the reagents in place to move quickly from identification to making a human vaccine?" he asks.

Last month, at a WHO meeting to determine next year's vaccine constituents, Webster presented his data on animal influenza and urged the development of vaccines for every HA subtype, to be used as a first defense should an unusual virus appear in the human population. WHO agreed and is developing a prioritized list of viral subtypes that it will send to all vaccine manufacturers, urging preparedness for the next pandemic. "It wouldn't provide total protection, but it could keep the virus from killing you," Webster says.

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HIV

Escape Artist Par Excellence

Recent findings are giving researchers new respect for HIV's Darwinian prowess in evading the body's immune defenses

BOSTON, MASSACHUSETTS—Move over, Darwin's finches. As examples of evolution in action, few organisms are better than the AIDS virus. Researchers have known for nearly 20 years that the virus gains a huge evolutionary advantage by targeting immune system cells that the body sends out to defeat it. But that's just its most obvious trait. New studies presented here last month during the largest annual U.S. AIDS conference* provide fresh insights into how the virus has deftly adapted to humans, hijacking, mimicking, and dodging defenses that otherwise might keep it at bay.

Few of these new findings captured headlines from the meeting, and some immediately kicked up controversies. But they prompted a buzz among AIDS researchers here, many of whom have been trying to take the measure of this virus for 2 decades. Every new piece of evidence seems to pro-

duce new respect for HIV's Darwinian prowess. And some of these new insights may also uncover weaknesses in the virus's highly evolved modus operandi. "Some years from now, people will look back and say 'These are the breakthroughs,'" predicts David Ho, the chair of the meeting, who heads the Aaron Diamond AIDS Research Center in New York City.

Evading innate immunity

HIV apparently has picked up clever tricks to get around an arm of the immune system known as innate immunity. Unlike the more refined adaptive immunity, which relies on white blood cells that learn to recognize foreigners and then attack them with exquisite precision, innate immunity uses "natural killer cells," biochemicals, and cellular proteins that behave with more brawn than brain. They take out whole classes of invaders with little discrimination and no memory of what they've done.

At the meeting, Paul Bieniasz, a virologist who works at Aaron Diamond, report-

*10th Conference on Retroviruses and Opportunistic Infections, 10 to 14 February.