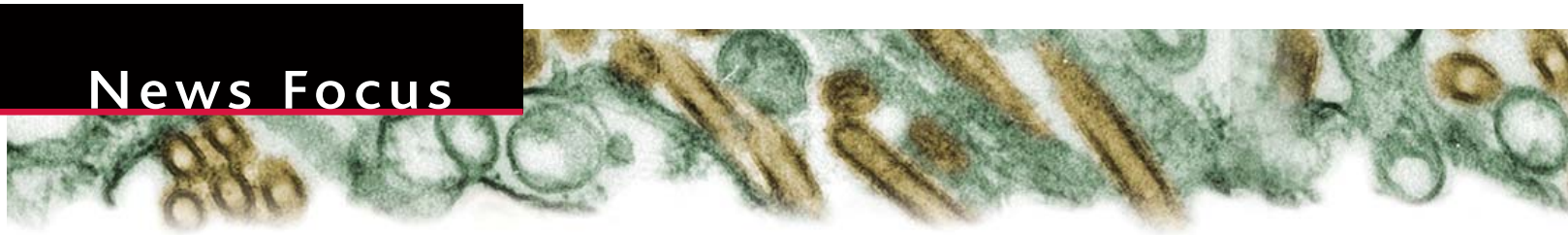


Researchers say crossing avian and human flu viruses is crucial to understanding the threat of a new influenza pandemic, but they admit that they might create a monster

Tiptoeing Around Pandora's Box

Once again, the world is crossing its fingers. The avian influenza outbreak in Asia, already one of the worst animal-health disasters in history, has flared up in four countries; tens of thousands of birds are being killed in desperate attempts to halt the virus's spread. And again, the unnerving question arises: Could the outbreak of the H5N1 strain spiral into a human flu pandemic, a global cataclysm that could kill millions in a matter of months and shake societies to their core?

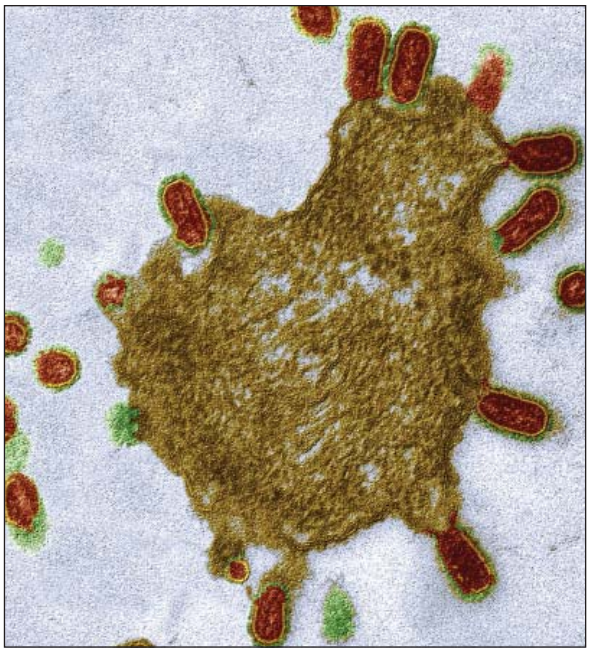
There is a way to find out, flu scientists say—but it's controversial.

Leaving nature to take its course, a pandemic could be ignited if avian and human influenza strains recombine—say, in the lungs of an Asian farmer infected with both—producing a brand-new hybrid no human is immune to. By mixing H5N1 and human flu viruses in the lab, scientists can find out how likely this is, and how dangerous a hybrid it would be.

Such experiments can give the world a better handle on the risks, but they could also create dangerous new viruses that would have to be destroyed or locked up forever in a scientific high-security prison. An accidental release—not so far-fetched a scenario given that the severe acute respiratory syndrome (SARS) virus managed to escape from three Asian labs in the past year—could lead to global disaster. Given their scientific merit, the World Health Organization (WHO) is enthusiastically promoting the experiments. But worried critics point out that there is no global mechanism to ensure that they are done safely.

Despite the concerns, such studies have already begun. In 2000, the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, started experiments to create crossovers between the H5N1 strain isolated during a 1997 outbreak in Hong Kong and a human flu virus adapted for the lab. The study was suspended when CDC's flu researchers became overwhelmed by SARS and the new H5N1 outbreak, both in 2003, says CDC flu expert Nancy Cox, who led the work. But the agency plans to resume the work shortly with the H5N1 strain now raging in Asia.

Others are exploring the options as well. Virologist Albert Osterhaus of Erasmus University in Rotterdam, the Netherlands, is eager to try not just H5N1 but also other bird flu strains, such as H7N7. The Netherlands won't have the required high-level biosafety lab until late 2005, so Osterhaus is talking to researchers in France who do. In the United Kingdom, researchers at the Health Protection Agency, the National Institute for Biological Standards and Control, and universities are also discussing the idea. There are no concrete plans yet—in part because of a lack of funds—but there's a consensus that the studies are important and that Britain is well suited to do them, says influenza researcher Maria Zambon of the Health Protection Agency.



Risk assessment. The H5N1 influenza strain is highly lethal to humans, but whether it could trigger a pandemic is still uncertain.

The aim of reassortment studies, as they're called, would not be to develop new countermeasures, says WHO's principal flu scientist, Klaus Stöhr, because researchers believe current drugs and an H5N1 vaccine in development would work against a pandemic strain as well. But the experiments would provide a badly needed way to assess the risk of a pandemic. If they indicate that a pandemic virus is just around the corner, health officials would further intensify their fight in Asia and go full-throttle in stashing vaccines and drugs; if not, they could breathe a little easier. "It's an extremely important question, and we have a responsibility to answer it," insists Stöhr.

The safety worries are legitimate, Stöhr concedes, and the work should be done only by labs with ample flu expertise and excellent safety systems—not the ones that let SARS out. "We don't want people just fiddling around," he says. He also downplays concerns that the results, when published, might help those who would unleash a pandemic on purpose. Anyone with the scientific smarts to do so can already find plenty of ideas in the literature, Stöhr asserts. Moreover, the studies are unlikely to produce anything that could not arise naturally, says Osterhaus: "You could create a monster. But it's a monster that nature could produce as well."

But critics beg to differ. "We've been debating whether to destroy the smallpox virus for years—and now we're planning to create something that's almost as dangerous?" asks Mark Wheelis, an arms-control researcher at the University of California, Davis. Wheelis also points out that there's no way to keep countries with poor safety records from getting in on the game. At the very least, there should be some global consensus on how to proceed, adds Elisa Harris, a researcher at the Center for International and Security Studies at the University of Maryland, College Park—although no formal mechanism for reaching it exists.

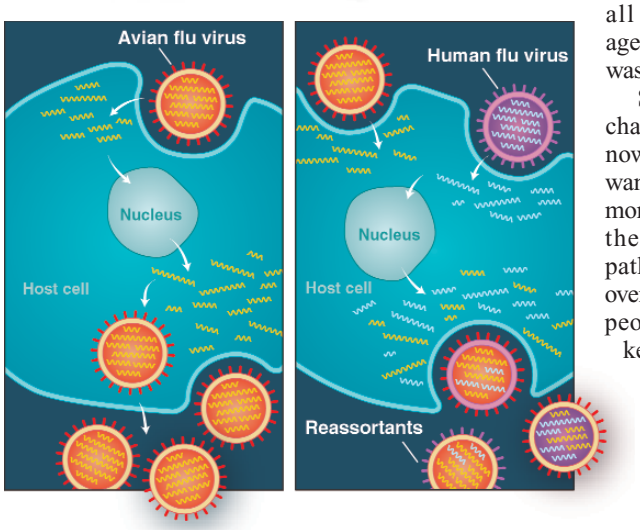
Mix and match

The H5N1 strain has been vicious to its human victims, killing 23 of 34 patients in Vietnam and Thailand this year. So far, however, every known patient had been in contact with infected birds; there's no evidence that the virus can jump from one person to the next—for now. But the virus could evolve inside one of its human hosts, acquir-

CREDITS: (TOP) C. S. GOLDSMITH AND J. M. KATZ/CDC; EYE OF SCIENCE/PHOTO RESEARCHERS INC.

ing mutations that make it possible to infect humans directly, Stöhr says. Another scenario—one researchers believe sparked several previous influenza pandemics—is reassortment with a human flu virus in a person infected with both.

Influenza has a peculiar genome that's divided into eight loose segments, most of them containing precisely one gene. Each segment is copied separately in the host cell's nucleus; at the end of the reproduction cycle, all eight meet up with one another—and with envelope and membrane proteins—to form a new virus particle that buds from the host cell membrane to wreak havoc elsewhere. When a cell happens to be infected with two different strains, homologous segments can mix and match into new, chimeric viruses.



Two can tango. Flu virus genomes consist of eight segments, each of which is copied separately by the host cell (*left*). When two strains infect one cell, they can reassort (*right*).

To create a worldwide outbreak, a newcomer must cause disease in humans and be transmissible between them, and its coat must look so new that no human immune system recognizes it. This is determined primarily by the two glycoproteins on the viral surface, hemagglutinin and neuraminidase—the "H" and "N" in names like H5N1. (Hemagglutinin comes in at least 16 different types, N in nine.) The current fear is that the Asian flu will keep its H5—which humans have never seen before—but swap enough of the remaining seven gene segments with those of a human strain to become more adept at replication in its new host.

During H5N1's first major outbreak in Hong Kong poultry in 1997, 18 people got sick and six died. But the outbreak was stamped out efficiently, and little was heard of H5N1 for 6 years—until it came roaring back last year. Given the magnitude of the current outbreak, the riddle is why reassortment has not yet taken place, says Stöhr. Reassortment studies could help explain whether the world has simply been lucky, or whether there's some barrier to reassortment of H5N1.

The experiments are straightforward. Researchers take a cell line such as MDCK or Vero cells, often used for virus isolation, and add both H5N1 and a currently circulating human strain, such as H3N2 or H1N1. Or they can use a slightly less natural technique called reverse genetics, with which virtually any combination of genes can be put into a flu virus. Any viable hybrid strains would be inoculated into mice; those that cause disease would move on to ferrets, a species very similar to humans in its susceptibility to influenza. Any strain that is pathogenic in ferrets and also jumps, say, from a sick animal to a healthy one in an adjacent cage could be humankind's next nightmare.

During its first round of experiments with the H5N1 strain, CDC managed to create several reassortants, Cox says, but it didn't get around to characterizing them; they're still sitting in a locked freezer in Atlanta.

Global risks, global review?

Most agree that such experiments are in a league of their own. Controversial flu studies were conducted in the past; for instance, researchers sequenced parts of the genome of the "Spanish flu" strain from 1918 (*Science*, 21 March 1997, p. 1793) and inserted its genes into other strains to find out why it was so deadly. But that didn't amount to a wholesale fishing expedition for pandemic strains. And because the 1918 strain was an H1 virus, just like one of the currently active ones, you'd expect at least some immunity to it in the human population, says Yoshihiro Kawaoka of the University of Tokyo and the University of Wisconsin, Madison, who studies the 1918 strain. With an H5 virus, in contrast, everyone would be vulnerable.

Yet although most countries have systems to review the safety and ethical aspects of run-of-the-mill scientific studies, none have formal panels to weigh studies that could, say, put the entire world at risk or be of potential help to bioterrorists. [The U.S. government has announced plans for a national biosecurity panel and a review system to fill that gap (*Science*, 12 March, p. 1595), but they have yet to be implemented.] So although CDC's first round of studies cleared all the usual review hurdles at the agency, Cox says, nothing beyond that was considered necessary.

Since then, "the times have changed," Cox says. The H5N1 strain now plaguing Asia, with which CDC wants to work this time, appears to be more virulent than the 1997 version, and the specter of nefarious use of pathogens looms much larger. Moreover, the mishaps with SARS have made people jittery about labs' abilities to keep bugs on the inside. That's why Cox says she has consulted more extensively with colleagues inside and outside CDC, including experts such as Nobel laureate Joshua Lederberg and WHO. She also plans to seek approval from colleagues at the U.S. National Institutes of Health and the U.S. Food and Drug Administration.

But flu researcher Karl Nicholson of the University of Leicester, U.K., says there should be a more formal, global consensus on the necessity of the studies, who should conduct them, and how. For any country to undertake them on its own, he says, "is like a decision to start testing nuclear weapons unilaterally." WHO would be the best organization to start such a process, says Harris: The destruction of the smallpox virus has been debated at WHO, and an international panel there is overseeing experiments with it at CDC and in Russia.

But Stöhr believes existing safeguards suffice. The studies have been discussed widely with scientists in WHO's global flu lab network and at a recent flu meeting in Lisbon, he says, and have met with nothing but "overwhelming agreement." "If there are other voices, we will take them seriously," Stöhr adds—but for now, it's up to the labs to have their plans rigorously vetted by national authorities and get started.

Eventually, any strain with pandemic potential should be destroyed, he says. But there's no way to enforce this, and skeptics point out that the smallpox virus was slated for destruction, too—until the threat of bioterrorism created a movement to keep it alive, perhaps indefinitely, for defensive studies. In a way this discussion is moot, says Richard Webby of St. Jude Children's Research Hospital in Memphis, Tennessee. With flu strains readily available, anyone with a good knowledge of molecular biology could recreate a pandemic virus once it's discovered, he says. "You can destroy this virus," Webby says, "but it will never really be gone."

–MARTIN ENSERINK

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