

Comment

H5N1

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The year 2005 might just go down in history as The Year Nature Struck Back. Earthquakes kill more than 50,000 in Pakistan and Kashmir (proving how stupid it is for people to fight one another when sooner or later they will need each other). More than 1,000 die in hurricane-spawned floods in Mississippi and Louisiana (exposing the sorry state of both the infrastructure and the emergency response system in the US). Eight straight days of rain dump a foot of water on New England, weakening ancient dams (there's that infrastructure problem again) and threatening numerous towns with severe flooding. And as I write this the 21st tropical storm of the Atlantic season, Wilma, is churning up the waters of the Caribbean, with a projected path towards Florida via the battered Gulf of Mexico. Wilma is also the 12th hurricane of this season, tying a record set in 1969 (the 21 tropical storms also tie a record, set in 1933).

How bad has it been this year? Well, we're about to run out of letters of the alphabet: tropical storms are named alphabetically each year, with boy's and girl's names alternating (it used to be only girl's names until someone suggested that this seemed a tad misogynist, as in fact it was). For some reason the letters Q, U, X, Y and Z are not used (no hurricane Quasimodo, Ursula, Xavier, Yves or tropical storm Zorro), so if more than 21 storms occur - and they might well; the storm season doesn't officially end until November 30 - the Greek alphabet will be used, starting with Alpha. That hasn't happened since they started keeping records in 1851.

But of all the natural disasters, actual or potential, that beset us this year, the one that seems to terrify people the most is the possibility of a flu pandemic. The H5N1 influenza strain, first identified in Asia (where it has killed 65 of the 117 people known to have been infected by it since 2003, 44 of them in Vietnam), has now turned up in dead birds in Turkey and Romania, and probably in Greece as well, although that hadn't been confirmed when this article was being written. These latest findings lay to rest a long-standing argument about whether influenza outbreaks that start

in domestic poultry can become a pandemic by the agency of migratory birds. Obviously, they can. And that is something to be very concerned about, as I'll explain in a minute. Normally, I don't get too worked up over widely publicized threats. The fact that everybody is worried about something makes it much less likely, in my experience, that things will turn out as badly as feared (remember the 'Y2K' computer bug?). It's the unexpected that usually produces the greatest consequences. So, since everybody is so worried about the possibility of a pandemic of influenza, doesn't that mean, *ipso facto*, that I'm not that worried? Unfortunately, it doesn't. Not in this case. I'm afraid an influenza pandemic is very much something to worry about.

The term pandemic refers to an outbreak of a disease that spreads over huge areas. Fortunately, there have been relatively few in recent history, largely because increased scientific understanding of the causes of disease has led to improved public health policies. But funding for public health is in decline in most countries, including the US, and it is worth recalling that there have been a number of pandemics in the past 100 years (starting with the great influenza pandemic of 1918), and nearly all have been outbreaks of flu. Major flu strains are named for the particular alleles of the two major viral coat proteins they contain. The outer shell of the membrane-enveloped influenza virus is studded with spikes of a sugar-binding protein called hemagglutinin (the 'H' in the strain designation) that is involved in target-cell recognition and fusion of the viral membrane with that of the cell, and a sugar-hydrolyzing enzyme called neuraminidase (the 'N'). Hemagglutinin seems to tolerate more mutational variability than does neuraminidase: there are about 15 different strain types of hemagglutinin and 9 of neuraminidase, making at least 135 potential major viral strains. Some infect birds; others infect pigs; still others, people, and so on. H5N1, which has hemagglutinin type 5 combined with neuraminidase type 1, is primarily an avian virus. It is particularly virulent for poultry, and has a high capacity for genetic change, both by single

mutations and by recombination with the genetic material of other flu strains.

The history of the H5N1 strain tells us a lot about the way flu works. It also tells us a lot about the responses of people and governments to it. The strain was known as a purely bird flu for decades, but in 1997 it passed from chickens to humans in Hong Kong, causing the death of 6 people out of 18 infected and leading to the destruction of some million chickens. That radical action squelched the epidemic, and it also taught scientists that the high avian virulence of H5N1 also applied to humans. Late in 2003 and early in 2004, outbreaks of H5N1 occurred in poultry farms in Cambodia, China, Indonesia, Japan, Laos, South Korea, Thailand and Vietnam. In February 2004, the strain was detected in pigs in Vietnam, showing that it had acquired the ability to infect non-human mammals. Fresh outbreaks occurred in China in July of that same year and in Malaysia in August. Ominously, also in August the first cases of human infection were reported in Vietnam and Thailand. These were almost exclusively poultry farmers. As had been the case in the 1997 Hong Kong outbreak, the mortality rate among infected humans was very high. In January of this year a major outbreak occurred among poultry in Vietnam: 33 of the 64 cities and provinces were affected. In an attempt to contain the epidemic, 1.2 million poultry were slaughtered. It is estimated that over 100 million birds died of the disease.

In May, it became clear that H5N1 had started to spread beyond southeast Asia. Migratory waterfowl began dying from the disease at Quinghai Lake Nature Reserve in Western China. Storks, geese and gulls are known reservoirs of avian flu virus but they rarely become ill from it. This variant of H5N1 was apparently even more virulent than usual. Public health officials became alarmed, because in the fall there are mass bird migrations from Asia to Africa, by way of Russia and Turkey. In July, the first human fatality in Indonesia was reported. In August, the virus was confirmed in birds in Mongolia and Kazakhstan; by the end of the month, it had spread to south-western Russia. In September, the United Nations health representative who would coordinate a worldwide response to a pandemic, David Nabarro, estimated that a pandemic among humans could kill up to 150 million people worldwide. And last month, analysis of dead birds in Turkey and Romania confirmed that H5N1 had reached continental Europe.

Simultaneously, a team from The Institute for Genome Research (TIGR) reported the complete sequence of 207 H3N2 isolates and two H1N2 isolates, giving the first comprehensive picture of influenza virus evolution (Ghedini *et al.*: *Nature* 2005, **437**:1162-1166). They observed point mutations, deletions and segmental exchanges. Their most dramatic finding is the discovery of an epidemiologically significant reassortment that explains the appearance, during the 2003-2004 season, of the 'Fujian/411/2002'-like strain,

for which existing vaccines had limited effectiveness. They conclude "not only that the influenza virus population contains multiple lineages at any given time, but also that alternate, minor lineages can contribute genetic variation to the dominant lineage, resulting in epidemiologically significant, antigenically novel strains."

The best example of what an "antigenically novel strain" can do to the world is the great 'Spanish flu' pandemic of 1918. No one knows exactly how many people died that year, worldwide, but the estimates range from 20 to 50 million, making the 1918 flu the deadliest single epidemic in world history. (More people may have died from the Black Death of the 13th century, but that was actually a series of epidemics stretching over decades. It killed a third of Europe, but spared most of the rest of the world. The 1918 'Spanish' flu hit almost every continent.)

It's funny about that 1918 pandemic. When I was a boy no one talked about it, even though there were plenty of people alive who had been through it. No books or newspaper articles were written about it, and every flu season didn't begin with panicky reminders of that devastating autumn. The 1918 flu spread around the globe in just a few months - in an era before regular airplane travel. It killed more young people than old people. On one day alone, October 10, it killed 342 people in New York and 514 in Philadelphia. It killed so many people in Boston that they ran out of coffins. It killed with alarming quickness, a trait not seen before in influenza epidemics. Religious folk thought the world was coming to an end. It was followed by an even more mysterious disease, an epidemic of sleeping sickness, encephalitis lethargica. Over a fifteen year period, 5 million people around the world came down with this malady. A third died quickly; one third never recovered, remaining virtually comatose for the rest of their lives. A bright young British pianist, Philip Leather, age 13, came down with encephalitis lethargica in 1933 and was admitted to the Royal London Hospital in Whitechapel. He never left, dying there on December 15, 2002, age 82, the last known survivor of the strangest epidemic in history. He, the disease that destroyed his life, and the flu pandemic that preceded it were all but forgotten until recent years. They've been rediscovered now, in part because scare stories about emerging diseases sell books and newspapers, and there's an appetite among the reading public for history that seems relevant to our times.

And there's another reason it makes a lot of sense to be thinking about the 1918 pandemic these days. In a remarkable feat of forensic genomics, teams of scientists have succeeded in genome sequencing (Taubenberger *et al.*: *Nature* 2005, **437**:889-893), and then reconstructing (Tumpey *et al.*: *Science* 2005, **310**:77-80) the influenza virus strain that caused the 'Spanish' flu. Their work shows that it was unquestionably an avian virus. But it wasn't, as people thought for years, the strain called H1N1. It appears to have

been a variant of H5N1. The 1918 pandemic virus genome was reassembled from fragments recovered from preserved tissues from 1918 victims. The reconstructed virus is as lethal as feared; it kills mice more quickly than any other human flu virus known. Three genes appear to be the chief contributors to its virulence: the hemagglutinin is unusually potent in latching onto the surface of cells; the virus doesn't need to rely on its host cells for the protease trypsin to cleave and activate the hemagglutinin - the neuraminidase appears to do that. (This may be why the 1918 virus, like some highly virulent bird flu strains, can grow in any cell type, not just trypsin-containing lung cells.) And finally, the 1918 strain has polymerase genes that allow it to replicate very efficiently in human bronchial cells. The polymerase genes are similar to those found in bird flu, including H5N1 in Asia, so the researchers conclude that the 1918 flu probably arose directly from a bird virus without combining with a flu strain already adapted to humans, unlike the strains that caused the much less lethal 1957 and 1968 flu pandemics. For example, there are only 10 amino acid positions (out of 2,232 total codons) that consistently distinguish the 1918 polymerase proteins (and those of other strains that infect humans) from their avian influenza counterparts. The present H5N1 strain making its way out of Asia already has some of the mutations it needs to look a lot like the 1918 strain. The rest could accumulate over time, but it would probably take quite a while. What is worrying public health officials is the possibility that, since influenza can also evolve through genetic rearrangement, time may be about to run out.

Currently, in humans, H5N1 has an extremely high mortality rate but a very low rate of bird-to-human infectivity and, as far as is known, a zero rate of human-to-human transmission - yet. It's the 'yet' that has everyone in a sweat, because the way avian flu strains were thought to acquire that capability is when they infect a mammal (a pig, say, or a person) that is also infected with a more human-like flu virus, and the two strains exchange genetic material, just the sort of recombination event the TIGR team describes. So the current efforts of public health officials to contain the virus are focused on culling flocks that contain infected fowl so that the probability of human infection is kept extremely low. That strategy will probably work in Europe, and maybe even in the Middle East, but the migratory birds that are now spreading this disease aren't planning to spend the winter in Nice, as attractive as that location may be: they're heading for East Africa. That's right, the same East Africa that is so beset by famine and war that two things seem certain: one, that no one there is going to kill domestic fowl herds en masse - food sources are just too valuable; and two, the local public health infrastructure is not likely to be able to prevent simultaneous infection of mammals with both avian and human viruses.

That's one reason there is such a fuss about the flu drugs oseltamivir (Tamiflu, by Roche) and zanamivir (Relenza, by

GlaxoSmithKline). These are a new class of antiviral compounds that work by inhibiting the neuraminidase. They are also among the first structure-based pharmaceuticals ever developed. They are the brainchild of an Australian named Graeme Laver, who works at the John Curtin National University in Canberra, Australia. He was one of the scientists who showed that the 1968 pandemic was caused by a virus that arose through genetic reassortment. Realizing that the best target for an antiviral drug should be an enzyme, he set out to determine the crystal structure of the neuraminidase in 1978. He couldn't get any major pharmaceutical company interested in his crystals so he took them to a young protein crystallographer at CSIRO in Melbourne named Peter Colman. Colman solved the structure in 1982. It made the cover of *Nature*. The structure immediately revealed a highly conserved cavity in the active site, a perfect target for drug development. Starting with a compound developed years before by two Viennese chemists that was a weak inhibitor, Colman and Laver began to use a series of neuraminidase-inhibitor crystal structures to produce an improved binder. They did this through a small company they formed called Biota, with the aid of chemist Mark von Itzstein. Once they had their drug, they tried shopping it to big pharma again, and again no one seemed interested. Finally Colman persuaded Glaxo to take a chance, and Relenza (Glaxo's name for zanamivir) was born. Once one major drug company decided that influenza drugs were a good idea, the rest of the pharmaceutical industry, with that creativity and daring that characterizes it, jumped right in. Next past the post was Roche, with a compound they called Tamiflu (oseltamivir, originally designed by a small company, Gilead).

Tamiflu is the major focus of drug stockpiling now, because it can be given orally (Relenza must be taken as a powder in inhaled form). In 1994, Fred Hayden of the University of Virginia was the first person to give a neuraminidase inhibitor (Relenza) to a human being. The clinical trial data show that these drugs shortened the recovery time of infected individuals by a couple of days, reduced the risk of complications, and when taken prophylactically seemed to protect against infection in many cases. Since they hit a highly conserved target, there is every reason to believe that they will work against most strains, including H5N1 variants, but to date their effectiveness against the reconstructed 1918 strain has not been established as far as I know, and there is too little experience with them in the field to know if resistance to them can develop rapidly. Certainly it can develop: a variant of H5N1 resistant to Tamiflu has just been discovered in Vietnam (*Le et al.: Nature* 2005, **437**:1108). Vaccine development for flu, as well as for other viral scourges, is still lagging way behind where it needs to be, making the neuraminidase inhibitors the front line defense against an H5N1 outbreak in a human population center. So, the fact that people now seem to be buying and hoarding these drugs in a panicky response to the scare stories about H5N1 is very troubling. Indiscriminate use of the drugs by ordinary

people could be a disaster: 100 million people around the world get 'ordinary' flu every year, and all it might take would be a few years of Tamiflu and Relenza use for such relatively harmless flu cases to produce a reservoir of resistant human virus. Resistant, and just waiting for a bird flu to exchange genes with.

So, for now the best thing for us to do is to wait, too. Wait, and hope that the H5N1 virus doesn't make it to East Africa. Because if it does, we may look back on the events of 2005 wistfully. Which reminds me of a story: two businessmen are sitting at lunch, bemoaning their lot. "What a time I've had," groans the first one. "Two years ago I lost \$50,000; last year I lost \$100,000, and this year I lost \$200,000." "That's nothing," says the second. "I spent a fortune sending my son to medical school. When he finished, he decided he didn't want to be a doctor; he wanted to be a lawyer. So I spent another fortune sending him to law school. Now that he's finished that, he says he doesn't want to be a lawyer. He wants to be a painter. What could be worse than that?" And the first man says, "Next year."