

## Waiting for the flu

Whether the problem is swine-based, fowl-dependent or civet-cat-related; epidemics of infectious respiratory diseases are in the news. Many of the informed take the problem of potential future pandemics very seriously—I have been involved with an exhumation in the month in which I write this in order to look for tissue to discover sequences that might inform us about what made the 1918 pandemic so devastating. Are you OK on this at parties? (flu, not exhumation). Here is a guide.

Influenza viruses are typed by their haemagglutinin (H) and neuraminidase (N) surface glycoproteins. It is the H2, H5, H6, H7 and H9 sub-types of influenza virus that are thought most likely to be transmitted to human beings from their multiple animal hosts (there are many of these and many more types—we cannot cover all the options in terms of potential vaccines). The currently widely distributed viruses H1 and H3 show remarkable antigenic drift—that is to say their glycoproteins are changing fast, and allow the viruses to produce annual outbreaks as acquired population immunity becomes ineffective. H2 are considered high risk for the future because they were the prime agent in the 1957 ‘Asian’ flu pandemic, and were the only influenza A sub-type circulating between 1957 and 1968; they are still around in duck populations. H2N2 could cause another pandemic, as no one under 30 has antibodies. H1, H2, and H3 are probably the only types that have caused serious trouble in the last 150 years.

In March 2003, there was an outbreak of H7N7 avian influenza in the Netherlands. Eighty of those who slaughtered the affected flocks developed a conjunctivitis, with evidence of human to human spread, and a vet died of respiratory disease. There was no further spread in Man but the lesson is clear—inter-species barriers are porous. In 1997, an outbreak of an H5 (H5N1) subtype killed 6 of 18 infected human beings (Hong Kong) and since then there have been human cases from H9N2, more H5N1 and H7N7.

The way in which H5 and H7 become deadly to chickens and ducks is by acquiring new amino

acids at the haemagglutinin cleavage site necessary for infectivity. Thus changes in the ‘epidemiology’ of animal infections may presage danger—these viruses have spread from wild bird populations to domestic poultry in the last 10 years. H9N2 viruses have been found in pigs and Man and have acquired human-like receptor specificity, but while they could not infect chickens before, they are now becoming endemic in the chickens in the Far East. Adaptation goes both ways.

The most recent outbreak of flu-like illnesses was in Vietnam, and was produced by a virus of subtype A (H5N1—the 1997 Hong Kong epidemic type). It appears to have leapt to human beings, and has killed at least five people. In Hong Kong, this virus has turned up in wild birds in parks, and in 2003, a nearly identical variant was found in a man who died of respiratory infection; his son became seriously ill but recovered. A daughter of the family had previously died of an uncharacterized respiratory disease in Fujian province in China. The existence of large animal reservoirs, the movement of people, the flexibility of the viral genome all identify potential risk factors for pandemics, if a strain develops that can be spread by human/human transmission. Fortunately, it appears to be a big if—but not so remote as to eliminate the need for planning.

What strategy should be used to deal with potential epidemics? Antiviral drugs do not seem to be promising in this context, and no one is stockpiling them, so vaccines look like the best bet. Current vaccine development depends on the capacity of the influenza genome to reassort when segmented and grown in eggs, thus giving rise to new segments which may be selected for the antigenicity required. Recombinant strains with the right HN combinations can then be exploited. The haemagglutinin and neuraminidase gene containing segments are married to the remaining six genes necessary—these from a H1N1 virus that is well tolerated by Man—and a vaccine seed strain can be grown in eggs. But all this takes time, and there are not enough eggs to make this

an effective strategy to deal with a pandemic. In any case, H5 and H7 cannot be grown in eggs. Their unique ability to accumulate basic amino acids at the site of haemagglutinin cleavage makes them lethal to chicken embryos (and enables them to spread systemically).

Webby and Webster (2003)<sup>1</sup> describe how plasmid-based reverse genetics can be used to produce vaccines more effectively. Viable viruses can be generated from individually cloned cDNA copies of each of the eight viral RNA segments, and re-assortment can be directed. The extra amino acids at the H cleavage site associated with high virulence can be removed, and a seed strain produced by cloning the appropriate H and N genes from the target virus and transfecting an appropriate cell line (the choice of cell line to use is not an easy one, but will not be discussed here). Antigenic combinations can be made.

All of this ignores the fact that as the immune response to vaccines has been disappointing in the past, the outcome in terms of protection may be inadequate. New vaccines, based on GM techniques, might not be acceptable to some, and would need to be trialed for safety and for the establishment of optimal immunization schedules. It is not clear what sort of safety testing should be carried out; a major factor to be considered, since the risk of failure or of adverse effects to manufacturers must be high.

From 1959 to 1998, there were only 17 flu outbreaks. Between 1998 and 2003 there have been six; this increase in rate has been blamed on the demand for chicken meat and the expansion of the commercial flock. So how long will it take for the human/human transmission problem to be cracked by the virus? It is difficult to guess, but it would be rash to say it will not happen. Is a rapid vaccine response likely? I don't think so. How about the antiviral drugs? Best taken just before you get infected. So? Take a couple of paracetamol and stay in bed.

Colin Berry

## References

1. Webby RJ, Webster RG. Are we ready for pandemic influenza? *Science* 2003; **302**:1519–22.

## Note added in press

Our exhumation was unsuccessful (nothing left to examine) but on the day it was carried out, *Science*, published two articles on the structure of the H1 haemagglutinin from the 1918 virus, and its receptor binding properties (*Science* 2004; **303**:838, 1866). Although phylogenetically the 1918 is an H1 virus, it retains an avian-like receptor binding site. Normally these bind to the gut in birds via sialic acids in the glycoproteins of the host; these acids attach to galactose sugars in the  $\alpha$ 2,3 linkage. In contrast, human-type viruses bind to respiratory epithelium using acids attached by an  $\alpha$ 2,6 linkage. The shift from the  $\alpha$ 2,3 linkage to  $\alpha$ 2,6 enables the switch from birds to man, and may involve single amino acid changes in the haemagglutinin. Avian virus haemagglutinins have glutamine at 226 and glycine at 228; H1 in Man has leucine at 226 and serine at 228. The H1 from 1918 has the avian pattern (glutamine at 226 and glycine at 228), so they have the antigenic characteristics of avian influenza A, but can spread in human populations.

The three-dimensional characterization of the receptor binding site adds more. There is an avian structure, but at the position where the haemagglutinin is cleaved into its sub-units (HA1 and HA2), the virus is able to form a conformation that binds to human cells despite the persistence of the avian amino acids. So they are virulent (from avian ancestry) and can bind to human cells. That's not the whole story, so more data will be sought.