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Australia tackles bird flu using RNAi

In May, scientists at Australia's Commonwealth Scientific and Industrial Research Organization's (CSIRO) Australian Animal Health Laboratory (AAHL) in Geelong moved to establish a real—and impervious—firewall between wild birds and the $300 billion global poultry industry, using DNA-delivered RNA interference (RNAi) technology to prevent virulent strains circulating in poultry, such as the influenza strain H5N1, from directly infecting humans. Advanced Technology Services Australia, a subsidiary of one of the world's largest poultry breeding companies, is sponsoring the five-year pilot project.

Chicken bones from Neolithic settlements in China's Huang He (Yellow River) Valley show that humans have been living cheek-by-beak with poultry for at least 7,500 years. Like humans, chickens are highly susceptible to influenza.

Virologists believed a natural firewall existed between the species that somehow protected humans. (The prevailing 'pig-in-the-middle' hypothesis of pandemic influenza, for example, proposed that pandemic strains originated in pigs, through recombination between human and avian influenza viruses.) But that notion was rudely dismissed when the deadly new influenza strain, H5N1, killed six people in Hong Kong in 1997. It emerged right under the noses of virus hunters on watch for a long-overdue pandemic. When it was traced to infected commercial poultry in local produce markets, alarm bells rang.

At CSIRO Livestock Industries' annual *Horizons in Livestock Science* conference on the Gold Coast in Queensland in 2005, virologist Laurence Tiley, from Cambridge University's Center for Veterinary Science, warned that poultry was the most likely source of a potentially devastating human influenza pandemic. He noted that the Hong Kong outbreak had been a "rude awakening," and the research community was "waiting [for] and dreading" a major outbreak. The H5N1 virus has infected 244 people since 1997, causing 177 deaths—a mortality rate of 72%. Evidence for direct human-human transmission remains inconclusive, but the pattern of spread has proven that the bird-human firewall was illusory.

A collaboration between Australia's CSIRO and a commercial poultry company aims to protect humans against pandemic strains of influenza by administering RNAi directly into birds.

Tiley said that economic models indicated that, even if a breakout H5N1 strain merely matched the 2% mortality rate of the 1918–1919 Spanish flu, which killed at least 50 million people on six continents, a 20% infection rate could cost the US economy alone $166 billion. Moreover, a vaccine would be too late—the H5N1 strain is so virulent that it kills the poultry eggs traditionally used to make attenuated-virus vaccines, and no vaccine would be available for at least six months after a pandemic began.

On the other hand, the industry's unique structure could facilitate total replacement of the global poultry flock with resistant stock in only four years, according to Tiley. Half a dozen major breeders supply the industry with its elite breeding stock, with two accounting for more than 50% of the market.

By using RNAi in birds, the CSIRO program aims to provide indirect protection for humans against pandemic strains of influenza. CSIRO was a pioneer in RNAi technology, and owns key international patents on DNA-delivered RNAi (**Box 1**).

Project leader John Lowenthal says the aim is to achieve proof of concept in the laboratory within three years for two RNAi-based strategies: one therapeutic, the other prophylactic.

He and AAHL colleagues conceived the idea of using RNAi in 2001, amid the rising threat of a pandemic of H5N1 avian influenza in Asia. The plan has evolved since then. The scientists will attempt to completely outflank the virus by targeting highly conserved gene sequences common to all subtypes and strains of influenza, including any that the virus might conjure from its wellspring of genetic diversity in wild shorebirds.

The therapeutic approach involves delivering small interfering RNA molecules (siRNAs) to chickens in drinking water or via an aerosol spray, priming the birds' innate RNAi defenses to recognize and destroy the virus. Although therapeutic RNAi would provide only transient protection in the event of an outbreak, it would give the birds breathing time to mount their own, durable RNAi defense against the virus.

Lowenthal says the challenge is to protect chickens from the early phase of infection, which triggers an overwhelming 'cytokine storm' that can be lethal in its own right. That was the case with the Spanish flu, which took a heavy toll among healthy young adults with strong immune systems. Many died within hours of exhibiting the first symptoms, drowning as the cytokine storm filled their lungs with fluids.

The alternative prophylactic RNAi strategy will use transgenes to permanently protect birds against influenza, according to Lowenthal. The transgenes will encode 'hairpin' RNA molecules with sequences complementary to fixed targets in highly conserved viral genes. The CSIRO plan is to constitutively express the RNAi transgenes in all of the birds' tissues, conferring 'always-on' protection against any strain or subtype of influenza.

Lowenthal acknowledges that consumer concerns about genetically modified organisms (GMOs) and opposition from anti-GMO nongovernmental organizations (NGOs) could be a hurdle for the transgenic solution. But he contends that anti-GMO activists' objection to 'alien' proteins in foods from GMOs would not apply—RNAi technology would not introduce any new proteins. It would merely augment the birds' innate RNAi defenses, by adding harmless RNA molecules to the rich assemblage already present in living cells.

Nonetheless, before commercialization, both strategies would face rigorous scrutiny by the national Office of the Gene Technology Regulator and community groups, and would be subject to Australia's GMO food–labeling laws, which are among the strictest in the world.

Rick Roush, dean of the School of Agriculture and Food Systems at the University of Melbourne, said it would be very difficult for anti-GMO NGOs to persuade consumers that an influenza pandemic that could cause tens of millions of human deaths and devastate the poultry industry was preferable to consuming genetically modified chicken meat and eggs.

Graeme O'Neill, Mildura, Victoria, Australia

Box 1 Australia's long history with RNAi

The patented DNA-delivered RNAi (ddRNAi) technology CSIRO and its collaborator are employing in the flu protection program uses transgenes that encode short snippets of double-stranded RNA, corresponding to selected sequences in the genomes of target viruses. Taken up by RNA-induced silencing complexes (RISCs), they act as templates to trap and destroy the virus's 'source code', blocking replication.

Designer ddRNAi transgenes activate the innate RNAi defenses of plant and animal cells. Peter Waterhouse and MingBo Wang, of CSIRO Plant Industry in Canberra, invented the technique. They began investigating enigmatic virus-quelling mechanisms in plant cells in 1993 and by 1995 had established that it was induced by the double-stranded RNA blueprints of invading viruses. The team went on to provide the first practical demonstration of RNAi-mediated viral resistance in a seminal experiment in tobacco in 1997, when they inserted transgenes containing DNA sequences corresponding to selected target sequences in the genome of tobacco ringspot virus. The resulting genetically modified plants were completely resistant to the virus.

In September 1998, Waterhouse and Wang published the first description of how RISCs protect plant and animal cells against viral infections.