# CSIRO explores protecting poultry from Avian Influenza

## CSIRO Livestock Industries (CLI) has announced a five-year pilot project to test RNA interference (RNAi) based strategies for protecting poultry against influenza.

At CLI's annual *Horizons in Livestock Sciences* conference in 2005, Cambridge University veterinary virologist Dr Laurence Tiley described how RNAi could be used to protect poultry from Avian Influenza (AI), indirectly protecting the world's 6.5 billion humans against the next deadly flu pandemic.

Dr John Lowenthal's research team at CLI's Australian Animal Health Laboratory (AAHL) in Geelong was already ahead of the game - they conceived the idea of developing 'flu-resistant poultry' years before.

At the time, bioscience researchers were just beginning to comprehend a prospect from the realms of science fiction: an anti-viral superweapon, capable of protecting all livestock, aquaculture and crop species against their major viral diseases.

Experiments in Australia and overseas have now established that DNA-delivered RNAi (ddRNAi) can completely block viral infections in laboratory plants and animals.

### Background on RNAi

CSIRO Plant Industry researchers Dr Peter Waterhouse and Dr MingBo Wang confirmed the existence of an RNA-based anti-viral mechanism in a pioneering experiment in 1997, by creating tobacco plants that were fully resistant to tobacco ringspot virus.

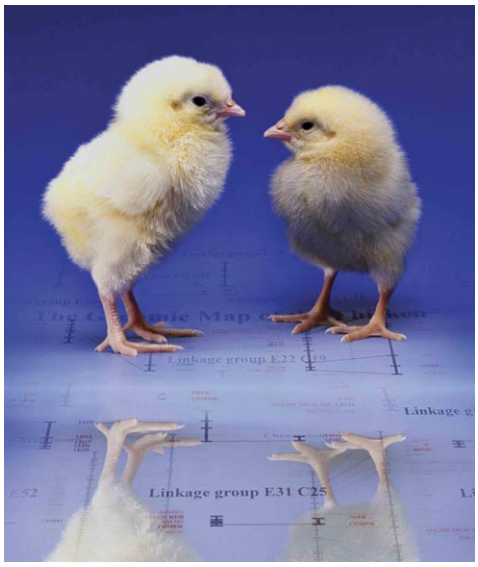
At around that time, US molecular geneticists Dr Andrew Fire and Dr Craig Mello discovered an RNAi-mediated gene-silencing effect in the nematode worm *Caenorhabditis elegans* – a discovery that won them the 2006 Nobel Prize for Medicine.

Drs Wang and Waterhouse were the first to provide a detailed description of how RNAi works to quell virus infections. They subsequently used ddRNAi to develop prototype cereal varieties with resistance to Barley Yellow Dwarf Virus (BYDV) and a complex of closely related viruses.

BYDV had defied decades of effort to breed resistant lines by conventional methods. Wheat and its "kissing cousins" offer no natural source of resistance to these viruses.

### Flu resistant poultry

Dr Lowenthal says that, similarly, there is limited potential to breed influenza-resistant poultry with conventional methods. His team, with funding from Advanced Technology Services Australia (ATSA), an Australian subsidiary of an international poultry-breeding company, will try two novel approaches – one therapeutic, the other prophylactic.




CSIRO Livestock Industries is testing RNAi-based strategies to protect poultry against influenza. Image source: Peggy Greb/US Department of Agriculture.

The therapeutic strategy involves designing small interfering RNA (siRNA) molecules that would be delivered to the birds via an aerosol, or in their drinking water. The siRNA strategy would provide transient protection against any outbreak of a rapidly lethal influenza strain, while the birds mounted their own protective RNAi response.

The prophylactic approach involves genetically modifying poultry to install an RNAi "firewall" – not just against the deadly H5N1 avian influenza strain, the "Bird 'Flu"- but all extant strains of the virus.

This approach would employ CSIRO's patented DNA-delivered RNAi technology. "Designer" transgenes would be introduced into foundation breeding lines of chickens to protect them and their billions of offspring against all strains of the influenza virus.

### Genetic modification

Technically, both the transgenic birds, and the therapeutic RNAi molecules, would be deemed to be genetically modified (GM).

Before commercialisation, both approaches would have to pass stringent safety and health checks, and community consultation procedures, required by the federal Office of the Gene Technology Regulator. Meat and eggs from the GM birds would also have to be labeled as genetically modified, to comply with Australian labeling regulations.

But, unlike the current generation of foods from GM crops and livestock, the GM birds would produce no new proteins. The end-product of an RNAi transgene is a set of small RNAi molecules that are not translated into protein.

## ddRNAi technology

RNA molecules are ubiquitous in the cells of all living organisms. In one role, they carry instructions from genes that guide the assembly of proteins from simple amino acids. Other RNAs constitute an elaborate system that coordinates the complex activity of plant and animal genomes.

With CSIRO's patented ddRNAi technology, researchers can custom-design genes that will arm plant and animal cells to recognize and destroy specific viruses, with high precision.

The prophylactic route uses transgenes that program the birds' cells to produce hairpin-shaped RNA molecules. These are taken up by tiny cellular structures called RISCs (RNA-Induced Silencing Complexes) and these "targeting sequences" allow the RISC to attach to and destroy the corresponding sequence of the genetic blueprint of the virus, preventing its replication.

## Target sequences

Dr Lowenthal says AAHL researchers have already identified target sequences common to all known major strains of the influenza virus. The virus has only eight genes, three of which are virtually identical across all strains because they are essential for its replication.

The other five genes, in particular those encoding the haemagglutinin and neuraminidase proteins of the viral coat, are not good targets because they vary considerably between strains.

The AAHL researchers will design gene constructs containing small, embedded sequences complementary to the highly conserved sequences in the genetic code of the influenza virus.

The plan is to assess whether these gene constructs can be presented in birds so that they will be permanently activated. If successful, the birds' cells would constitutively express the anti-viral RNAi molecules and therefore be able to mount an immediate, overwhelming response, quelling any influenza infection.

### A history of Avian Influenza

Through recorded history, deadly influenza epidemics have emerged episodically from Asia to take a huge toll on human life.

In 175BC, an Egyptian scribe recorded a "fiery fever" that ran through the ancient city of Luxor. Pain-racked victims sneezed, coughed, and died in droves. The virus appears to have vaulted down the ancient Silk Route extending from Shanghai in China, to Cadiz in Spain.

A "burning fever" decimated Charlemagne's army in the late 8th century, delaying the Frankish king's conquest of Europe.

But the great Spanish 'Flu pandemic of 1918-19 previewed the potentially catastrophic consequences of another lethal influenza pandemic on a world of 6.5 billion people, many of whom live in the megacities of Asia, Latin America and Africa.

Caused by a strain later designated H1N1, it killed at least 40 million people on six continents. It immobilized 20 per cent of the US workforce, and cost the US economy $2 billion. Doctors and nurses were among the first to die of the infection, which killed healthy young adults within six hours of exhibiting the first symptoms of fever.

The virus infects the lining of the respiratory tract, causing a high fever and a sore throat. The immune system responds with a "cytokine storm", flooding the patient's body with cell-signalling molecules called cytokines, that cause the acute pain in the muscles, spine and joints typically associated with an influenza infection.

The lungs become congested as infected epithelial cells leak fluid. Victims of the Spanish 'Flu literally drowned as fluid filled their lungs.

Uncontrollable sneezing and coughing spreads billions of virus particles in fine aerosols, infecting healthy individuals.

Nobel Laureate virologist Joshua Lederberg has described the influenza virus as the world's most dangerous microbe, noting that in 1995, an epidemic of "an ordinary, garden variety" killed 70,000 people in the US alone.

Humans and chickens have lived almost cheek-by-beak throughout Asia for millennia, but Western virologists considered pigs – also an integral part of traditional Asian farming systems the most likely conduit for a new human pandemic strain. Pigs serve as a 'mixing vessel' for human and bird strains of the virus to swap gene segments, a process that occasionally produces highly virulent strains capable of causing pandemics.

So the emergence in Hong Kong in 1997 of a lethal H5N1 strain capable of direct bird-to-human transmission had come as "a rude shock", Dr Tiley told the *2005 Horizons* conference. He warned that poultry were now the most likely source of the next human pandemic of influenza.

## A changing target

The influenza virus mutates rapidly, and constantly spawns new strains – an evolutionary strategy that prevents its animal hosts developing a protective immune response after previous infections.

Dr Lowenthal says the targeted sequences are designed to confer broad-spectrum protection against all known influenza virus strains, making it very difficult for the virus to mutate and evade the bird's RNAi defences.

He points out that adding a few tiny RNA molecules to the vast complement of RNA molecules already present in cells is most unlikely to create any novel risk to the birds themselves, or to human health. They will merely augment the natural activity of the birds' own, natural RNAi defences.

## The way forward

Dr Tiley told the *2005 Horizons* conference that poultry were now the most likely source of a devastating pandemic of human influenza.

Migratory waterbirds are the main reservoir for influenza viruses, and have been implicated in the spread the current H5N1 strain from East Asia as far west as the UK and EU. Many victims died after being infected during mass-culls of poultry to contain outbreaks of "bird 'flu" in Vietnam, Thailand and Indonesia.

There is no effective vaccine yet, and it could take at least six months to produce one – too late to contain an emergent pandemic strain. Dr Lowenthal says the H5N1 strain is so virulent that it kills the embryonated eggs traditionally used to produce vaccines.

The AAHL team hopes to achieve laboratory proof-of-concept for both techniques within three years, and it will take another two years to determine whether the strategies are commercially practicable.

"The risk of a pandemic is sufficiently high to at least make the attempt – it's no longer just a commercial issue, it has spilled over into human health," Dr Lowenthal says.

CSIRO scientists at the Australian Animal Health Laboratory in Geelong will use two novel approaches in the bid to protect poultry against infection by the Avian Influenza virus. (Photo: Frank Filippi, CSIRO)

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