



## At CSIRO: Studying a Specific Change in One Gene of the Avian Flu Virus

Karel A. Schat, Microbiology and Immunology, went to Australia in April 2006 to study a genetic mutation in the avian flu virus at the Australian Animal Health Laboratory (AAHL), which is part of the Commonwealth Scientific and Industrial Research Organization (CSIRO). After two three-month stints (April–June and October–December 2006), Schat hopes that his group is on the right track to finding answers to some puzzling questions about the bird flu.

When researchers isolated the virus in chickens that had infected humans in Asia, they found that its RNA, which is involved in copying the virus's genes, differed in an amino acid in the PB2 gene. In chickens this was glutamic acid, but when the virus was isolated in humans, this amino acid was changed to lysine in about half the cases.

Will this change shift the pathogenicity of the virus? Does the mutation that leads to the presence of lysine have an effect on ducks and chickens? Does the virus with lysine change again once it is reintroduced to birds? Can birds become vectors of the virus with lysine, which might then infect mammals, including humans?

### The Research at CSIRO in Australia

In Australia I studied a very specific change in the polymerase PB2 gene of the H5N1 avian influenza virus, which is often referred to as bird flu. The avian influenza virus is an RNA virus, and the polymerase complex, which includes PB2, makes new RNA needed for the production of the new virus. We are interested in the mutation of the PB2 gene at amino acid 627, which changed from glutamic acid in the chicken to lysine in humans.

When an RNA virus replicates, many errors may be made by the polymerase complex resulting in changes in the virus. To allow us to study the specific importance of this change at position 627, Ruben Donis, my collaborator at the Center for Disease Control (CDC), provided us with a reverse genetic-generated influenza virus. Reverse genetics means that he cloned the eight segments of viral RNA as cDNA in bacterial plasmids. The plasmids are inserted into *E. coli*, which provides a stable form for each influenza gene as DNA. The virus is retrieved by introducing these eight genes as DNA into susceptible cells to obtain the influenza virus for experimental work.

In order to study the importance of the specific change at amino acid 627 in PB2, Donis generated by reverse genetics the virus, isolated from a person who died from influenza. Then he mutated one nucleotide in that virus so that the amino acid changed from lysine to glutamic acid, as had been present in the original chicken isolate. So now we have two identical viruses that are different only in one amino acid. This allows us the opportunity to determine any specific biological effects associated with the change.

### New Developments

We did one experiment in which chickens were infected with one or the other form of the virus. These chickens were closely searched for differences of all kinds. There were no differences in mortality—all chickens died within 24 to 48 hours of infection. However, our results suggested that there is a major *biological difference* between the two viruses. Our next step is to conduct other experiments to confirm what look like unique changes between the two viruses in the chickens, which did not influence mortality. The changes may have profound importance in

our understanding of what happens in humans when infected. We need to confirm the findings of a second experiment in chickens and an experiment in ducklings, which we conducted during my second visit. We are now doing the laboratory analysis of these two experiments, and I am applying to the NIH and USDA for funding to continue the research.

**What Is the Fear**

The virus may change. Then there are two possibilities. It may mutate to behave like the regular flu virus (known as human flu) and become transmittable like it—with easy

transmission, for example, just by sneezing—but retain its pathogenicity. If this happens, we will have a big problem. Alternately, other changes may occur, making the virus appear less pathogenic or dangerous. It looks like the regular flu causing mortality and is treated as such. It is important to note that, every year in the United States, 30,000 to 35,000 people die with the regular flu.

We are interested in the mutation of the PB2 gene at amino acid 627, which changed from glutamic acid in the chicken to lysine in humans.

The virus may also mutate by small changes, or a reshuffling of the genes may occur. If a person had the regular flu, and at the same time became infected with the bird flu, the genes of the two viruses could mix in the same cells. Genes of the human flu could end up in the particles of the avian flu—and vice versa—resulting in the generation of a new flu virus.

At the CDC, researchers did an artificial reshuffling in a cell culture. They took genes from the bird flu and genes from the regular human flu and made new viruses that have been tested in animal models. They found that those strains were not as pathogenic, showing that it is not easy to get a flu virus that will cause a pandemic with high levels of mortality through the reshuffling of the genes.

I believe a riskier possibility, however, is that a small change could occur, like the one I

am studying. If a number of small changes coincide, they could create a virus that maintains its pathogenicity as it infects humans.

In the case of an outbreak of bird flu among humans, treatment and education would become crucial. The treatment of bird flu in humans is problematic because of the very nasty pneumonia that one gets with it. Many lung machines would be needed. Most of the mortalities have occurred in countries like Indonesia, where the care is less adequate than in Western Europe or the United States. Also, in countries like Indonesia, 20 chickens

are a small farmer’s livelihood. The farmer may not be forthcoming in revealing sick chickens to the authorities, because proper reimbursement for them may not be made. This is also a problem of education.

Even if the bird flu did not cause high levels of mortality in humans, but spread like a regular flu, it would have a profound economic effect. The World Bank has estimated that a pandemic could reduce world income drastically—by up to 20 percent—causing a tremendous economic downturn.

Several companies are working on vaccines, some recently released with promising results. The problem with making the vaccine for the bird flu is that it kills chicken embryos very rapidly—within 24 to 48 hours. Currently all flu vaccines are made in embryonated chicken eggs, and embryos need to survive for four to five days to get a high yield for human vaccines. The vaccine industries are looking for different technologies that do not depend on chicken embryos.

**What Has Been Known for Several Years**

If a human has extensive contact with chickens, ducks, or geese that are sick with H5N1, the virus can infect the human. In order for a human to get sick with the bird flu, the virus has to get deep into the lungs

Alexis Wernick-Roberts



Karel Schat

**How the Avian Flu Virus Got Its Name**

There are two proteins on the cell surface: one is hemagglutinin and the other is neuraminidase. There are sixteen known hemagglutinin genes and nine known neuraminidase genes. The H5N1 virus has the fifth hemagglutinin gene, and the first neuraminidase gene.



## Unwinding



Schat with a group of CSIRO colleagues at the finish of the bicycle tour, "Around the Bay in One Day." (l. to r.) Matt Bruce, Ton Schat, Mark Tizard, and Kristen Morris.

where there are receptors on the cells similar to those in the chicken. The regular flu virus in humans recognizes receptors in the upper respiratory system—nostrils and upper trachea, for example. You catch it if someone across from you sneezes. To become infected with the bird flu, the virus not only has to travel to the upper respiratory system, but also deep into the lungs, which is more difficult.

The H5N1 virus may mutate to behave like the regular flu virus (known as human flu) and become transmittable like it—with easy transmission, for example, just by sneezing—but retain its pathogenicity.

The people who have become ill with the bird flu lived and worked closely with their chickens. They may have butchered sick chickens trying to save some of the meat. Indonesia had the highest number of avian flu mortalities in 2006 (52, at last count), and 75 percent of the people who were infected died.

Thus far, there is very little evidence of human-to-human transmission. One case that occurred in Indonesia in April 2006 suggested that the virus went from human to human to human, but then it stopped. The transmission to the second and third persons was most likely because these people were intimately involved in the care of the first and second persons.

### What Slips through the Cracks

My fear is this: if we get bird flu in the United States, it will be through illegal importation. Currently, all importation to the United States of poultry products from China or other places with bird flu is forbidden. Last summer there was an incident in Detroit, where authorities confiscated illegally imported frozen bird products (geese and chicken), hidden under all sorts of products from China. The same thing happened in Venice. A couple of years ago in Brussels, airport authorities confiscated two eagles hidden in tubes, which had been smuggled from East Asia. It turned out that they were positive for H5N1. Fortunately, we have

excellent controls in place, but we do not know what slips through the cracks.

The strain of H5N1 that killed birds like herons and gulls in a nature reserve in China had the same mutation of the PB2 gene at position 627 as the virus that was found in humans. The virus with this mutation had spread from there to Mongolia and other

parts of Asia among the same kinds of birds. The H5N1 virus killed swans in Europe, particularly mute swans; tigers in Asia, which were probably fed sick chickens; and cats in Germany, which probably ate dead swans.

Migratory birds might also introduce bird flu into the United States. An exchange between migrating birds in northeast Asia and the northwest United States carries a potential hazard. Extensive testing of migratory birds in the United States has thus far shown all tested birds to be negative for H5N1.

People often ask if it would be safe to eat chicken in the case of an outbreak of bird flu. The answer is yes—cooking destroys the virus. In addition, there are systems in place to ensure that all birds in the United States are free of the virus before they get to the processing plant.

*Karel A. Schat*  
*Microbiology and Immunology*

**For more information:**  
E-mail: kas24@cornell.edu



## At the AAHL

AAHL is a high-security disease center. In order to handle pathogens, I work under the conditions of Biosecurity Level 3–Zoonotic, meaning that the disease can be transferred from animals to humans. These secure areas have low pressure and all kinds of barriers.

It takes a lot of time to work in a BioSecure Lab. Every day I work, I go through a rigorous security routine procedure.

I work very slowly and carefully. When I take my samples, I make sure I do not cut myself or stick myself with needles. I felt very comfortable working with the air hood from the first time I went for training. During my training, the lab people told me, "If you don't feel comfortable with this, don't do like a fellow did who went in, panicked, and ripped off his hood in the unit. Just tell us, and we'll get you out safely!" What is comforting is that the airflow goes right into the face. It gives me confidence that the system is working.