Peter Doherty's pioneering research into human immune systems earned him the Nobel Prize for Physiology and Medicine in 1996. He was Australian of the Year and awarded a Companion of the Order of Australia in 1997 and currently divides his professional time between the University of Melbourne and St Jude Children's Hospital in Memphis, where he is helping unravel the mystery of childhood cancer.

PETER DOHERTY

SENTINEL CHICKENS

WHAT BIRDS TELL US ABOUT OUR HEALTH AND THE WORLD

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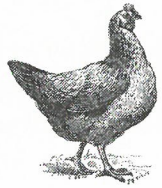
CONTENTS

books about science. Some of his last experiments used the chick embryo chorioallantoic membrane to explore questions about tissue graft rejection and immune tolerance.

Sir Macfarlane Burnet chatted with Ghandi, met Kings and Queens, and received the highest orders of the old British Empire. He gave every prestigious lecture and was generally applauded as a very great man, an opinion that was evidently agreeable to him. On the other hand, he stayed honest and kept on with his 'day job' of drilling holes in eggs and dropping stuff onto chick embryo membranes. One of the best photographs of Burnet is in a white coat, demonstrating his egg inoculation techniques to students and faculty at the University of Wisconsin. There we have the public and the private face of science, the perception and the practice. Staying grounded in reality is everything, and some of our current political leaders might learn from Burnett's example. But maybe such groundedness belongs to a past era—an era when chick embryos were at the cutting edge of infectious disease research.

4

Sentinel chickens



THE IDEA OF 'SENTINEL chickens' seemed pretty incongruous when I first heard the phrase as a young undergraduate. My reaction was no doubt conditioned by recollections of the scatty and fussy hens that scratched about in the dusty chicken run in my grandmother's backyard. The notion of the humble chicken waiting like a trained soldier, alert and focused, for some unseen and approaching enemy just didn't seem likely. Hens *en garde*!

Like most students in that distant era, I knew everything and knew nothing. Nowadays, any reasonably sophisticated young person would go immediately to the internet and find that way back to mythological times, guard duty has been part of the avian job description. Gods with the body of a man and the head of a

bird, like the ibis, falcon, hawk or heron, watched over the ancient Egyptians. In the Western tradition, the cockerel, or rooster, symbolises vigilance and has been widely used as a French heraldic device. Adopted as the national symbol at the time of the 1789 revolution, the proud, colourful rooster of France (*le Coq Gaulois*) went beak to beak with the black eagle of Germany during World War I.

When it comes to warning us of imminent danger, sentinel geese have long been associated with the human story. Geese go on the attack and make an enormous noise if they perceive an incursion into what they regard as their patch. The trick is to provide feed and nurture so that they make our patch their patch. According to the Roman historian Titus Livius—better known as Livy—sacred geese in the temple of the Goddess Juno alerted the exhausted defenders of ancient Rome to a nocturnal attack by marauding Gauls. In modern times, Scottish whiskey distilleries are sometimes guarded by gaggles of geese that raise a loud hue and cry if a thief tries to make off with what many consider the most spiritual of all aqua vitae. Whiskey may be part of the local religion, but I doubt whether the pragmatic Scots would regard the birds as sacred.

Then there's the story of the ravens that somehow guarantee the integrity of the Tower of London and, beyond that, the continuity of the crown of England. Legend has it that the monarchy will fall when the ravens leave the tower. Following the spirit of a decree by Charles II, there are always at least ten ravens available, six on duty and four active reservists. Cared for by a raven master drawn from the ranks of the Beefeaters, the medievally attired tower guards, clipping one wing ensures that the resident ravens can't fly away.

* * *

I first heard the term *sentinel chicken* from an older cousin, Ralph Doherty, a medical scientist who was then building a substantial reputation in the study of the insect- or arthropod-transmitted

viruses, known collectively as the arboviruses, also called togaviruses because they have an outer 'envelope' or 'coat'. Among the major achievements of his research group at Brisbane's Queensland Institute of Medical Research was the discovery that the mosquito-borne Ross River virus (RRV) is the cause of the human disease epidemic polyarthritis with rash, a painful and debilitating condition that can persist for several months. With more than 4000 cases every year, this non-fatal disease is all too familiar to those who live in the northern parts of Australia and has been rapidly spreading away from the tropics.

Like all viruses, the arboviruses can only reproduce themselves within living cells. What makes the arboviruses special is that they replicate in the tissues of very different types of animals, though the individual viruses in this very large group do vary considerably in their overall host range. The 'virus production factories' include biting insects, particularly mosquitoes and ticks, which, as they take their blood feed, either become infected or (if already carrying the virus) transmit the infection to warm-blooded species, including human beings and a whole spectrum of furry and feathered vertebrates.

And that's why we have sentinel chickens. The progressive spread of many arboviruses is monitored by placing caged chickens around the countryside at sites where they are likely to be bitten by mosquitoes. The widely distributed birds are sampled regularly, a comparatively non-intrusive process that involves taking a small amount of blood from the prominent wing vein. The blood is allowed to clot, and the yellowish serum supernatant is either frozen or taken on ice to a specialist laboratory, where the samples are analysed for seroconversion. That is, the technician uses a well-established assay to detect newly acquired (since the previous test) circulating antibodies specific for the virus in question. (That doesn't work for all mosquito-borne infections. Chickens aren't very susceptible to RRV, for example, which seems to prefer mammalian hosts, and they're of no value for tracking malaria, for

which we humans are the most sensitive sentinels. As explained in Chapter 11, birds have their own distinct malaria parasites.)

If, for example, the birds were seronegative when taken to their guard station, then seropositive for some arbovirus six months later, it's obvious that they were exposed to an infected mosquito at some time over that period. The relatively few virus particles injected by the feeding mosquito will have travelled via the circulation to invade susceptible cells in one or other organ of the new chicken host. Successive cycles of virus replication then lead to the presence of a great deal more virus in blood (viremia), a process that terminates somewhere over the next 7–12 days or so, when the developing immune response will lead to the production of specific, neutralising antibodies. Those antibodies will continue to be made for the life of the bird. Once antibody-positive for the infection of interest, the chicken veteran is both permanently immune and eligible for honourable retirement and replacement with a new recruit.

* * *

Virologists further sub-classify the arboviruses into alphaviruses and flaviviruses. The alphaviruses include RRV and Barmah Forest virus (in Australia), eastern equine encephalitis virus (in the USA) and the Chikungunya virus that has lately been spreading from the Indian Ocean region to South-East Asia and the Mediterranean. Human infection with Chikungunya, RRV or Barmah Forest virus can lead to the development of persistent polyarthritis with rash, while chickens, at least, remain asymptomatic.

All the flaviviruses are broadly related to yellow fever virus (YFV), the terrible pathogen that kills humans by a combination of haemorrhagic disease and liver destruction. That's where the 'flavi' (Latin for yellow) comes from, describing the severe jaundice that characterises the lethally compromised patient. The main vector is the mosquito *Aedes aegypti*, which is present in

tropical North Queensland, though there have been no cases of yellow fever in Australia. A vaccine was developed in the 1930s by the South African medical scientist Max Theiler, an achievement recognised by his 1951 Nobel Prize. There are, however, 70 known flaviviruses, with 30 of these being found in southern Asia and the Australasian region. Some are 'orphan' viruses that are not associated with any known disease.

Way back in the 1960s, cousin Ralph's involvement with sentinel chickens reflected the broad interests of his research group in arbovirus epidemiology, the study of how this diversity of infections spreads and is maintained in nature. Some arboviruses, particularly the tick-borne ones, can 'overwinter' by vertical transmission through the successive stages of an insect life cycle, but even when this does occur, it's unlikely to be the main mechanism that keeps the virus going in nature. Though infectious disease epidemiologists search for the vertebrate 'maintaining hosts' that continue the mosquito–animal transmission cycle, the identity of the key species can be incredibly hard to nail down. Antibodies (the footprints of prior infection) to RRV have, for example, been found in marsupial and placental mammals and, less often, in birds, but that doesn't prove that the levels of virus in blood were sufficient to cause widespread infection of the mosquito vectors. This two-way insect–vertebrate interchange probably continues throughout the year in the warmer parts of a continental landmass, particularly in forested areas where there is no effective mosquito control. Migrating birds are, of course, likely culprits for any north or south spread away from the tropics with the onset of spring and summer.

* * *

Over the years, one of the medically important functions of Australia's valiant sentinel chickens has been to serve as 'birds of record' for measuring the southern spread of Murray Valley

encephalitis virus (MVE), a flavivirus that's also called Australia encephalitis virus. This infection becomes a problem when the combination of warm weather and an abnormally wet season leads to a massive increase in mosquito numbers. If MVE is somewhere in the neighbourhood, perhaps at high enough levels in the blood of susceptible birds, then mosquitoes become infected and sporadic cases of encephalitis are seen in humans, particularly those living along the banks of major water courses like the Murray River. Though MVE has also been found in Papua New Guinea and Indonesia, the main threat to our north is the closely related, but much more dangerous, Japanese encephalitis virus (JEV), which causes severe disease in a relatively high proportion of infected people. Pigs, rather than birds, are known to be a major maintaining host for JEV, and one way of protecting humans is to decrease the 'multiplier' factor by vaccinating pigs. There are also effective human vaccines for JEV. According to public health doctors, JEV is not a cause of locally acquired disease in the USA, perhaps because of the lack of the main vector, *Culex tritaeniorhynchus*. This mosquito is also absent from Australia, but an alternative vector, *Culex gelidus*, has been identified in the tropical north, where there have been two fatal JEV cases.

Staffed by successive generations of avian 'volunteers', at least some of those sentinel chicken outposts that were located around the country to inform us about the spread of MVE in the 1960s and 1970s still house birds on active duty as part of a continuing Australian surveillance network. Sentinels in the cooler south seroconvert to MVE from time to time, though most evidence of infection is found in tropical northern Australia where occasional human outbreaks continue to occur. The Australian chickens also pick up evidence for the circulation of the closely related (to MVE) Kunjin virus, an occasional cause of human encephalitis, and Barmah Forest virus. Kunjin recently (2011) caused a number of deaths in Australian horses, and is very closely related to the West Nile virus discussed in the next chapter.

The use of sentinels depends, of course, on knowing the identity of the virus that's being looked for. Otherwise, it isn't possible to set up a specific antibody test to determine if any individual—whether poultry or person—has indeed been infected. Though human outbreaks of what was then called Australian X disease had been recognised as early as 1917, it wasn't till 1951 that Eric French, then working at the Walter and Eliza Hall Institute in Melbourne, reported the isolation and initial characterisation of the MVE virus (see Chapter 9).

Apart from the information from sentinel chickens and human cases, what else is known about MVE? The mosquito vector, *Culex annulirostris*, has been identified, but there are only indirect antibody results that implicate several species of cormorants and the Nankeen night heron as possible maintaining hosts. The Nankeen night heron is common in the wetter regions of southern and northern Australia and is generally regarded as a non-threatened species. It does depend heavily on access to fresh water, and there was some cause for concern during the recent long drought, now broken by the return of an unprecedented La Niña climate system, bringing severe flooding and massive cyclonic activity. That, of course, is also likely to increase the incidence of mosquito-borne infections.

Sometimes it's a relatively straightforward matter to establish that a particular species of bird is susceptible to a given arbovirus infection and is capable of circulating the virus, thereby functioning as a maintaining host. For example, eastern equine encephalitis virus, an alphavirus that circulates in the USA and causes disease in both horses and humans, also kills significant numbers of ibises, starlings and emus. Both the birds that eventually die and the survivors can have very high levels of virus in blood.

In general, though, it's been easier to identify the insect vectors that transmit these infections than to establish which particular wild birds or mammals support their overwintering. One reason for this is that arboviruses generally persist longer in

mosquitoes, as they lack the type of adaptive, or highly specific, immune system that is characteristic of birds, mammals and the other bony vertebrates. Even when vertebrates suffer a severe infection, the virus is usually eliminated from the blood of survivors within 8–12 days. The other reason is that trapping and handling wild birds takes a lot of effort, while it's relatively easy to catch large numbers of mosquitoes using light traps that emit CO_2 and other chemical attractants (like octenol), simulating the presence of warm-blooded animals. A more primitive technique is to allow them to bite, say, a tethered horse or your own arm, then capture them using some sort of suction device that may be as simple as a skilfully used drinking straw.

Once trapped, the mosquitoes are classified by a medical entomologist, then those of the same type are pooled, frozen and later ground up in saline for injection into some detection system (such as tissue culture or suckling mouse brain), which will then grow any virus the mosquitoes were carrying. The freshly isolated viruses can then be identified by sequencing to determine their characteristic genetic code, using essentially the same technique that forensic experts employ to identify DNA from a rapist or murderer.

* * *

The capacity to produce highly specific antibodies following natural infection in the field or forest is the basis of the sentinel chicken's role. We feed and nurture these doughty guardians because birds have both a thymus that produces the immune T lymphocytes (including the killer T cells, which I've worked on for almost four decades) and the B lymphocytes or plasma cells that produce the specific antibodies we detect in blood. As discussed in more detail in Chapter 14, the avian and mammalian immune systems have evolved somewhat differently over the eons, but they do the same job of controlling infection. Furthermore,

this shared capacity for generating long-term immune memory is the reason why, in the past century or so, we have seen the development of numerous protective vaccines for both domestic birds and chickens.

Arboviruses aren't, however, on the chicken vaccine list, as they don't affect commercial producers. How vaccines are used is always determined by practical considerations, and the fact that a product is used in one vertebrate but not another doesn't reflect some sort of discriminatory 'speciesism'. For obvious reasons, it's pretty much impossible to vaccinate wild birds against anything. In the USA, valuable horses are vaccinated against the Venezuelan equine encephalitis alphavirus, while humans are not. People who live in the more prosperous countries are protected by the environmental control of mosquitoes that's practised in most of the larger, warmer cities, by a more indoor lifestyle and by the judicious application of mosquito repellent when venturing into the countryside. We've never made a vaccine against MVE because the incidence is too low, but such a vaccine could be developed if, for example, the warming associated with anthropogenic climate change led to MVE becoming a more substantial threat to large numbers of humans.

A more likely danger for Australians is that infections like JEV and malaria will simply migrate south as ambient temperatures rise, birds modify their migration patterns, and mosquitoes extend their host range. That is already happening in parts of Africa, as infected mosquito populations move inexorably into the cooler and higher regions of the continent, which were formerly malaria-free. In Europe, Chikungunya virus has now penetrated as far north as Ravenna. As land, air and water temperatures increase, the shift of viruses that depend on a mosquito–vertebrate (bird or mammal) lifecycle into what were temperate regions will inevitably continue.

5

Falling crows

TALKING CASUALLY WITH AN immunologist colleague over coffee, the conversation wandered to the delights of golfing on a beautiful course overlooking the sea. Somewhere in California, as I recall—maybe Torrey Pines at La Jolla?

'An odd thing,' he says. 'Hitting into the rough, I came face to face with a cage full of chickens! Chickens on a golf course! What's that about?'

'Hah, sentinel chickens,' I replied. 'They've been placed there to monitor the spread of WNV, West Nile virus.'

My scientific world is largely split between two disciplines, virology and immunology. Any virologist would have known immediately what those birds were for. The fact that my

immunologist golfing friend hadn't made the chicken–WNV connection reflects the ever-increasing depth of specialisation in the sciences and the enormous expansion in knowledge. We working scientists increasingly find ourselves living in a kind of Tower of Babel, where it's harder and harder to stay abreast of what's going on in even closely related fields. In the research and writing of this book about the interface between birds, humans, diseases and environmental degradation, I've been forced to confront my own colossal ignorance of a number of related areas, particularly zoology and ornithology.

But I do know something about West Nile virus—indeed I have worked with similar pathogens. Everyone with any interest in the epidemiology of infectious disease is very aware of what began in New York City in the summer and fall of 1999. Crows fell from the sky; a whole spectrum of exotic and native birds (including Chilean flamingos and bald eagles) died at the Bronx and Queens zoos, and some 62 people developed neurological symptoms, with seven deaths. First isolated in Uganda in 1937, WNV is a flavivirus that was a known cause of periodic encephalitis and meningitis outbreaks in human populations in parts of the Old World. The invading New York WNV strain, isolated from one of the dead flamingos, was identified by genetic sequencing as being essentially identical to a virus that infected at least 500 people in Bucharest in 1996, caused some 400 cases in Israel in 1998 and was responsible for more than 40 fatalities in the Volgograd region of Russia in 1999.

Classified as a member of the Japanese encephalitis group that also includes the Australian Kunjin virus, the New York WNV epidemic marked the first time that this virus had caused problems in North America, though it turns out that there had been earlier cases of human WNV infection on Manhattan Island. Back at the beginning of the 1950s, Chester Southam and Alice Moore injected a spectrum of viruses, including WNV, into people with inoperable cancer. The hope was that these viruses, which killed

similar cells in tissue culture, might grow selectively in, and eliminate, the rapidly dividing tumours. However, though they were able to show that WNV did indeed cause asymptomatic infections in five of their 21 patients at New York's Memorial Cancer Center, there was no suggestion of any clinical benefit. Today, of course, nobody would even think of deliberately injecting very sick people with potentially lethal viruses. There were no ethics committees to monitor such activities in those distant days of human experimentation.

In fairness to Southam and Moore, though, it is important to recognise that they acted in good faith at a time when we knew so much less about infectious diseases and cancer. Also, their small study has ultimately proven to be of value, as it established definitively that the level and duration of WNV circulation in human blood can be sufficient to infect biting mosquitoes. Perhaps the 1999 WNV outbreak strain crossed the Atlantic in a viremic traveller who was incubating the disease. Only about 30% of those infected with WNV develop noticeable symptoms, and younger people, who are more likely to be adventurous in ways that might bring them into contact with infected mosquitoes, are much less likely to be clinically affected.

Then there's also the possibility that WNV traversed the ocean barrier in imported birds. Either way, exposure to local mosquitoes on the ground at JFK or Newark airports could have led to that initial transmission and, ultimately, to the establishment of WNV as a cause of endemic infection in the Americas. As with many situations where a species suddenly 'breaks out' and spreads more widely, the outcome might simply be the sum of a number of essentially random events.

Both the distance across the Atlantic to New York City and the lethality of WNV for wild birds would seem to minimise any possibility that the virus was carried from Europe or Africa during some normal process of avian migration, though not all species

are as susceptible to this disease as American crows. And there's always the remote possibility of a laboratory escape, but it seems extremely unlikely that a mosquito could have taken a blood meal from an infected vertebrate, perhaps a researcher or a laboratory mouse, and then carried WNV out into the wild. Anyone working with such a virulent virus would be extremely careful, and all laboratory animals, especially those infected with exotic pathogens, are kept in rigorously inspected, high-security facilities that certainly exclude mosquitoes.

Bioterrorism is also a possibility, but no group claimed responsibility. While there was some 'weaponisation' of such viruses during the Cold War, they were never considered to be a very practicable component of any military arsenal. In this case, weaponisation simply meant making lots of the stuff to spray at some unfortunate enemy. Laboratory infections caused by accidental exposure to aerosols containing high virus concentrations are known to occur, but free-floating arboviruses are too labile to spread far through the air. The USA did, though, grow vast numbers of yellow fever virus–infected *Aedes aegypti* mosquitoes at their Pine Bluff Arsenal before we finally regained our senses and such dangerous idiocy was banned, along with everything else of that type, under the 1975 Biological Weapons Convention. Any US stockpiles had been destroyed by the early 1970s.

A prominent characteristic of the New York and other recent WNV outbreaks has been very high mortality rates in some wild bird species. One possibility is that this reflects a mutational change to greater virulence for, in particular, corvids like crows and magpies. That may be true, but Telford Work and Dick Taylor showed years back in Egypt that the WNV strain they were studying was extremely lethal for the local hooded crows. And both the Israeli and the New York viruses have also proved deadly for domestic geese. On the other hand, experimentally infected chickens circulated the virus in blood for up to ten days

and developed a few signs of pathology, but none died or showed neurological symptoms.

* * *

As my golfing friend discovered, sentinel chickens continue to do a sterling service, monitoring WNV activity across the USA for the various state health departments and the national Centers for Disease Control and Prevention (CDC). The massive CDC laboratory complex in Atlanta provides technical and field response backup for the US Public Health Service (USPHS) Commissioned Corps, which has regional offices across the nation.

When the personable and flamboyant C Everett Koop was surgeon general of the USA in the Reagan administration, he mandated that USPHS officers wear their elegant white uniform at least once each week. During that time, I had the odd experience of visiting Tom Monath at Fort Collins, Colorado, to find him dressed like an admiral. A prominent arbovirologist, Tom is now in the private sector and has recently been involved in developing a live, attenuated WNV vaccine. The main use of this vaccine so far has been to protect horses. Right at the beginning of the New York outbreak, some racehorse owners moved their prized stock south to Florida. As viruses do, though, WNV soon caught up with them, so vaccination is the better option.

The task of coordinating what is happening with WNV in the USA is the responsibility of the CDC Division of Vector-Borne Diseases. Data is collected weekly from wild birds, sentinel chicken flocks, human cases, veterinary cases and mosquito surveillance. The CDC officers have to be paid, of course, unlike the sentinel chickens, which don't even need to be checked every day. Given the state of contemporary technology and the professionalism of the CDC and associated organisations, this must be one of the best-analysed epidemics in history, challenged only, and for

much the same reasons, by what happened in 2009 with the swine influenza pandemic.

Though there is some history of human-to-human spread, through blood transfusion—the US blood supply is checked for WNV—or mother's milk, for example, there is no doubt that WNV is normally spread by the bite of mosquitoes. The principal vectors are various *Culex* species but more than 50 strains of mosquitoes can be infected with this virus in the USA alone. In addition, there is evidence that WNV can be transmitted vertically through the various insect larval stages, and that the virus can overwinter in infected hibernating mosquitoes.

While horses and humans are occasional hosts, it is very clear that WNV will now be maintained permanently in North America by a bird–mosquito life cycle. Right from the outset in July 1999, the virus was detected in mosquitoes, crows and humans. As early as June 1999, the residents of the New York suburb of Queens noticed an unusual number of dead and dying crows. Dead birds were collected over the ensuing months, and of 280 or more that were shown definitely to be WNV positive between August and December, about 90% were American crows. By the end of 2000, evidence of human WNV infection was found in the contiguous states of New Jersey and Connecticut, and it continued to spread north (into Massachusetts and Ontario) and south (into Florida and Louisiana) through the following year. The CDC figures then show a progressive westward distribution, with the first years of high incidence in Colorado and California being 2003 and 2004 respectively. Cases of WNV infection have now been reported from all the states of the Continental USA, north to Alberta and Saskatchewan in Canada, as far east as the Caribbean, and down to Mexico and Argentina, though the disease has not spread to Alaska or Hawaii. Working from the annual CDC figures that have been published since 1999, epidemiologists had (by December 2009) identified neurological symptoms associated with WNV

infection in more than 17 000 people, and the virus is considered to have caused at least 1100 deaths.

That rate of geographical spread is completely consistent with the idea of a slow, progressive mosquito-borne transmission from viremic hosts (mainly birds) to previously uninfected avian populations. American crows and raptors (eagles, hawks, owls and vultures) are the likely maintaining vertebrate hosts. While the majority of infected crows are thought to die within three weeks, that still allows plenty of time for mosquitoes to feed, especially if the birds are debilitated by the infection. This is likely to be affecting crow numbers, though it is hard to generate solid figures on overall mortality for any wild bird population. That type of information generally comes from volunteer bird watchers and amateur ornithologists. Such data are, for obvious reasons, much harder to collect and collate in the absence of the databases and resources that are available to salaried public health officers for analysing human populations.

A telling account is, however, available for another corvid, the yellow-billed magpie, a California native that local Audubon Society members recently named as their 2009 bird of the year. According to Holly Ernest, who directs the Wildlife Health and Ecological Genetics Unit in the Veterinary Genetics Laboratory at the University of California, Davis, yellow-billed magpie numbers declined substantially following the arrival of WNV. Field surveys by Ernest and her graduate student Scott Crosbie indicated that there was at least a 20% drop in numbers between 2004 and 2006. The difficulty in coming to a more precise conclusion reflects the fact that earlier estimates of population size are not supported by such solid data. According to the CDC, evidence of extensive, human WNV infection was first detected in California in 2004, when case numbers of meningitis and encephalitis went from two (in 2003) to 289. Over the same interval, 12 000 magpie carcasses were reported to the Californian Department of Health, with almost 80% of those tested being WNV positive.

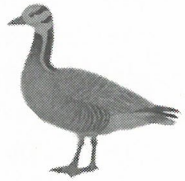
By 2009, observers from the Audubon Society had the sense that yellow-billed magpies may be coming back, while only 67 reported cases of likely human WNV-associated neurological disease were recorded in California. This could indicate several things. It had been very dry in California, which should mean that there were fewer mosquitoes around. A second possibility is that WNV itself has provided the selective pressure to drive the rapid emergence of a more resistant strain of yellow-billed magpie. If so, given the short time frame, the resistance genes must have already been present in a subset of the population that was first hit by the virus. The third alternative is that the virus itself has mutated to a form that is less lethal for the yellow-billed magpie. Perhaps, as bird numbers fell, it may have been advantageous for the virus to select variants that allowed the maintaining host to be more mobile and thus, perhaps, encounter greater numbers of mosquitoes over a broader geographic range. The tools to differentiate between these different scenarios are there, and I expect that the virologists, on the one hand, and the wildlife geneticists, on the other, are actively pursuing the various possibilities.

written in stone that tells us how to predict novel influenza pandemics. Though we can be pretty sure that influenza A viruses will occasionally jump into human populations, we don't know which particular strain will make the transition, when this might occur, or how severe and extensive the disease might be in us. Chance rules, and as with the risk of war, we need to be both vigilant and to keep our defences in good shape. That might sound a bit over the top, but bear in mind that the 1918–19 Spanish flu claimed many more lives than were destroyed by the collective insanity of World War I. Our general level of awareness was raised for a time by the 60% human mortality rate associated with the H5N1 bird flu epizootic (an epidemic in animals rather than people), but we tend to forget about such possibilities when they're no longer in the news.

In December 2011, though, that changed, with extensive reporting on the controversy associated with the publication of genetic changes in H5N1 viruses that are lethal for chickens and, after being passaged serially in ferrets, acquire the capacity to transmit naturally in that species. When it comes to spreading flu, ferrets and humans are very similar. Combine that with the fact that the relatively mild 2009 'swine' H1N1 went round the world in less than five months, and it's obvious that we cannot afford to be relaxed and comfortable when it comes to influenza.

8

Bird flu: from Hong Kong to Qinghai Lake and beyond



HAVING DISCUSSED INFLUENZA IN general, it's time to focus on the birds. The first thing we have to realise is that, being natural and generally mild infections of a whole range of waterfowl, there is no way that these potentially lethal pathogens can ever be eliminated unless our small planet turns into a dry, lifeless rock. So long as we share the earth and oceans with our feathered cousins, mammals such as ourselves (and seals, whales, pigs, horses, leopards, racing greyhounds and so on) have to live with the certainty that novel influenza A viruses will occasionally 'jump' from wildlife reservoirs, sometimes with disastrous consequences.

Aquatic birds play a key role because, unlike many viruses, influenza survives happily in fresh water, which means that ponds, lakes and dams are a major source of cross-infection. Ducks, geese, flamingos, cranes, waterhen and so on all have to drink. Even passerines that aren't normally swimmers and divers are at risk and can, for instance, become infected and spread the virus to poorly protected chicken houses. Cross-infection becomes more likely under dry or drought conditions, when sparrows, swallows, starlings and waterfowl come close together at reduced bodies of water.

Avian diversity is an essential part of the mix. The same virus that kills one bird species very quickly may cause a subclinical, long-term, persistent infection in another. In general, it's a very bad idea for commercial chicken producers to keep a pond where they raise a few domestic geese or ducks. Visits from their free-flying, migrating relatives can introduce an unwelcome influenza guest that first infects the local water birds, then spreads to cause chicken mayhem.

Maintaining very large numbers of free-range chickens and turkeys is also risky, as the probability of exposure to wild birds is much greater under field conditions. That's thought by many to describe what happened with a 2003 H7N7 outbreak in the Netherlands. Public concern for the welfare of chickens that are caged for life had led to a 'deinstitutionalisation' of the local poultry industry, the emphasis being on allowing the birds a more natural lifestyle. The disease spread to poultry in Belgium and Germany, was responsible for one human death and led to the culling of some 33 million domestic birds.

Looking, say, at wild-caught Canada geese before the fall migration, as many as 30% of juveniles can be shedding one or other strain of influenza virus. You've never seen, or heard, of a goose or a duck with what looks like the flu or even a bit of a cold? The reason is that influenza is generally a relatively mild infection of the avian gastrointestinal tract (rather than respiratory tract). While the goose or duck immune system works perfectly well and

eventually gets rid of the pathogen, the virus can be detected in gut contents for 5–10 days without causing any obvious problem for the birds. And it's not only wild birds that are involved: influenza virus can, for instance, be excreted from the common cloacal opening of healthy domestic ducks for as long as 17 days after experimental infection. The pasty, white mixture of faeces and urinary tract excretions that we've all experienced sometime or other as a 'gift' from on high is highly infectious when voided into water, and survives well in wet bird droppings on the ground.

The duration of virus excretion, together with both the variety and the enormous numbers of wild birds that are involved, means that there is little cause for the viruses to change in order to maintain their avian transmission cycle. That's very different from the situation with long-lived humans, where durable antibody protection in those who have been vaccinated or recovered from influenza exacerbates the problem of limited numbers in the potentially susceptible pool. The virus will just die out if it can't keep transmitting from person to person. The net consequence is that influenza viruses that are being maintained in human populations are under constant selective pressure to alter their outer-coat H and N proteins and escape from antibody-mediated immune control. That 'antigenic drift' effect is normally minimal for influenza infections of wild birds, and the viruses can remain essentially unchanged for decades.

The same can also be true for domestic birds: relatively innocuous, 'low-pathogenicity' (low-path) influenza A viruses can circulate in chicken populations for years without causing much of a problem. Producers don't generally bother about vaccination, but these viruses sometimes mutate to a 'high-pathogenicity' (high-path) form and cause massive, lethal outbreaks. High-path influenza in birds is completely different from the normal situation for ducks, geese and chickens infected with the low-path strains. These high-path viruses are systemic, meaning that they spread in the blood and grow in all organs, including the brain

and the lungs. Large numbers become very debilitated, show haemorrhages in the skin and legs, turn blue (cyanotic due to lack of oxygen) and die very quickly. The disease can be extraordinarily severe and is quite terrifying to watch. The very natural response is to ask: what if that were us? Those who deal with such avian outbreaks have been the first to warn about the potential danger that influenza poses to crowded, and highly mobile, human populations.

According to the World Health Organization (WHO), there were 21 avian high-path flu incidents reported between 1959 and 2003, occurring in Europe, Asia, Australia, and North and South America. This low- to high-path 'switch' has been seen only for H5 and H7 viruses, though low-path H9N2 and H6N2 viruses can sometimes cause problems as co-infections with something else. The WHO figure for 'high-path' transitions is undoubtedly an underestimate, as the likelihood that such an event will be recognised depends on the sophistication of both farmers and the local veterinary services. Five of the 21 were in Australia (caused by H7N7, H7N3 and H7N4 viruses), where general awareness among producers and regulatory authorities that exotic infections can cause enormous economic loss means that there are well-established state and national animal virus diagnostic laboratories.

For the virologists and other flu specialists, perhaps the most dramatic wake-up call prior to the H5N1 bird flu story came with the 1983 outbreak caused by a high-path H5N2 virus in Pennsylvania chicken flocks. Suddenly, seemingly out of nowhere, large numbers of chickens started to die in commercial production facilities. The disease spread to the neighbouring states of New Jersey and Maryland, and was ultimately handled by culling more than 17 million birds at a direct cost of some US$60 million. Humans did not become infected, and though the original virus may have come from wild birds, live bird markets were thought to be the likely focus of dissemination throughout the chicken industry. Ducks, geese and so forth were infected experimentally

under laboratory conditions, but none of those species either developed severe symptoms or excreted unusually large amounts of virus. Then the high-path chicken experience was repeated in Mexico in 1993, and in 2006, 60 ostriches died from an H5N2 infection on a farm in South Africa. Thinking about this, it becomes very obvious that the process of genetic change that causes any influenza A virus to switch from being mild to severe can occur any place at any time.

What frightened the virologists was that the transition from low-path to high-path resulted from a single point mutation in the viral RNA. That is, the 1983 H5N2 virus became extraordinarily lethal for chickens as a consequence of just one amino acid change in the virus H protein. This was long before Jeff Taubenberger and Johan Hultin reconstructed the 1918 pandemic virus, and it was at the forefront of everyone's mind that such a minimal modification could have occurred back then to cause the virus to 'jump' from some other species (pigs or birds perhaps) into humans. The serial-passage H5N1 ferret study mentioned in chapter 7 indicated that five mutational changes may be sufficient to allow this avian virus, which is highly lethal for the relatively few people who have been infected, to transmit readily between humans. Then, though, there's also the possibility of reassortment to allow the emergence of a virus that has some genes from a strain that infects birds, and others from an isolate that transmits readily between people. That clearly happened at least twice during the twentieth century. When the Dutch authorities had to deal with the 2003 H7N7 outbreak in chickens and turkeys that also caused some human infections, the first thing they did was to make sure that those 'in contact' were given the standard 'seasonal' influenza vaccine, to minimise the risk of concurrent infections with a human and a bird strain, the necessary prerequisite for gene reassortment. Some of those handling the infected birds were initially reluctant to take the influenza antiviral drug Tamiflu, but that changed after one of the veterinarians died from the infection.

The realisation that a lethal flu outbreak in domestic poultry can constitute a direct threat to human wellbeing first entered the broader consciousness when, between May and December 1997, a high-path H5N1 virus caused six deaths and otherwise severe disease in a total of 18 Hong Kong residents. This was the first time that an H5N1 strain had been shown to make the jump and cause major clinical problems in humans. All the evidence indicated that the virus, which had been causing deaths in domestic chickens from March, was in some way contracted from infected birds and did not spread between people. Comprehensive testing of birds in the wild, on farms, from zoological gardens and in the live bird markets that bring city people into direct contact with ducks, geese, chickens and so forth showed evidence of widespread involvement. A report by Ken Shortridge, who was then the senior resident influenza researcher in Hong Kong, relates that the H5N1 virus was isolated from 2.4% of ducks, 2.5% of geese and 21% of domestic chickens.

The Chinese tradition is to eat meat that is as fresh as possible. As a consequence, Shortridge reckoned that in Hong Kong in 1997, there were about 1000 urban live-bird markets. Apart from the obvious dangers associated with killing and dressing an infected bird for the table, the possibility of infection from contaminated bird droppings and local water sources was also suggested by the fact that some of the cases clustered in neighbourhoods near the bird markets. From late December, the epidemic was brought to an end with the culling of some 1.5 million domestic poultry.

During 1998, I visited my US-based colleague, the avian influenza expert Rob Webster, who was then spending a few months every year helping with the 'influenza virus watch' program operated by Shortridge and his colleagues at the University of Hong Kong. Rob took me along to one of the live-bird markets. By that time, ducks and geese had been banned, but there were many cages of chickens and quite a few 'loose' birds roaming free. Then there were cages of tiny quail, which were often placed right next to, or

even directly under, the chickens. Looking closely, all the quail had ruffled feathers, and even for a lapsed veterinarian like me, it wasn't hard to see that they were not in optimal health. Rob assured me that he could pick any one of those fluffed-up little birds, take a swab from the cloaca and isolate one or other influenza virus.

It was a lesson in epidemiology or, to be more correct, epizootiology. The chickens and quail sell fast, but they in turn infect pheasant, chukar and guineafowl that move more slowly (in the commercial sense) and hang around to infect the next batch of chickens freshly arrived from the farm. At that stage, there was no H5N1 flu anywhere in Hong Kong, at least so far as anyone was aware, but H5N1 was never the whole game for the local avian world. The widespread testing during the 1997 outbreak also turned up several H9 strains that were circulating at a lower level (0.9% in ducks, 0.6% in geese, 4.1% in chickens and 3 isolates from pigeons). One sampling of a live-bird market showed that 36% of the birds were H9 positive! The same situation no doubt applies in many situations where large numbers of birds are brought together at close quarters, but the H9 influenza strains were low-path viruses that were not causing problems, so nobody was too bothered.

The kill-off in Hong Kong was not, sad to say, the end of the story for the high-path H5N1 viruses. An April 2010 news story, for instance, reported that a 27-year-old man had died in Cambodia from the H5N1 bird flu virus. He was the tenth person in the country to be infected with the virus, and the eighth person to die from it. The report went on to caution people to be on the lookout for sick poultry and to report such incidents to the authorities.

As of March 2010, the WHO had recorded 486 human cases of H5N1 bird flu, with 287 deaths, a mortality rate of around 60%. By October, the figures were more like 500 and 300. Apart from Cambodia and the 1997 outbreak in Hong Kong, people have died on the Chinese mainland and in Azerbaijan, Egypt, Indonesia, Laos, Indonesia, Nigeria, Pakistan, Thailand, Turkey and Vietnam.

Clearly this virus, though highly lethal, is not very infectious for humans and has yet to make that 'jump' that allows it to spread between people. But, despite the low infectivity, any virus that causes 60% mortality raises obvious concern. That's especially true for influenza, which has a well-known proclivity for mutation (or gene reassortment) to extend its host range.

The current high-path H5N1 virus does, for example, cross readily from birds into cats. Domestic cats show a high incidence of infection in areas where the H5N1 virus is killing chickens, and both tigers and leopards have died in zoos, perhaps as a result of being fed infected chicken carcasses. There is as yet no evidence that cats spread the disease to humans, but there is concern that they could act as a 'mixing vessel' (as was long suspected for pigs) to allow strains that are circulating concurrently in birds and people to meet up in the same infected lung cell.

When it comes to human infections, what is thought to have happened with the relatively few unfortunates who have developed H5N1-induced disease is that they received a very large dose directly from infected birds. It now seems that this form of influenza only happens when aerosolised virus penetrates to the farthest ends of the human lung, the small bronchi and alveoli. The team led by virologist Yoshi Kawaoka (who has labs at both the University of Madison, Wisconsin, and the University of Tokyo) has shown that while cells with the 'human' form of sialic acid dominate our upper respiratory tract, some of our deep lung epithelium also expresses the 'avian' form, the preferred receptor for the H5N1 virus.

A classic scenario involves a small farmer in an Asian village who knows that his chicken and duck flocks will be killed and then burnt or buried by regulatory authorities if they show any signs of high-path H5N1 bird flu. At the first hint of infection, he decides to distribute the birds around his extended family so that everyone can enjoy at least one good dinner. His young son stuffs a couple of the better-looking chickens down the front of

his shirt, and hops on his bike to take them to Auntie's place. The infected birds breathe almost directly into his face, exposing him to a massive dose of high-path H5N1 influenza virus. Pedalling vigorously, he draws the virus-tainted air into the depths of his lungs. The appalling result is that the child dies of pneumonia 5–10 days later.

Apart from the death of the boy, there are many elements of personal tragedy in this little tale. Among them is that killing large numbers of infected and in-contact birds reduces the supply of high-value protein to people who live on a nutritional knife-edge. Currently, in a world where Westerners are dying prematurely of obesity, there are thought to be just under a billion people who simply do not get enough to eat each day. At least 500 million chickens have been destroyed during the continuing high-path H5N1 epizootic, either by the infection itself or as a result of culling to control virus spread.

Then there's the loss of income as bird populations are slaughtered, reducing, among other things, the financial flexibility that frees men and women from unrelenting drudgery and allows children to be educated. In poorer countries, people's fates and lifestyles are still intimately linked to the health of their animals. According to an old African saying, 'If the cattle die, the people die also.' That may be just as true for chickens, particularly in Asia. As flu spreads, so does a great deal of human suffering, and it is not necessarily associated with people contracting the disease. As we wait for the virus to make the 'jump' that will allow global spread, those of us in the West keep watch, but only for a time; once the journalists and editors tire of the story and switch to something else, we soon lose interest. Media images of jumpsuit-clad, masked men slaughtering chickens and burying them in pits can't rival the visual drama of flood, fire, earthquake or tsunami.

The absence of TV and newspaper coverage does not, though, mean that the problem has gone away. Fortunately, we have organisations like the WHO, the CDC, the US National Institutes of

Health, and the US Department of Agriculture, which continue to watch the situation very closely. Those who attack organisations like the United Nations (UN) and the idea of global cooperation think in terms of parochial politics, not international science. By choice or from ignorance, they are clearly unaware of the massive risk posed by diseases like influenza and the good job that UN agencies, like the WHO, do in looking out for our interests.

In general, both our capacity to monitor infectious disease outbreaks and our understanding of the basic science have been evolving with incredible speed. Rapid technological advances mean, for example, that genomes can be expanded by PCR and sequenced in a day or two to identify an influenza A virus that is causing, say, an outbreak in poultry or a severe human case. Much of that work is done within the six WHO Collaborating Centres, located in Britain, China, Japan, Australia and the USA. These in turn link back to a much larger network of government- and university-based laboratories. Our capacity to follow what is happening globally in 'real time' is probably more sophisticated for influenza than for any other infection, with the possible exception of HIV/AIDS. As things stand, though, influenza is potentially much more dangerous, because of its proclivity for extremely rapid respiratory spread.

* * *

The origin of the 1997 high-path H5N1 Hong Kong outbreak was ultimately traced back to a 1996 virus isolated from domestic geese in Guangdong, China. Since then, despite massive efforts at control, including some ill-advised bird-vaccination programs, lethal H5N1 strains (or 'clades') have continued to spread and to evolve genetically. Hui-Ling Yen, Guan Li, Malik Peiris and Rob Webster summarise the situation for commercially raised birds as follows:

> Previously reported high-path outbreaks of H5 and H7 in domestic poultry have either been stamped out or burnt out and disappeared. The current high-path H5N1 has been stamped out in Japan, South Korea, Thailand and many other countries in Asia, Africa and Europe—only to return again during the cooler months.

After the 1997 Hong Kong outbreak, lethal H5N1 viruses continued to spread south as far as Indonesia. To what extent that distribution reflected the (often illegal) movement of domestic birds rather than transmission by wildlife is not clear, though human transport was undoubtedly a factor. The fact that H5N1 has not jumped from Indonesia to north-western Australia can probably be attributed to the lack of any trade in poultry between those two regions, and to 'Wallace's line', which follows the deep-water channel along the interface between different tectonic plates. Running north to the Philippines, it effectively divides the fauna of some of the Indonesian islands and separates South-East Asia ecologically from Australia and New Guinea. This is undoubtedly a key barrier that has helped to keep Australian wildlife and domestic species free of many exotic infections.

No such divide exists between Asia and Europe. It seems likely that the westward movement of high-path H5N1 viruses as far as Egypt, Scandinavia and the United Kingdom has largely been due to migrating waterfowl, though the distribution indicates that there is much we don't understand about those routes. Further research using radio tracking is currently under way. The first case of H5N1 viruses causing widespread fatal disease in wild birds occurred in 2002, when sparrows, pigeons and many aquatic species were found dead in Hong Kong's Penfold Park and Kowloon Park. Then, in 2005, a lethal outbreak killed more than 6000 migratory waterfowl at Qinghai Lake, a nature reserve that is a major breeding site in western China. This affected bar-headed geese, great

black-headed gulls, ruddy shelducks and great cormorants. Other species that have since been found to be highly susceptible include whooper swans, black-necked cranes and pochards.

Long-distance westward spread of the Qinghai Lake strain was detected as early as 2005, the likely culprits being migrating ducks. Geese were exonerated because the infection was 100% lethal. Even in the mildly affected ducks, the virus changed from being a predominantly gastrointestinal-tract to a respiratory-tract infection. What had happened to make this high-path H5N1 virus so virulent for many species of wild birds and, incidentally, for mammals as represented by laboratory mice, ferrets, cats and us? The key modification was a single point mutation in one of the influenza polymerase (PB2) genes, again emphasising the fragility of the genetic relationship between these viruses and the bird species that maintain them in nature. If the infection had not been so much less severe in ducks, the disease would quickly have burnt out in wildlife and this virulent H5N1 virus would soon have disappeared.

Why this mutated pathogen emerged in wildlife living on Qinghai Lake, which is a protected, national reserve, was a little puzzling. In general, influenza A viruses maintain a reasonably amicable relationship with waterbirds living under natural conditions. A plausible theory is that the mutation occurred in a nearby intensive, domestic poultry operation and was then spread to the lake by bar-headed geese. Aiming to provide some variety in the diet of railroad workers in western China, the authorities initiated a commercial breeding program for bar-headed geese. Spread from chickens to geese could have been either direct or as a consequence of exposure to contaminated water sources. It only needed the escape of one recently infected goose, then a relatively short flight before symptoms set in, for the virus to reach Qinghai Lake.

The road to hell is paved with good intentions. As a consequence of initiatives by many agencies, including US and Australian aid organisations, the numbers of domestic poultry in Asia, particularly South-East Asia, have grown more than fiftyfold

since the end of World War II. The aim has been to increase prosperity and to improve lives by developing local industries that provide affordable, high-quality protein to a rapidly expanding human population. The number of people on the planet has more than doubled since I graduated from the University of Queensland in 1962, with most of this growth being in poorer, developing countries. More mouths to feed also mean more pigs. House all these species at close quarters in a warm, wet environment and we have the ideal conditions for the emergence of a novel influenza A virus that will jump into humans.

One way to drive the emergence of mutant influenza viruses is to crowd large numbers of chickens together under conditions where there is rapid turnover. Even worse is the construction of 'high-rise' bird markets that bring many avian species together for differing times. Add the exposure of migrating birds to that equation and the situation becomes even more dangerous. That contact may not necessarily be direct. The waste from chicken houses is, for example, used to fertilise rice fields, a crop grown under the type of wet conditions that favour visits by waterfowl. The lesson is that if we are to change the basic ecology of natural systems, we must also be prepared to manage the consequences, informed by the underlying science. At least when it comes to birds in warmer, wetter climates, the influenza A viruses are an important factor to be considered. This isn't necessarily 'rocket science'. The solutions can lie with approaches as basic as proper waste management.

1 SEARCHING FOR PUFFINS: AN INTRODUCTION

Armstrong, RH et al. 1980, *A Guide to the Birds of Alaska*, Alaska Northwest Publishing Co., Anchorage.

Butchart, SHM et al. 2010, 'Global biodiversity: indicators of recent declines', *Science*, vol. 328, no. 5982, pp. 1164–8.

Goldsworthy, SD, Gales, RP, Giese, M & Brothers, N 2000, 'Effects of the *Iron Baron* oil spill on little penguins (*Eudyptula minor*) 1: estimates of mortality', *Wildlife Research*, vol. 27, no. 6, pp. 559–71.

Golet, GH et al. 2002, 'Long-term direct and indirect effects of the "Exxon Valdez" oil spill on pigeon guillemots in Prince William Sound, Alaska', *Marine Ecology Progress Series*, vol. 241, pp. 287–304.

2 DISTANT RELATIVES

Feduccia, A 1999, *The Origin and Evolution of Birds*, 2nd edn, Yale University Press, New Haven.

Maina, JN et al. 2010, 'Recent advances into understanding some aspects of the structure and function of mammalian and avian lungs', *Physiological and Biochemical Zoology*, vol. 83, no. 5, pp. 792–807.

Tudge, C 2009, *The Bird: A natural history of who birds are, where they came from and how they live*, Three Rivers Press, New York.

3 CHICK EMBRYOS AND OTHER DEVELOPING LIFE FORMS

Beveridge, WIB 1947, 'Simplified techniques for inoculating chick embryos and a means of avoiding egg white in vaccines', *Science*, vol. 106, no. 2753, pp. 324–5.

Burnet, FM 1969, *Changing Patterns: An atypical autobiography*, American Elsevier, New York.

Dunbrack, RL & Ramsay, MA 1989, 'The evolution of viviparity in amniote vertebrates: egg retention versus egg size reduction', *American Naturalist*, vol. 133, no. 1, pp. 138–48.

Goodpasture, EW, Woodruff, AM & Buddingh, GJ 1932, 'Vaccinal infection of the chorio-allantoic mebrane of the chick embryo', *American Journal of Pathology*, vol. 8, no. 3, pp. 271–82.

Marmion, BP and Feery, B 1980, 'Q-Fever', *Medical Journal of Australia*, vol. 2, pp. 281–2.

Patten, BM 1946, *The Early Embryology of the Chick*, 3rd edn, Blakiston, Philadelphia.

Sexton, C 1999, *Burnet: A life*, Oxford University Press, Melbourne.

4 SENTINEL CHICKENS

Doherty, RL 1974, Arthropod-borne viruses in Australia and their relation to infection and disease, *Progress in Medical Virology*, vol. 17, pp. 136–92.

Eron, C 1981, *The Virus that Ate Cannibals: Six great medical detective stories*, Macmillan, New York.

Fenner, FJ (ed.) 1990, *History of Microbiology in Australia*, Australian Society of Microbiology, Canberra.

Komar, N, Dohm, DJ, Turell, MJ & Spielman, A 1999, 'Eastern equine encephalitis virus in birds: relative competence of European starlings (*sturnus vulgaris*)', *American Journal of Tropical Medicine and Hygiene*, vol. 60, no. 3, pp. 387–91.

MacKenzie, JS & Williams, DT 2009, 'The zoonotic flaviviruses of Southern, South Eastern and Eastern Asia and Australasia: the potential for emergent viruses', *Zoonoses and Public Health*, vol. 56, nos 6–7, pp. 338–56.

Nathanson, N (ed.) 1997, *Viral Pathogenesis*, Lippincott-Raven, Philadelphia.

Peters, CJ & Olshaker, M 1998, *Virus Hunter: 30 years of battling hot viruses around the world*, Anchor Publishing, Canada.

Theiler, M & Downs, WG 1973, *The Arthropod-borne Viruses of Vertebrates: An account of the Rockefeller Foundation virus program 1951–1970*, Yale University Press, New Haven.

Van den Hurk, AF et al. 2010, 'Vector competence of Australian mosquitoes for Chikungunya virus', *Vector-Borne and Zoonotic Diseases*, vol. 10, no. 5, pp. 489–95.

5 FALLING CROWS

CDC, Division of Vector Borne Diseases, 'West Nile virus', www.cdc.gov/ncidod/dvbid/westnile/surv&control.htm#surveillance

Crosbie, SP et al. 2008, 'Early impact of West Nile virus on the yellow-billed magpie (*Pica nuttalli*)', *Auk*, vol. 125, no. 3, pp. 542–50.

Eidson, M et al. 2001, 'Crow deaths as a sentinel surveillance system for West Nile virus in the northeastern United States, 1999', *Emerging Infectious Diseases*, vol. 7, no. 4, pp. 615–20.

Ernest, HB, Woods, LW & Hoar, BR 2010, 'Pathology associated with West Nile virus infections in the yellow-billed magpie (*Pica nuttalli*): a California endemic bird', *Journal of Wildlife Diseases*, vol. 46, no. 2, pp. 401–8.

Kuno, G & Chang, G-J 2005, 'Biological transmission of arboviruses: reexamination of and new insights into components, mechanisms, and unique traits as well as their evolutionary trends', *Clinical Microbiology Review*, vol. 18, no. 4, pp. 608–37.

Southam, CM & Moore, AE 1951, 'West Nile, Ilheus and Bunyamwera virus infections in man', *American Journal of Tropical Medicine and Hygiene*, vol. 1, no. 6, pp. 724–41.

Work, TH, Hurlbut, HS & Tylor, RM 1955, 'Indigenous wild birds of the Nile Delta as potential West Nile virus circulating reservoirs', *American Journal of Tropical Medicine and Hygiene*, vol. 4, no. 5, pp. 872–8.

6 TICKS, SHEEP, GROUSE AND THE GLORIOUS TWELFTH

Gilbert, L et al. 2004, 'Ticks need not bite their red grouse hosts to infect them with louping ill virus', *Proceedings of the Royal Society B: Biological Sciences*, vol. 271, supplement 4, pp. S202–5.

Reid, HW 1999, 'Monks, ticks and the molecular clock', *Biologist*, vol. 46, pp. 197–200.

7 FLU FLIES

Andrewes, CH, Laidlaw, PP & Smith, W 1935, 'Influenza—observations on the recovery of virus from man and on the antibody content of human sera' *British Journal of Experimental Pathology*, vol. 16, no. 6, pp. 566–82.

Barry, JM 2004, *The Great Influenza: The story of the greatest pandemic in history*, Viking Press, New York.

Crosby, AW 2003, *America's Forgotten Pandemic: The influenza of 1918*, 2nd edn, Cambridge University Press, Cambridge.

Mullis, K 1998, *Dancing Naked in the Mind Field*, Pantheon, New York.

Neiderman, CS, Sarosi, GA & Glassroth, J (eds) 2001, *Respiratory Infections*, 2nd edn, Lippincott, Williams & Wilkins, Philadelphia.

Shope, RE 1931, 'The etiology of swine influenza', *Science*, vol. 73, pp. 214–15.

Taubenberger, JK, Hultin, JV & Morens, DM 2007, 'Discovery and characterization of the 1918 pandemic influenza virus in historical context', *Antiviral Therapy*, vol. 12, no. 4 (part B), pp. 581–91.

Yuen, KY et al. 1998, 'Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus', *Lancet*, vol. 351, no. 9191, pp. 467–71.

8 BIRD FLU: FROM HONG KONG TO QINGHAI LAKE AND BEYOND

Feare, CJ, Kato, T & Thomas, R 2010, 'Captive rearing and release of bar-headed geese (*Anser indicus*) in China: a possible HPAI H5N1 virus infection route to wild birds', *Journal of Wildlife Diseases*, vol. 46, no. 4, pp. 1340–2.

Lebarbenchon, C, Feare, CJ, Renaud, F, Thomas, F & Gauthier-Clerc, M 2010, 'Persistence of highly pathogenic avian influenza viruses in natural ecosystems', *Emerging Infectious Diseases*, vol. 16, pp. 1057–62.

Salomon, R & Webster, RG 2009, 'The influenza virus enigma', *Cell*, vol. 136, no. 3, pp. 402–10.

Webby, RJ, Webster, RG & Richt, JA 2007, 'Influenza viruses in animal wildlife populations', *Current Topics in Microbiology and Immunology*, vol. 315, pp. 67–83.

Yen, H-L, Guan, Y, Peiris, M & Webster, RG 2008, 'H5N1 in Asia', in H-D Klenk, MN Matrosovich & J Stech (eds), *Avian Influenza*, Monographs in Virology, vol. 27, Karger, Basel, pp. 11–26.

9 BIRD FLU GUYS

Downie, JC, Webster, RG, Schild, GC, Dowdle, WR & Laver, WG 1973, 'Characterization and ecology of a type A influenza virus isolated from a shearwater', *Bulletin of the World Health Organization,* vol. 49, no. 6, pp. 559–66.

Mandavilli, A 2003, 'Profile: Robert Webster', *Nature Medicine*, vol. 9, no 12, p. 1445.

Pereira HG, Tumova, B & Webster, RG 1967, 'Antigenic relationships between influenza A viruses of human and avian origins', *Nature*, vol. 215, pp. 982–3.

Webster, RG 2010, 'William Graeme Laver', *Biographical Memoirs of Fellows of the Royal Society*, vol. 56, pp. 215–36.