

## CHAPTER SEVEN

### CONCLUDING REMARKS

Highly pathogenic avian influenza and velogenic Newcastle disease are the two most serious diseases of poultry worldwide and cause massive mortalities during outbreaks. While NDV is recognised as the most important disease threatening backyard poultry production for sustainable rural livelihoods in developing countries, HPAI has the status of having zoonotic potential. Asian HPAI H5N1 (genotype Z) has recently been at the receiving end of much media attention, most of it hype surrounding the theoretical possibility that HPAI H5N1 could be involved in the next human pandemic, in which millions of immunologically-naïve humans are predicted to die. South Africa regained its HPAI-free status in October 2005, but this was once again suspended in July 2006 when an outbreak of HPAI H5N2 was reported on an ostrich farm in the Riversdale area of the Western Cape Province.

Similarly, South Africa is not free of NDV and therefore trade restrictions apply. During 2004/2005, the South African poultry meat industry again made the largest contribution to the national gross value of agricultural production with 15.6% of R68 026 million (~\$9 700 million). In 2004, almost 600 million broilers were slaughtered for consumption in South Africa (Trends in the Agricultural Sector 2005, NDA). The ostrich farming industry in South Africa is the single largest in the world producing 65% of the world's ostrich meat and export income amounts to R1,2 billion annually with 90% of both leather and meat products being exported (South African Ostrich Business chamber). Although AI and ND outbreaks can potentially have a huge impact on the economy, very little was known about the status of these two diseases in South Africa. It has for many years been assumed that NDV is enzootic here, periodically spilling over from an unknown reservoir. Similarly, the origin of the sporadic AI infections of ostriches was unknown. Therefore, the objectives of my investigation were to analyse genetically and phylogenetically the AIV and NDV viruses that were isolated across South Africa since the early 1990s. During this time, South Africa also experienced its first outbreak of HPAI since the 1960s. This was the first time that molecular techniques were applied in South Africa to analyse AIV and NDV strains.

In Chapters Two and Three, I demonstrated for the first time that South African AIV strains share recent common ancestors with viruses isolated predominantly in Russia (particularly Lake Chany and Primorje), northern Europe (Germany, the Netherlands, Norway, Denmark and Sweden), Italy and China. No South African viruses have yet been isolated in other regions, according to published reports. Although LPAI viruses have been isolated in 12 bird orders, the large majority of isolations were reported in the orders Anseriformes (in particular in the family Anatidae: ducks, swans and geese) and Charadriiformes (shorebirds and relatives). While anatidae certainly seem to play the major role in the ecology and transmission of LPAI viruses to domestic poultry in Europe and Asia (Brown *et al.*, 2006), southern African ducks and geese are only short-to-medium distance migrants, and do not come into direct contact with the wild duck and goose populations of Europe and Asia. They are thus unlikely to be directly involved in the initial introduction of LPAI into South Africa. However, about two dozen species of the orders Ciconiiformes (storks) and Charadriiformes (waders and terns) are Palaearctic migrants that over winter annually in Southern Africa (Underhill *et al.*, 1999) (Appendix 4). Siberia, and in particular the West Siberian Lowland, is the breeding area of many different migratory species in the Palaearctic, where birds arrive during springtime, following different routes from Europe, Asia, and Africa. The breeding season in these northern most regions of Eurasia is usually very brief, and migratory bird populations start migrating southward with their newly-hatched juveniles to escape the first frosts in early autumn. This results in the build up of pre-migration concentrations of many species of waterfowl in the south of the breeding areas, where maturation and moulting take place before the main southern migration starts. This is a critical stage in the LPAI virus transmission and redistribution process because of the density of waterfowl and the presence of immunologically naïve individuals, which may undergo several subsequent infection episodes, shedding large quantities of the virus in their faeces (Halvorson *et al.* 1985; Stallknecht and Shane 1988). As the autumn migration involves juveniles, it is characterised by short flights and several stopover sites along the way, which enhances the risk of AI virus transmission, when compared to the spring migration (northwards) that involves much longer distance flights. There is thus a strong seasonality, both in the prevalence of LPAI viruses in the wild bird populations, and in the timing of the high risk period on the migration route. Three main routes for Palaearctic migrants from Siberia to Africa have been described viz. the Black Sea/Mediterranean, West Asian/East African, and East Atlantic flyways (Appendix 3). In the northern hemisphere, these flyways

intersect with the Central Asian flyway and the East Asian/Australasian flyway. The possible persistence of AI viruses in cold water (Stallknecht *et al.* 1990) in the Arctic region and the transmission within populations during pre-migration concentrations also contributes to the large-scale redistribution of AI viruses along the different migration paths. Based on the sequence data presented in this study, it seems likely that the East Atlantic flyway is at least one of the major routes used by the specie(s) that carries LPAI into South Africa on southbound migration. This flyway utilizes Lake Chany, one of the largest freshwater lakes in Russia. From there, migrants move through northern Europe, via important stopover sites like the Wadden Sea. The Wadden Sea ranks among the most important wetlands to migratory waterbirds in the world. Its vast area of intertidal mudflats hosts numerous bird species breeding in the tundra from arctic Canada in the west to northern Siberia in the east. This may also be the ecosystem where the North American lineage H5 gene described in Chapter Three was introduced into the gene pool. From there the journey continues down the West African coast, where birds would fly for one week at a time and stop for about two to rest and refuel before continuing (Les Underhill, personal communication). Birds start to arrive in South Africa around August and September, and most have arrived by November. At the end of summer they start preparing to depart on the spring migration northwards, that usually starts around April. Alternatively, some populations may make use of the Black Sea/Mediterranean flyway and the West Asian/East African flyway, moving southwards through Eastern Europe and down the fertile African Rift Valley (Underhill *et al.*, 1999). At some point in their lifecycle in the overwintering grounds of southern Africa, pelagic migrants shed viruses into the environment where they are ingested by and infect sympatric species, since LPAI has been isolated on several occasions from wild waterfowl in South Africa.

In Chapters Two and Three, molecular data for the first time provided conclusive evidence that wild ducks are linked with ostrich influenza outbreaks. The interaction between wild ducks and ostriches on the winter lucerne grazing pastures of the Klein Karoo is well-documented but the conversion of an LPAI virus originating from wild ducks to an HPAI virus in ostriches in 2004 was a seemingly rare event. Consequently, biosecurity awareness among Cape Ostrich farmers led to changes in farm management practises to minimise the contact between free-ranging ostriches and wild waterfowl, by fencing off rivers on ostrich farms, preventing contact with wild birds at ostrich feed points and by regular disinfection

of feed and water troughs (Adriaan Olivier, personal communication). Hopefully these biosecurity measures will help to reduce the risks.

Very little is known about the length of time that ostriches are able to maintain LPAI viruses. The results of Chapter Two suggested that ostriches possibly act as mixing vessels for LPAI strains and as a reservoir pose a threat to the chicken-producing industry when biosecurity breaks down. LPAI H6N2 still seems to be circulating in South Africa, based on PCR-detection of the H6 gene in ostriches, doves and NDV-infected chickens in four provinces, but interestingly, preliminary phylogenetic evidence suggests that this H6 gene is identical to the original H6 gene from the 1998 ostrich virus (A/Ostrich/South Africa/KK98/98) instead of that from the 2002 H6N2 chicken viruses (Abolnik *et al.*, Avian Diseases, in press). However, the NA type was confirmed to be N2 by NI tests performed at Allerton Provincial Veterinary laboratory. These findings (not presented in this work) strongly suggest that the progenitor H6N2 virus to sub-lineages I and II is still circulating in ostriches and/or poultry.

South Africa therefore seems to be at the end of a “migratory funnel” for pelagic shorebirds, where birds from a wide geographic distribution and vast wetland ecosystems eventually converge at much smaller water bodies in South Africa. Why then are outbreaks caused by LPAI and HPAI viruses such relatively rare events in South Africa? I propose that a combination of the following factors might play a major role:

**i. The primary vectors of AI viruses to South Africa (Charadriiformes) are not the major hosts**

Anatidae harbour the highest diversity and prevalence of avian influenza viruses (EFSA 2005; Stallknecht and Shane 1988) and historical outbreaks of HPAI in poultry have been linked to LPAI strains circulating in ducks (Campitelli *et al.* 2004; Munster *et al.* 2005). Domestic ducks have been shown to be able to excrete large amount of H5N1 virus while remaining relatively healthy (Hulse-Post *et al.* 2005), and direct contacts especially between wild Anatidae and domestic wildfowl are believed to be more common than with other wild bird groups. None of the Anatidae from Europe and Asia migrate as far South as southern Africa (Underhill *et al.*, 1999).

## **ii. Ostriches, seemingly the link between wild birds and domestic poultry in South Africa, are not ideal hosts**

Ostriches are generally not considered to be highly-susceptible to AIV. Clinical disease and mortality usually seen where isolations of LPAI have been made were usually associated with concomitant infection with other pathogens, high population densities, inadequate ventilation and bad hygiene resulting from poor management practises. Green urine syndrome has not been conclusively or exclusively tied to AIV infection (Adriaan Olivier, personal communication). Transmissibility of AIV between ostriches also seems to be poor as they have to be in very close contact, and a higher viral load may be required to infect an ostrich than other smaller poultry species. Furthermore, the IVPI of the HPAI H5N2 virus from 2004 was initially low for chickens, and only increased with serial passage in chickens (Manvell *et al.*, 2005) which suggests that virulence determinants in ostriches do not evolve as they do in chickens. The results of Chapter Two also suggest that the evolutionary rates of AI viruses are slower in ostriches than in other poultry.

## **iii. Ostrich farming and poultry-producing areas are geographically separated**

Appendix 1 illustrates the distribution of poultry production in South Africa. Ostriches thrive in drier climates thus the main ostrich-producing areas are concentrated in the arid regions of the Cape Provinces. The Klein Karoo has low (288 mm per year) and unreliable precipitation that falls mainly in the winter months. It is bordered by the Outeniqua, Langeberg and Swartberg mountain ranges and is thus geographically separated from the major chicken-producing industries that are concentrated in KwaZulu/Natal, Gauteng and North-West provinces and to a lesser extent Mpumahlanga, Orange Free State and Western Cape provinces. As a result, there are not many opportunities for ostriches and chickens to mix.

## **iv. Consumer preference for chicken over duck meat in South Africa**

Consumer demand for duck meat in South Africa is not as large as in other countries, and chicken remains the poultry staple. The raising of ducks by subsistence farmers is

uncommon, probably because the scarcity of water in the country. This is in comparison to the large formal and informal scale of domestic duck farming in other regions, such as Europe and East Asia. Recent evidence showed a strong association between free-grazing ducks in rice paddies, and incidence of HPAI H5N1 in Thailand (Gilbert *et al.*, 2006). Therefore, an important link in the ecology of HPAI viruses, viz. intensively and widespread farmed domestic ducks is absent in South Africa.

#### **v. Lack of live bird market systems and integrated farming practises**

Unlike the developing countries in Southeast Asia and the rest of Africa, live bird markets are limited in South Africa and most poultry is kept as backyard flocks. Integrated farming is not practised at the scale as in Asian countries. The cull-buyer industry, that basically trades spent hens of the backs of open trucks into townships and rural areas could be one of the factors that limit the number of large live bird markets in SA.

#### **vi. Climate**

Generally, South Africa has far less surface water than countries in the northern hemisphere, and therefore wild waterfowl congregate at smaller, fewer water bodies distributed across the country. It was recently demonstrated that in chicken manure (with a pH of 8.23 and 17.7% moisture), HPAI H5N1 lost infectivity within 24 hours at 25°C and within 15 minutes at 40°C (Chumpolbanchorn *et al.*, 2006). A virus in waterfowl faeces will probably not remain viable for very long on a hot and dry Karoo day, where surface temperatures can reach the upper-thirties in summer, but this could also explain why AIV outbreaks occur mainly in the wintertime in ostriches, as cool wet winter weather allows viruses to survive for longer in the environment, and immuno-suppressed, hungry ostriches are more susceptible to microbial infections. In South Africa (and Australia), the highest UV radiation values in the world are attributed to a thinner ozone layer and less cloud coverage. A combination of all these factors would possibly decrease the viral load in the environment, and further reduce the availability of an infectious load that is required to infect ostriches.

However, despite the supposedly rare introduction of a notifiable strain with pathogenic potential into the ostriches, the 2004 HPAI H5N2 outbreak in ostriches had devastating economic consequences. A total of 7232 ostriches or 32.2% of the population on the five infected farms died between June and mid August 2004, and 26 000 Eastern Cape Ostriches were culled to curb the spread of the infection. This, coupled with ban on the export of ostrich meat from South Africa that lasted thirteen months, caused a financial loss of R600 million (~\$86 mil) and 4000 jobs losses within the industry (Anton Kruger, South African Ostrich Business Chamber).

It is accepted that migratory birds played a role in moving HPAI H5N1 from eastern Asia into Europe, and this has emphasised the fact that no country on the migration paths of wild waterfowl can consider themselves to be free of the disease or free from the risk of introduction. But what about Newcastle disease in South Africa? Is it really enzootic? According to the definition on page 1, an enzootic disease should be *peculiar to* or *constantly present* in a locality. Since the 1970s, the government, poultry industry, and international community have believed that ND is enzootic in South Africa, based on the perception that it is more or less constantly present in the region (disappearing on average every four years to some unknown reservoir before sporadically reappearing). However, if ND was *truly* enzootic then surely a unique South African strain or lineage should exist that has persisted here for decades?

In Chapters Four, Five and Six I explored the molecular epidemiology of avian paramyxoviruses isolated in South Africa since the early 1990s. I demonstrated that distinct genetic lineages of virulent ND viruses were periodically introduced into South Africa, completely replacing their predecessors in each case, and that they were always prevalent in other geographic regions first. In two cases during the current outbreak of goose paramyxovirus (lineage 5), and genotype VIII of the 1970s and 1980s, the Far East was the recent and direct source of infection. However, in the case of the panzootic strain of the 1990s (VIIb) and pigeon paramyxoviruses (on at least two recent occasions), the infections appear to have been European in origin (UK, Spain and Portugal). Therefore, introduction of ND into South Africa appears to occur via a variety of mechanisms. These could be:

### **i. Illegal importation of exotic birds and poultry products**

All birds entering South Africa legally are quarantined and tested at state laboratories and must be accompanied by a veterinary health certificate from the country of origin, proving freedom of infection from NDV (and AIV). Unfortunately, anecdotal evidence suggests that exotic birds such as parrots and racing pigeons illegally enter South Africa from time to time. Cock-fighting, particularly with birds brought from East Asia, or belonging to ship crew, is another illegal activity that poses a risk for the introduction of avian diseases. A significant source of infection could be via the approval of importation of frozen chickens from infected countries by corrupt officials. A recent example involved the importation of frozen chickens from China (personal communication, Kevin Lovell, South African Poultry Association)

### **ii. Contaminated harbour swill**

By law, swill off international ships must be destroyed, but cases of illegal swill dumping are still reported in South Africa. Humans, wild birds and animals feeding off or handling contaminated ship galley waste are potential vectors for virulent strains, particularly from the Far East in the case of NDV. However, ND outbreaks in South Africa have either started in Durban or Hartebeesport (Gauteng province), but never from other major harbours such as Richards Bay, Port Elizabeth or Cape Town. Infected swill has definitely played a role in the introduction of infectious diseases into South Africa before. In 2000, Foot and Mouth disease was introduced into KZN with contaminated swill (Brückner *et al.*, 2002), and Classical Swine Fever was probably also introduced into the Western Cape in 2005 by illegal swill feeding of pigs (Truuske Gerdes, personal communication).

### **iii. Potential introduction by wild migratory birds**

Paramyxoviruses isolated from wild birds are usually categorised within the highly divergent lineage 1 (Aldous *et al.*, 2003). However, some birds have also been able to carry velogenic strains without showing clinical symptoms. Some examples include the isolation of five virulent NDVs with ICPIs ranging from 1.68 to 1.72 from Semipalmated Sandpipers (*Calidris pusilla*) and Ruddy Turnstones (*Arenaria*



*interpres*) in Brazil from April to May 2004. The latter shorebird specie is a frequent Palaearctic visitor to South Africa (Appendix 3). In another case, a lineage 5b (VIIb) virus, *Sterna/Astrakhan/Z275/2001*, was isolated from a shorebird in the Volga River Delta (Usachev *et al.*, 2006). At the time of writing the sequence was not available for comparison with South African strains. Therefore, like LPAI, virulent NDVs may be brought to South Africa periodically by migratory shorebirds.

KZN seems to be the hot spot for NDV outbreaks. The commercial poultry-producing region in KZN lies adjacent to the “Valley of 1000 Hills”, a remote area with poor infrastructure that is well-populated by Zulu villages, most of which house backyard chicken flocks. Detection of ND in such regions relies on reporting. Although indigenous village chickens have been suggested to be the asymptomatic reservoirs of velogenic NDV, there is very little evidence to support this, especially since they are unvaccinated and highly susceptible to velogenic NDV strains. I suspect that there is no true asymptomatic reservoir for NDV in South Africa, but that outbreak strains have either (a) circulated unreported in Zulu chickens and somehow avoided sampling in serological surveys (village chickens are essentially wild and almost impossible to catch, and there are thousands of villages in rural KZN), and/or (b) been present in low levels in vaccinated spent layers bought off cull-buyers or immune survivors of outbreaks (a small percentage of birds do survive outbreaks of velogenic NDV).

Whatever the initial source of infection is, once the disease is introduced into commercial poultry it does not remain localised for long. I was able to demonstrate by mapping of specific genetic variants, that outbreaks were sustained for years (for example throughout the 1990s in the case of genotype VIIb) via road transportation of infected poultry and/or their products. This is facilitated firstly by the large commercial producers themselves, who transport hatching eggs and day-old chicks between their operations, and secondly by the cull-buyers.

The spent-hen industry in South Africa is large and lucrative. Broilers (table chickens) do not play a large role in this industry, because their lot is eventually the abattoir. In contrast, commercial layers have a commercial lifespan of about 70 weeks, and broiler/breeders of about 65 weeks. After their peak productivity has passed commercial farmers sell them to cull buyers (a.k.a spent hen vendors). In Europe and elsewhere, commercial chickens have

almost no resale value after their usefulness has expired, however, in South Africa, a bird that was initially bought for R25 (~\$3.50) can be sold for R17 (~\$2.40), allowing the farmer to recoup some of his expenses. Therefore, in South Africa a layer hen still has a value at the end of her production life, and this makes the layer market very competitive, as about 16 million layer hens are in production in South Africa at any given time.

Cull buyers pick up the culls in large batches of thousands of birds with trucks, and transport them to central depots. Some large commercial operations even have their own cull depots, and in this way, birds from different farms are brought together. From the depot, fowl (perhaps several hundred or less) are sold to smaller buyers, who then supply birds to informal markets, including townships and rural areas. Where the market is good, large cull buyers will often travel over long distances, for example, between Gauteng and the Western Cape provinces, or between Gauteng and KZN. Therefore, culls that originate in the Cape can very easily, and are commonly, sold in KZN. KZN seems to be a net buyer of cull birds. This is apparently due to the demand for these birds by Indian and black buyers. Not only are they less expensive, at R17 per bird, but older chickens are coveted because they apparently make better curry. The cull buyer industry is legal, although largely unregulated and uncontrolled. Most large commercial farms conduct exit bleeds to ascertain the disease status in the birds, but this is mostly surveillance for AI and not NDV. Furthermore, these tests are performed by contracted laboratories and veterinary services and not state facilities. The South African Poultry Association (SAPA) recognised the role that cull buyers play in the dissemination of NDV in South Africa some time ago, and attempted to control and regulate the industry, without success. The main problem is that the South African poultry industry allows the cull buyer industry to exist, because it's lucrative (S Bisschop, personal communication).

Molecular epidemiology applied for the first time in South Africa to assess the status of NDV has shown that NDV in South Africa should be considered to be *exotic* and not *enzootic*, and this finding may have important implications for the industry. It is clear that if better control is enforced, particularly in the regulation of the cull buyer industry, South Africa may be able to regain its NDV-free status. This should be done at government-level, possibly by restricting the movements of poultry to those that have been tested and issued with a movement permit by the Directorate of Animal Health. Furthermore, better control

can be enforced during periods of outbreak by restricting the movement of poultry between provinces or infected areas. It is ironic that the formal poultry industry, that is most heavily impacted by ND outbreaks and the resultant trade restrictions, is the main contributor to the problem by moving infected birds and their products across the country and by selling off vaccinated spent hens that perpetuate and spread the infection in rural areas.

### **Current, ongoing and future research**

The genetic link between South African and Eurasian AI and ND viruses raises many questions. One of our immediate needs is to conclusively prove that pelagic shorebirds are indeed the vectors of these viruses to South Africa, to determine which species or populations are involved, and exactly which routes they follow. As a spin-off of this work a national surveillance project funded by the DoA was initiated at OVI to monitor the presence of AIVs and APMV-1 in wild waterfowl. In this program, faecal samples from migrants are tested as birds arrive in the spring and throughout summer, and in winter, the indigenous wild ducks and geese are sampled as indicators of which viruses were recently introduced and which are still circulating. It is hoped that by monitoring our wild bird species that they will act as an early warning system for the entry of HPAI H5N1 and other notifiable AIVs into South Africa. Data of the prevalence and distributions of AIVs will also be used in the generation of prediction and risk models in collaboration with other institutes. It is also unknown whether the shorebird and wild duck reservoirs shed constantly or intermittently, and in the latter case which factors trigger shedding. Physiological stressors may be involved. For example, migration restlessness (triggered by changes in photoperiodism) was experimentally linked to the shedding in migratory birds of a spirochete that causes Lyme disease (Gylfe *et al.*, 2000). This information will be critical for the design of effective future surveillance programs. Since the incursion of HPAI H5N1 into Europe, it has become vital to monitor the Russian and northern European waterfowl populations of the wetlands that South African shorebirds are likely to use as stopover sites during their southbound migrations. The data presented here predicts that once HPAI H5N1 becomes established in those shorebird populations, that it's likely to reach South Africa within a year or two. Of course, the risk of introduction of HPAI H5N1 via illegal poultry or exotic bird importation from one of the African or other states is also a cause for concern.

Characterisation of the 2006 HPAI H5N2 Western Cape ostrich outbreak virus is underway, and preliminary results indicate that the virus is not directly linked to the 2004 Eastern Cape outbreak strain, since the 2006 strain was produced by multiple reassortments with wild duck viruses, and furthermore contains three amino acids less at H<sub>0</sub>. A probable LPAI H5N2 precursor was isolated from a Western Cape ostrich farm. Many unanswered questions surround the interaction between AI viruses and the ostrich host, and this is a research area in which South Africa is well-positioned to take the lead. For example, what is the biological link between green urine and AIV infection (if any)? Why are ostriches less susceptible to HPAI strains? And most importantly, what biological factors are involved in the conversion of LPAI to HPAI in ostriches, again highlighted by recent events in the Western Cape. A reverse genetics system recently developed at OVI will assist us in answering this question, and others related to the molecular determinants of virulence of AIV, for example what contribution does the NS1 deletion in the 2004 HPAI H5N2 virus make to the pathogenicity of the virus for ostriches? Reverse genetics technology will furthermore enable us to genetically alter local strains for enhanced growth and reduced pathogenicity for use as inactivated vaccines.

It is likely that HPAI H5N1 will continue to spread and cause periodic outbreaks, similar to H9N2 in the 1990s, and it is evident that when uncontrolled, ND is able to spread rapidly within months throughout the whole of South Africa through the movement of infected poultry. Using NDV as a model for the spread of HPAI H5N1 in chickens is clear that we could face a humanitarian disaster of epic proportions if biosecurity were to break down. The largest densities of commercial poultry producers are clustered around heavily-populated areas in Gauteng and KZN, and both provinces are home to high incidences of HIV-positive immune-compromised individuals. With a mortality rate of slightly above 50% in relatively healthy people, the effect of HPAI on an immune-compromised population could be disastrous. Only if biosecurity and control of avian diseases in South Africa is drastically improved in a partnership between government and private industry, can future disasters be averted.