WILDLIFE AND EXOTIC DISEASE PREPAREDNESS PROGRAM

Australian Government

Department of Agriculture, Fisheries and Forestry

REPORT TO THE DEPARTMENT OF AGRICULTURE, FISHERIES AND FORESTRY

Targeting surveillance for avian influenza in wild birds: a pilot investigation in New South Wales

John Tracey

VERTEBRATE PEST RESEARCH UNIT
NSW DEPARTMENT OF PRIMARY INDUSTRIES

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1. PROJECT OUTLINE

1.1 PROJECT INFORMATION

1.1.1 Project Name

Targeting surveillance for avian influenza in wild birds: a pilot investigation in NSW

1.1.2 Details of Applicant

(a) Organisation Details

Vertebrate Pest Research Unit (VPRU),
NSW Department of Primary Industries
Orange Agricultural Institute, Forest Rd., Orange, NSW 2800

(b) Project Managers

	John Tracey	Glen Saunders
Tel:	0263913952	0263913980
Fax:	0263913972	0263913972
Email:	john.tracey@dpi.nsw.gov.au	glen.saunders@dpi.nsw.gov.au

(c) Collaborators

Franz Zikesch	NSW Department of Primary Industries (NSW DPI)
Peter West	NSW DPI Orange
Steven McLeod	NSW DPI Orange
George Arzey	NSW DPI Camden
Peter Kirkland	NSW DPI Camden
Simone Warner	Primary Industries Research Victoria
David Roshier	Charles Sturt University Albury
Rupert Woods	Australian Wildlife Health Network
Chris Bunn	Department of Agriculture Fisheries and Forestry

1.1.3 Period of Project

Commencement date: 1 / July / 2004   Completion date: 31 / August / 2005

1.1.4 Project Aim

To investigate a method for detecting avian influenza in wild bird populations using information on: the species most likely to carry the virus; their relative occurrence; seasonal fluctuations of virus prevalence and their proximity to commercial poultry operations.

1.1.5 Project Objectives

1. Examine the role of wild birds in the transmission of avian influenza in an Australian context. (Chapter 2)
2. Identify the species that are most likely to transmit avian influenza. (Section 2.2, 3.1)
3. Identify the optimal timing for sampling with consideration of predicted trends in avian influenza occurrence in wild birds based on previous sampling in Australia and overseas. (Section 2.2, 3.1)
4. Identify the priority areas for the surveillance of avian influenza in wild birds in New South Wales (Section 3.2) by:
   a. mapping poultry establishments according to the size of operation;
   b. mapping the abundance of bird species identified with highest prevalence of the virus using Birds Australia data; and
   c. identifying regions and localities where high numbers of Anseriformes occur in close proximity to poultry.
5. Review protocols for field sampling for detecting avian influenza in wild birds in NSW, including techniques for the capture, handling, euthanasia and sampling regimes. (Section 3.3)

1.1.6 Acknowledgements

1.1.7 Summary

- Highly pathogenic avian influenza (HPAI) has the potential for very serious and rapid spread, which is of serious socio-economic and public health concern, and is of importance in the international trade of domestic poultry. Low pathogenic avian influenza (LPAI) virus can multiply readily in avian species and some subtypes (H5

and H7) have a propensity to mutate into HPAI and produce severe epizootics in poultry. The reservoir for all avian influenza virus haemagglutinin (H) and neuraminidase (N) subtypes is waterbirds, particularly Anseriformes.

- Due to the natural low prevalence of AI virus in wild birds in Australia broad-scale surveillance of wild birds is logistically difficult and cost prohibitive. Specifically directing sampling of wild birds according to: the species that are most likely to carry the virus, their relative abundance, seasonal fluctuations in virus prevalence, and at locations where these species have the greatest risk of interacting with poultry has the potential to substantially increase sampling efficiency.

- To evaluate the risks associated with the transfer of LPAI to poultry in New South Wales surveillance of Anseriformes is recommended within designated wetlands, with priority given to the major wetlands in Central Coast and Sydney basin. Sampling is likely to be most efficient between December and February, based on information of breeding behaviour and seasonal patterns of virus prevalence.

- To evaluate the risks of wild birds introducing foreign subtypes of avian influenza surveillance of Anseriformes is recommended on the Central and South Coast regions and in other wetlands of importance for Charadriiformes. For this purpose sampling between August and October is recommended to coincide with the arrival of Charadriiformes in Australia.

- Future priorities for research include; targeted surveillance of Anseriformes at priority areas, investigating suitable field sampling techniques, improved predictions of waterbird density and movements and how these influence virus transfer, and further information on virus subtypes present in other bird species.

2. BACKGROUND

Although information is available on the relative abundance of wild birds and poultry establishments, and several extensive sampling programs for avian influenza have been conducted in Australia there remains considerable uncertainty as to the role of wild birds in the transmission of avian influenza to poultry (Bunn 2004; Turner 2004; Tracey *et al.* 2004; Arzey 2004a; Arzey 2004b). The risks associated with wild birds introducing H5N1 or other subtypes from overseas and their function in maintaining endemic strains are virtually impossible to quantify with current information. There is insufficient knowledge of the epidemiology and transmission of avian influenza viruses. These viruses are also highly unpredictable and have a documented propensity for mutation.

The relative occurrence of avian influenza is known to vary significantly between bird species and genera (Stallknecht and Shane 1988; Kawaoka *et al.* 1988), region (Astorga *et al.* 1994), and season (Hanson 2001). Review of strategies for the surveillance of avian influenza in wild birds in Australia is required.

2.1 AVIAN INFLUENZA

The biology and ecology of avian influenza viruses have previously been reviewed (Alexander 1993). Influenza viruses are members of the Family Orthomyxoviridae and are characterised into types A, B or C on the basis of the antigenic character of the internal nucleoprotein antigen. Avian influenza is an infectious disease of birds caused by type A strains of the influenza virus (WHO Expert Committee 1980). Only influenza A viruses have been isolated from avian species. The disease occurs worldwide and was first identified in Italy more than 100 years ago (Alexander 1987). Avian influenza viruses normally do not infect species other than birds, but have been recorded infrequently in a range of other animal species including humans (Hinshaw *et al.* 1981) (Alexander 1982; Claas *et al.* 1998; Katz 2003).

Influenza A viruses are divided into subtypes determined by haemagglutinin (H) and neuraminidase (N) antigens. At present, 16 H subtypes and 9 N subtypes have been identified. Each virus has one of each subtype in any combination. The reservoir for all avian influenza virus H and N subtypes is aquatic birds, particularly waterfowl (Suss *et al.* 1994), in which they multiply in the gastrointestinal tract producing large amounts of virus (Webster *et al.* 1978; Hinshaw *et al.* 1980) usually without producing clinical signs (Kida *et al.* 1980). In this environment, new combinations of H and N genes are generated and dispersed (Scholtissek *et al.* 1993). This process of exchanging genes between virus strains is called re-assortment within influenza viruses and occurs when single cells of the host become co-infected with two genetically different viruses (Hinshaw *et al.* 1980). In wild waterbird hosts, the H and N subunits appear to be stable, and do not mutate (Sharp *et al.* 1997) like they do when the viruses infect domestic poultry and mammals. New virus combinations multiply readily in avian species and, in chickens and turkeys a proportion have a propensity to mutate and produce severe disease which in turn produce epizootics in poultry enterprises.

Infection in birds causes a wide spectrum of symptoms, and viruses can be divided into two groups according to their pathogenicity (Office International Epizooties. 2001). Some forms of these viruses, known as highly pathogenic avian influenza (HPAI), can cause severe illness and mortality approaching 100% (Alexander 1993; Swayne and Suarez 2000). However,

most strains of the virus are non-virulent, do not produce clinical signs or cause only mild respiratory or reproductive disease. These are known as low pathogenic avian influenza (LPAI) viruses which are commonly isolated from wild birds, particularly Anseriformes (swans, ducks and geese) (Slemons and Easterday 1972; Stallknecht and Shane 1988). Highly pathogenic influenza viruses, however, are not maintained by wild bird populations, but are occasionally isolated from wild birds during outbreaks in domestic poultry (Nestorowicz *et al.* 1987). The ability of LPAI to mutate into HPAI (Perdue *et al.* 1998), particularly in poultry, and the diversity of viruses circulating in wild bird populations (Webster *et al.* 1992) emphasises the potential importance of wild birds as a primary source of infection.

Epizootics of avian influenza may occur when a HPAI virus (with either a H5 or H7 haemagglutinin) is introduced to a naïve poultry population. Severe pandemics in humans occur when a major "antigenic shift" has occurred such as when the haemagglutinin is changed in influenza viruses that infect humans. Severe disease epidemics occur when there is "drift" with significant antigenic change in the haemagglutinin gene. The presence of avian influenza viruses in wild birds thus has significance primarily for its potential to infect domestic poultry and humans, within which it can then undergo re-assortment to produce pathogenic forms (Webster *et al.* 1971; Webster *et al.* 1973). In addition, if humans are concurrently infected with both human and avian strains of influenza there is an increased risk of a new subtype emerging, which could result in the direct transmission between humans with the possibility of a pandemic (Webster 1998; Snacken *et al.* 1999; Baigent and McCauley 2003; Katz 2003).

There have been five known outbreaks of avian influenza in commercial bird flocks in Australia. Outbreaks occurred in 1976 (Turner 1976), 1985 (Barr *et al.* 1986), and 1992 (Selleck *et al.* 1997) in Victoria; 1994 in Queensland (Westbury 1998); and in 1997 in Tamworth New South Wales (Selleck *et al.* 2003). Viruses identified have all been of subtype H7 (H7N7, H7N3 and H7N4). The 2003-2004 Asian epidemic of HPAI (subtype H5N1) commenced in August 2003 and by March 2004 was confirmed in China, Cambodia, Indonesia, Japan, Laos, South Korea, Taiwan, Thailand and Vietnam. H5N1 has also caused disease and death in humans (Claas *et al.* 1998; Subbarao *et al.* 1998; Yuen *et al.* 1998) via direct avian-to-human transmission.

The potential transmission of the H5N1, and other influenza A viruses from Asia to other countries via wild birds is of concern. There are many bird species known to undertake movements between Asia and Australia; the species involved, their movement behaviour, ecology and susceptibility to disease are all of importance when assessing the risk of introducing foreign disease into Australia (Tracey *et al.* 2004).

2.2 SPECIFICITY AND SEASONALITY

There are a large number and variety of influenza viruses maintained in wild bird populations. Avian influenza viruses have been isolated from more than 88 species of wild birds from 12 orders comprising most of the major families (Stallknecht and Shane 1988; Alexander 2000). The first isolation from wild birds occurred in South Africa from Common Terns (*Sterna hirundo*) in 1961 (Becker 1966). An increase in surveillance during the late 1970s revealed ducks and geese (Anseriformes) as the main reservoir of the viruses, where prevalence exceeded 60% in some studies (Hinshaw *et al.* 1980). Overall isolation rates estimated from over 20,000 samples indicate around 15% of Anseriformes and approximately 2% of all other species are infected with the virus at any one time (Stallknecht and Shane

1988; Alexander 2000). However, many of these studies are only based on regular samples of Anseriformes and may be unrepresentative of region and species. In other studies Charadriiformes (shorebirds, plovers and lapwings) (Kawaoka *et al.* 1988) and spoonbills (Astorga *et al.* 1994) have also been found to have a high prevalence of the virus, with isolation rates of up to 20% and 32% respectively.

In Australia, prevalence of the virus is found to be lower (Mackenzie *et al.* 1984; Mackenzie *et al.* 1985; Peroulis and O'Riley 2004). In recent sampling in Victoria, Peroulis and O'Riley (2004) isolated AI virus from 1.4% (n=284) of anatids. Mackenzie *et al.* (1984, 1985) examined samples from over 8 000 birds of 67 species in Western Australia and found avian influenza in approximately 0.6% of birds. However, in some species the prevalence was higher. For example in MacKenzie *et al.*'s (1984) initial study AI virus was isolated from 5.7% (n=211) of Pacific Black Duck (*Anas superciliosa*); 4% (n=74) of Australian Shelduck (*Tadorna tadornoides*) and 2.8% (n=105) of Grey Teal (*Anas gracilis*).

Isolation rates and subtypes vary considerably over time, region and between species (Kawaoka *et al.* 1988; Sharp *et al.* 1993). This has been identified for Charadriiformes where sampling along the Atlantic coast and the Gulf of Mexico revealed 78% of isolates from Ruddy Turnstones (*Arenaria interpres*), with concentrations of the virus during one season (spring) and in one location (Delaware Bay) (Hanson 2003). Anatini tribes of Anseriformes also exhibit higher prevalence of avian influenza than other species of the same order (Stallknecht and Shane 1988). Other species normally not associated with the maintenance of avian influenza viruses are also occasionally infected (Table 1; Stallknecht and Shane 1988). This has also occurred during outbreaks of HPAI, for example, Starlings (*Sturnus vulgaris*) (Nestorowicz *et al.* 1987), ratites (Selleck *et al.* 2003) and flamingos, falcons and crows during previous epidemics in Asia.

In the northern hemisphere, seasonal infection patterns have emerged in Anseriformes, with the greatest prevalence during late autumn and winter (Sinnecker *et al.* 1982; Halvorson *et al.* 1985). This trend is consistent with the timing of outbreaks of human influenza, but differs from the spring epidemics evident in Charadriiformes (Hanson 2003). Movements and age of birds also appear to be important and correlated with seasonal effects. For example, a significantly higher prevalence of the virus was recorded for juvenile mallards before migrating south for the winter (Deibel *et al.* 1985; Hinshaw *et al.* 1985; Hinshaw *et al.* 1986). There has been insufficient sampling of wild birds in Australia to investigate seasonal fluctuations in virus prevalence.

With the exception of H7, most subtypes have been detected in Australian wild birds (H1, H3, H4, H5, H6, H11, H12, H15) (Downie and Laver 1973; Downie *et al.* 1977; Mackenzie *et al.* 1984; 1985; Nestorowicz *et al.* 1987; Rohm *et al.* 1996; Peroulis and O'Riley 2004). In Australia, circumstantial links have been made to wild birds during the five previous HPAI outbreaks in poultry. In addition Australian isolates of HPAI have been found to be distinct from those in other parts of the world including Asia (Rohm *et al.* 1995; Banks and Alexander 1997), which suggests an endemic rather than exotic source of infection. However, there is circumstantial evidence that waterfowl may have been involved in previous outbreaks; and sampling has been limited and on one occasion may have occurred too long after the epidemic (Westbury 1998; Selleck *et al.* 2003). Direct and in-direct contact with waterfowl has been reported and has been suggested as a potential cause of initial infection (Westbury 1998). During the 1997 outbreak in Tamworth New South Wales, HPAI was isolated from an adjacent Emu (*Dromaius novaehollandiae*) farm which was suggested to

have played a role in the transmission of the virus (Selleck *et al.* 2003). Some evidence also suggests the transmission of HPAI can occur between domestic poultry and passerines, following reports of the virus infection in starlings (Nestorowicz *et al.* 1987; Westbury 1998).

The unpredictability inherent in avian influenza viruses; the variation in prevalence between species (Becker 1967); and temporal and spatial variation in virus occurrence makes generalisations across families difficult. However, to allow targeted surveillance, Tracey *et al.* (2004) described the relative occurrence of avian influenza virus using four prevalence classes (Table 1). Of the 27 families known to move between Australia and Asia, avian influenza infection was suggested to: commonly occur in Anatidae; occasionally occur in Charadriidae (plovers, dotterels and lapwings), Laridae (skuas, jaegers, gulls and terns) and Scolopacidae (snipe, godwits, curlews, sandpipers, stints and phalaropes); rarely occur in Ardeidae (herons, egrets, night-herons and bitterns), Threskiornithidae (ibis and spoonbills) and Procellariidae families (petrels, shearwaters and prions); and was suggested to be extremely rare or unknown in the other 18 families.

Table 1. The relative occurrence of avian influenza in families of birds known to move between Australia and Asia

From Tracey *et al.* (2004). Families were classified according to the relative occurrence of avian influenza within species using a subjective increasing scale (Unknown, Extremely Rare, Rare, Occasional, Common), derived from information in Downie and Laver 1973 (1); Downie *et al.*1977 (2); Hanson 2003 (3); Kawaoka *et al.*1988 (4); Lipkind *et al.* 1982 (5); Mackenzie *et al.* 1984; 1985 (6); Morgan and Kelly 1990 (7); Peroulis and O'Riley 2004 (8); Romvary and Tanyi 1975 (9); Roslaya *et al.* 1974 (10); Slemons *et al.* 1973 (11); and Stallknecht and Shane 1988 (12).

Order	Family	Common Family	Relative occurrence of avian influenza	Source
Anseriformes	Anseranatidae	Magpie Geese	Unknown	6
	Anatidae	Waterfowl	Common	6, 8, 12
Procellariiformes	Procellariidae	Shearwaters/Petrels	Rare	1, 2, 6
	Hydrobatidae	Storm Petrels	Unknown	
Pelecaniformes	Phaethontidae	Tropicbirds	Unknown	
	Sulidae	Boobies/Gannets	Unknown	
	Phalacrocoracidae	Cormorants	Rare	2
	Pelecanidae	Pelicans	Unknown	
	Fregatidae	Frigatebirds	Unknown	
Ciconiiformes	Ardeidae	Herons/Bitterns	Rare	2, 10
	Threskiornithidae	Ibises	Rare	2
Gruiformes	Rallidae	Rails	Unknown	
Charadriiformes	Scolopacidae	Turnstones/Sandpipers/Phalaropes	Occasional	3, 4
	Charadriidae	Plovers	Occasional	3, 4
	Glareolidae	Pratincole	Unknown	
	Laridae	Gulls/Terns	Occasional	3, 4
Columbiformes	Columbidae	Pigeons/Doves	Extremely Rare	9
Cuculiformes	Cuculidae	Cuckoos	Unknown	
Coraciiformes	Halcyonidae	Kingfishers	Unknown	
	Meropidae	Bee-eaters	Extremely Rare	11
	Coraciidae	Dollarbird	Unknown	
Passeriformes	Meliphagidae	Honeyeaters	Unknown	
	Dicruridae	Drongoes	Unknown	
	Campephagidae	Cuckoo Shrikes	Unknown	
	Oriolidae	Orioles	Unknown	
	Sturnidae	Starlings	Extremely Rare	5, 7

2.3 ROLE OF WILD BIRDS

The role of wild animals in the introduction, maintenance and transmission of disease is largely dependent on a range of ecological factors, including the distribution and density of susceptible wild animal disease hosts. The risks associated with wild birds introducing H5N1 or other subtypes of avian influenza are virtually impossible to quantify with current information. There is insufficient knowledge of the epidemiology and transmission of avian influenza viruses and a lack of reliable information on the interchange of many birds between Asia and Australia, particularly of the Anatidae and Ardeidae. Moreover, avian influenza viruses are highly unpredictable and have a documented propensity for mutation. Review of current knowledge of bird movements and avian influenza in Australia is important for identifying the focus for future research and targeting species, timing and regions for surveillance (Tracey *et al.* 2004).

Ninety-nine bird species are known to move between Asia and Australia (Tracey *et al.* 2004). Sixty-three of these undertake frequent migration, 20 travel occasionally and 16 rarely visit. Shorebirds (Charadriiformes) regularly migrate to Asia, but are mainly aggregated along Australian coastlines and at specific inland wetlands (Figure 1). In contrast ducks and geese (Anseriformes) and other nomadic waterbirds are widely distributed but rarely move from the Australian mainland. Pelagic birds (Procellariiformes and Pelecaniformes) are annual or partial migrants, and are occasionally known to carry the virus but are rarely observed inside the continental shelf. Other migratory species of northern and north-eastern Australia, travel through the Torres Strait Islands during winter but are unlikely to carry avian influenza.
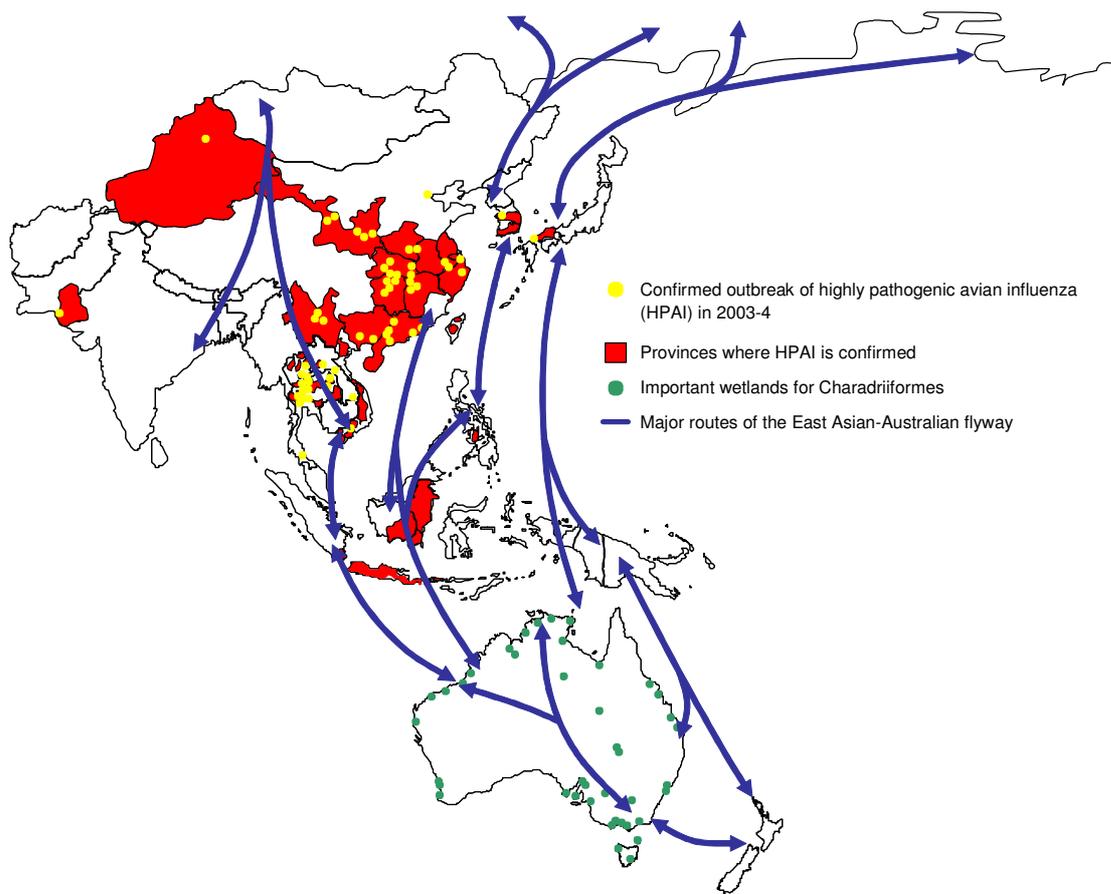
Figure 1. Major routes of the East Asian-Australian flyway in relation to the 2003-4.
(Source: Tracey *et al.* 2004; Wetlands International Oceania)

The risk of wild birds introducing foreign subtypes to Australia appears to be in the association between shorebirds, which are potentially harbouring avian influenza viruses, and Australian ducks and geese. If infected, these ducks and geese could potentially spread virus to poultry farms as they disperse from coastal areas. If affected, poultry would then have the ability to transmit virus to humans. However, to date the transmission of avian influenza from poultry to humans is rare and has only been associated with a small number of viruses, mainly of Asian origin (Horimoto and Kawaoka 2001; Baigent and McCauley 2003; Katz 2003). Although there is insufficient information to assess the risks of wild birds initiating a pandemic in Australia, the threat to human health is more likely to be a result of virus transfer via humans that have been infected elsewhere than from wild bird hosts. However, existing

subtypes in Australian wild birds also represent a risk to the poultry industry. In addition there is limited surveillance and currently no vaccine available for endemic avian influenza in poultry within Australia.

By definition, highly pathogenic avian influenza (HPAI) has the potential for very serious and rapid spread, irrespective of borders, which is of serious socio-economic and public health consequence, and is of importance in the international trade of livestock and livestock products. In Australia, current procedures for the management of incursions of HPAI within the poultry industry involve eradication. The five previous outbreaks of HPAI in the Australian poultry industry were eradicated by 'stamping out' - a procedure involving the destruction of all potentially susceptible birds. However, the destruction of wild birds is very unlikely to be effective, useful or practical in preventing the spread of the virus. Management of the virus should instead focus on ensuring wild birds do not come into contact with domestic birds, either by direct contact or by contaminated water; and by improving our understanding of the epidemiology of naturally circulating viruses in wild birds.

The Australian poultry industry is small in comparison to many other countries, including Hong Kong and China (Animal Quarantine Policy Branch 2001). The main areas within Australia for poultry production are usually sufficiently isolated from one another to provide some protection against widespread transmission of exotic disease. Where poultry (and susceptible animals) exhibit a contiguous or near-contiguous population, the risk of widespread disease transmission may increase substantially.

3. PRIORITIES FOR SURVEILLANCE IN NEW SOUTH WALES

3.1 APPROACHES TO SURVEILLANCE

To efficiently contribute to our understanding of the epidemiology of avian influenza viruses circulating in wild birds we need to carefully consider the following when planning for surveillance:
- the purpose of the surveillance program (Why),
- the target species (What),
- the timing and frequency of sampling (When),
- the location (Where)
- sample sizes (How), and
- sampling procedures (Section 3.3) and resources (How)

3.1.1 Purpose of surveillance

An improved knowledge of the epidemiology of avian influenza viruses circulating in Australian wild birds by field sampling will enable us (1) to assess the risk of endemic low pathogenic avian influenza viruses in wild birds becoming highly pathogenic through interactions with poultry; and (2) to assess the risk of wild birds introducing foreign subtypes of avian influenza. Surveillance of wild birds can be specifically targeted to address these aims.

3.1.2 Target Species

Although many species of wild birds can carry avian influenza viruses, Anatidae are considered the major reservoirs (Section 2.2). Hence targeting these species is likely to significantly improve the probability of detecting the virus (e.g. Suss *et al.* 1994). Most Australian anatids are restricted to Australasia, with irregular movements of some species through the archipelagos of south-east Asia (Tracey *et al.* 2004). In contrast there are around 3 million shorebirds (Charadriiformes: Family Scolopaciadae and Charadriidae) consisting of 35 species, which regularly migrate from Australia each year including provinces where HPAI has been confirmed during the 2003/2004 outbreaks (Tracey *et al.* 2004; Section 2.3). Hence Charadriiformes may represent the greatest risk of introducing foreign viruses. Sampling of these species is difficult due to the magnitude of migrating flocks and the expected low prevalence of the virus. Hence while these species may be contributing to the interchange of foreign viruses sampling should perhaps instead focus on Anseriformes where they interact with Charadriiformes (Figure 3).

3.1.3 Timing and Frequency

In Australia, there has been insufficient sampling in wild birds to investigate seasonal patterns in the occurrence of avian influenza. Many viruses including avian influenza (Deibel *et al.* 1985; Hinshaw *et al.* 1985; Hinshaw *et al.* 1986) can infect a higher proportion of juveniles. In Australia, breeding of Anseriformes is known to vary with available water. However during regular rainfall events peak breeding time for most anatids typically occurs between August and November. With incubation of 27-28 days, fledging occurs approximately 3 months later. Hence if age is important, a higher prevalence of the virus

would then be expected to occur during the summer months (December- February), after juveniles have fledged.

When assessing the risks of wild birds introducing foreign subtypes the optimal time for sampling would occur when shorebirds first arrive in Australia (August- October). This time period also coincides with suggestions that higher prevalence of avian influenza for these species occurs in spring in the northern hemisphere. To investigate seasonal patterns of virus prevalence sampling should occur across all seasons. However, if resources are limited preliminary sampling could focus on the periods that have the maximum likelihood of detecting the virus to address the specific aims of the surveillance program (Section 3.1.1).

3.1.4 Location

Broad-scale surveillance is logistically difficult and cost prohibitive due to the natural low prevalence of the virus (Mackenzie *et al.* 1984; Mackenzie *et al.* 1985; Stallknecht and Shane 1988; Peroulis and O'Riley 2004). Surveillance may be more appropriate if set up where Anseriformes have a greater risk of interacting with poultry, such as around free-range poultry establishments, 'backyard' operations or where biosecurity measures are lacking. The interaction between these farms and other commercial operations is also important in understanding the persistence of avian influenza viruses and their contact with poultry. Surveillance in remote aggregations of Anseriformes in Australia may therefore be less important than where concentrations of domestic poultry occur, for example, near capital cities and key regional areas of NSW, Victoria and Queensland (Animal Quarantine Policy Branch 2001) (Figure 2; Section 3.2). As discussed, targeting Anseriformes where they share habitat with Charadriiformes may also be appropriate to detect the interchange of foreign subtypes. Focussing sampling on Anseriformes at specific locations over longer time frames is likely to provide greater benefits than one-off broad-scale surveillance programs. Priority areas for surveillance in New South Wales are identified in Section 3.2.

3.1.5 Sample sizes

An appropriate number of birds must be sampled to ensure the calculated prevalence of the virus is accurate within an investigation area and investment in surveillance is justified. Broad-scale surveillance of wild birds is logistically difficult, and collecting and processing large numbers of samples is expensive. There are various tools to assist in calculating the required number of samples to achieve a reasonable level of accuracy (Cannon and Roe 1982; Cameron and Baldock 1998; Rahme *et al.* 2000; Johnson *et al.* 2004), including a variety of on-line calculators (e.g. 'Pooled Prevalence Calculator': www.ausvet.com.au/pprev). The more information that is available on the desired level of precision, and the expected prevalence of the virus, and how this varies with a range of factors, the more accurate the estimates. More recent models allow additional parameters such as differences in prevalence between species, age and season.

A simple method to estimate the sample size required to detect disease was proposed by Cannon and Roe (1982):

$$n = \log (\text{error}) \div \log (1 - \text{disease prevalence}) \qquad (1)$$

This method uses an expected disease prevalence and assumes samples are taken from a large population (>3000). The number of samples required varies according the prevalence of the disease (Table 1).

Table 1. Number of samples required to detect avian influenza in a large population with a probability of 95% (Cannon and Roe 1982)

Prevalence (%)	50	30	25	20	15	10	5	4	3	2	1	0.5	0.1
Number of samples	5	9	11	14	19	29	59	73	98	149	299	596	2995

To minimise the number of individuals obtained to achieve an accurate estimate of disease prevalence (under- or over-sampling) the sampling rate can be adjusted when more accurate information becomes available.

Johnson *et al.* (2004) provides an approach that could prove useful for surveillance of avian influenza in Australia. In this study a Bayesian approach is used to provide evidence of disease freedom. An area is considered 'disease free' if the probability of disease is less than a specified threshold value (Johnson *et al.* 2004). However, further information is required to determine how the level of disease prevalence in wild birds influences the risks of transfer to poultry. Alternative approaches that involve increase sampling effort once virus is detected could also be attempted following methods for adaptive sampling for estimating wildlife abundance (Thompson 1991a; Thompson and Seber 1996)

Pooling samples may also improve the efficiency of surveillance programs. However, the difficulties determining individual-level prevalence and reductions in sensitivity need to be considered. There is a trade-off between the accuracy and sensitivity and the cost of the surveillance program.

3.2 IDENTIFYING PRIORITY AREAS FOR SURVEILLANCE

A geographic information system was used to identify priority areas for surveillance. Two models for surveillance were investigated: (1) to assess the risk of endemic low pathogenic avian influenza viruses in wild birds becoming highly pathogenic through interactions with poultry and (2) to assess the risk of wild birds introducing foreign subtypes of avian influenza.

3.2.1 Commercial Poultry Operations

The major centres for commercial poultry operations in New South Wales are Newcastle and the Sydney basin. Poultry farms with large numbers of birds also occur in Griffith (up to 970 000 birds), Tamworth, Lismore, Young and Canberra (Figure 2). Targeting surveillance of Anseriformes in these regions will improve our understanding of the risks associated with naturally circulating low pathogenic avian influenza and potential transfer to poultry. Biosecurity measures are fundamental to reducing the risks of virus transfer, and the type (free-range, broilers, layers) and size of the operation may also influence the risk of an outbreak of HPAI. A minimum number of poultry of around 10 000 birds and a threshold of 40-50 000 has been suggested before avian influenza viruses rapidly mutate into highly pathogenic forms (Woods *et al.* 2005).
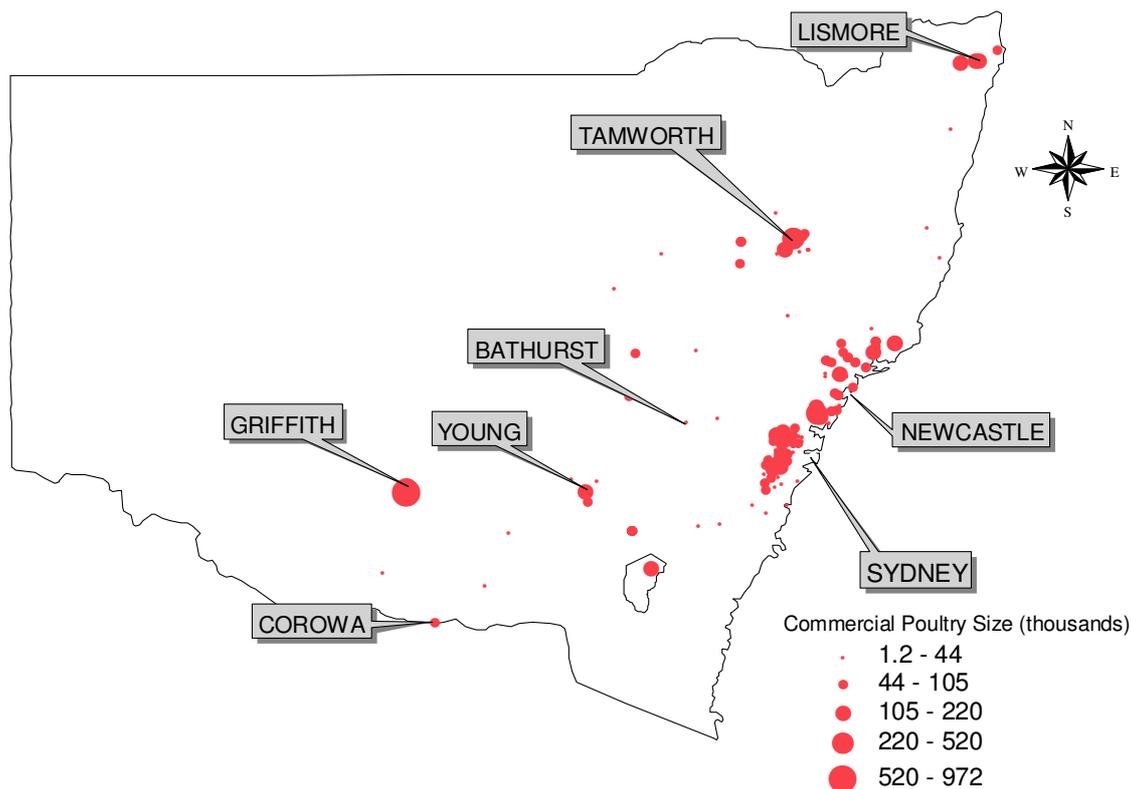
Figure 2 : Locations of commercial poultry operations in New South Wales

There are many small-scale "backyard" poultry sheds in NSW not presented in Figure 2. These sheds may play a role in the epidemiology of avian influenza as they are likely to have few biosecurity measures in place and interactions with wild birds may be higher. However there is uncertainty of the ability of a small population of poultry to initiate an outbreak of HPAI. Some researchers have suggested (Ito *et al.* 2001; Turner 2004) that extensive passaging and selection is required to generate a HPAI virus. Conversely, other studies indicate that mutation into HPAI can occur with as few as 1 or 2 passages (Arzey 2005).

3.2.2 Occurrence of Anseriformes

The Anseriformes order includes members of the Anatidae (Ducks and Geese) and Anseranatidae families (Magpie Geese). Movements of Anseriformes in Australia are less predictable than their counterparts in the northern hemisphere and many populations are nomadic (Lawler and Briggs 1991). Their movements and distribution in New South Wales, and Australia is largely determined by available water (Briggs 1992; Lawler *et al.* 1993; Kingsford 1995; Roshier *et al.* 2001; Roshier *et al.* 2002). Anseriformes are widespread and abundant in NSW (Figure 3). Populations along the east coast of NSW and the tablelands environs are typically more constant than in drier areas. However large influxes of birds occur into these regions periodically as a result of lower availability of surface water inland.

In the current investigation the reporting rate was calculated using the number of species observed of the Anseriformes order and the number of surveys conducted at 10' localities as follows:

$$\text{Reporting Rate (RR)} = \frac{\text{Number of species of Anseriformes}}{\text{Number of surveys}} \qquad (2)$$

The estimated reporting rate used here differs from the species specific reporting rate used by Birds Australia (Barrett *et al.* 2003) as multiple species of the same family can be observed on each survey, hence the values are displayed as a proportion rather than a percentage and can be greater than 1. The mean number of surveys per 10' (0.167 degree) area was 30 (S.E = 2.0, range 1-1986, n=2414).
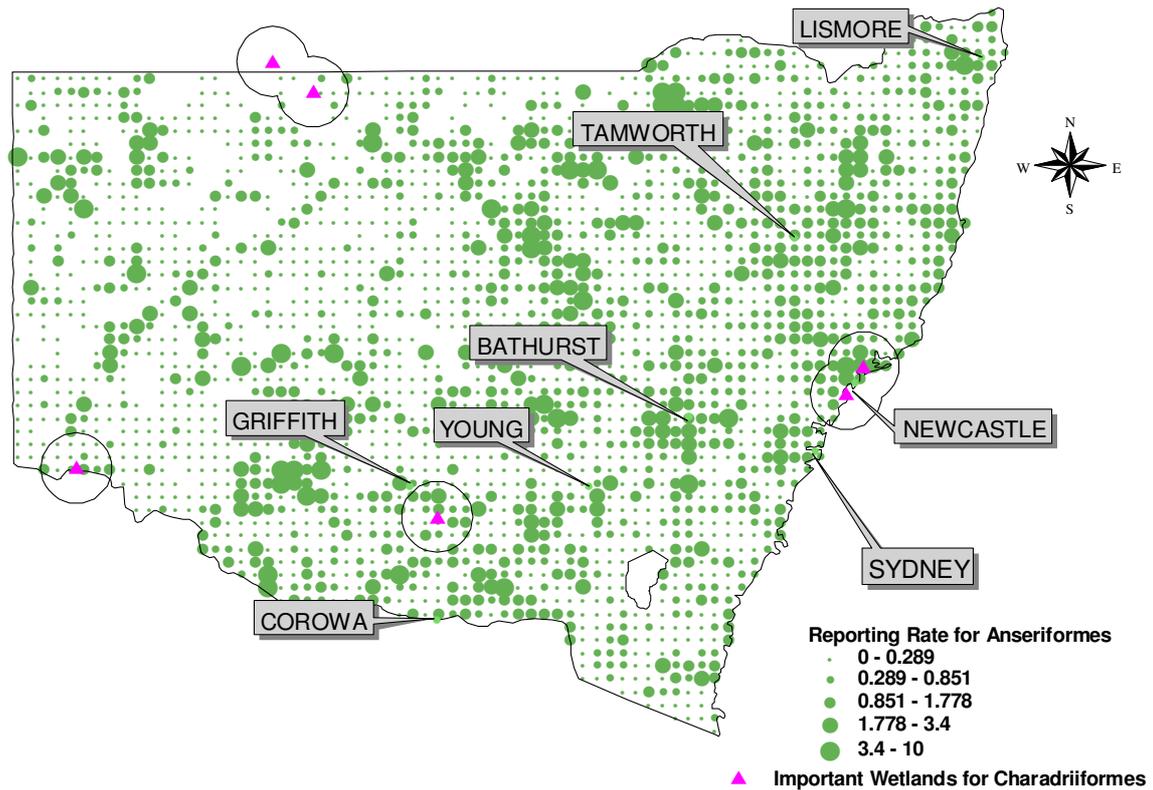
Figure 3 : The occurrence of Anseriformes and important wetlands for Charadriiformes in New South Wales. Reporting Rate was calculated using the number of species observed and the number of surveys conducted within a 10' (0.167 degree) area.

3.2.2 Identifying Wetlands for Surveillance

To identify the regions and wetlands of highest priority the reporting rate was calculated using formula (2), but included all observations within 15km of commercial poultry operations. The mean number of surveys per 15km buffer area was 774 (S.E = 393, range 4-9241, n=29).
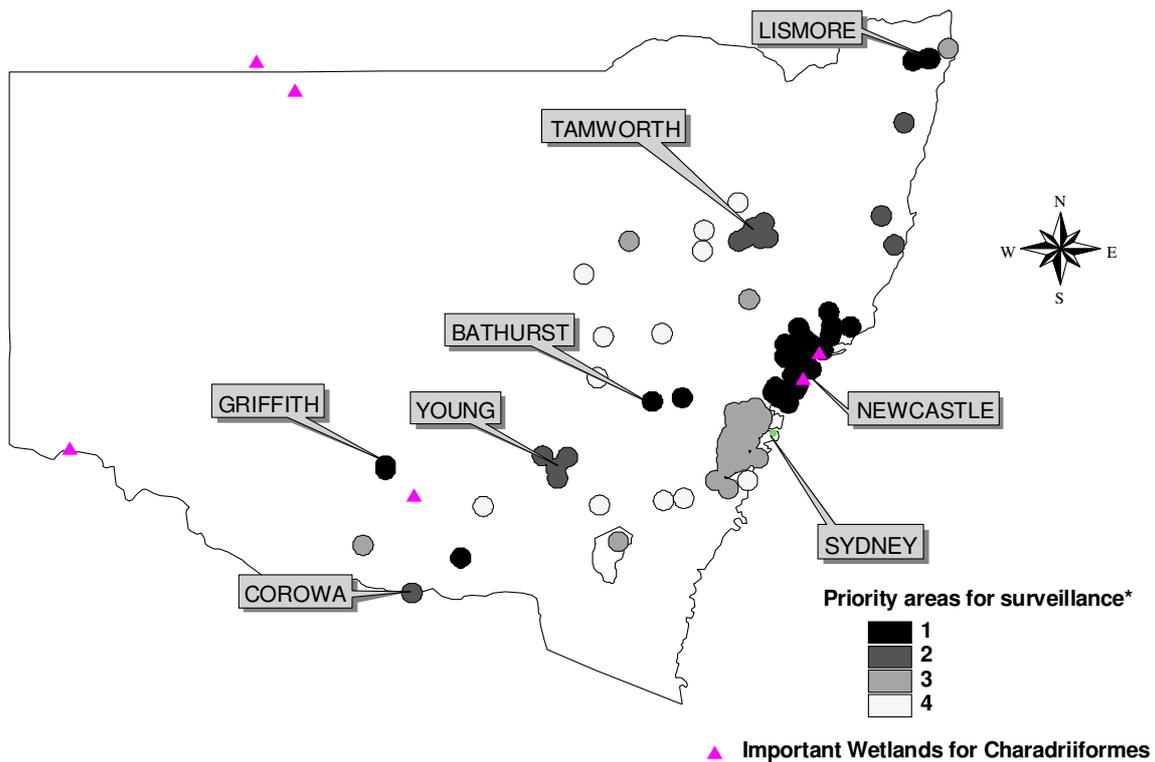
Figure 4: Priority areas for surveillance in New South Wales.

*Rankings are based on the proximity (within 15km buffer) of major wetlands to commercial poultry operations, and the occurrence of Anseriformes, where *RR* is the reporting rate for Anseriformes (average number of Anseriformes observed per survey); *n* is the number of surveys within 15km of a commercial poultry operator; and Rank 1=RR >1.25; Rank 2=RR 1 – 1.25; Rank 3=RR 0.5-1; Rank 4=RR<0.5.

Large numbers of Anseriformes are evident around Newcastle and key regional centres for commercial poultry including Tamworth, Lismore, Young, Canberra and Griffith (Figure 3). Major wetlands in these areas (Appendix 1; Figure 4) should be targeted for surveillance for addressing (1) (Section 3.1.1). On the Central Coast key wetlands including Lake Macquarie, Tuggerah Lake and Grahamstown Storage Reservoir could be targeted in the first instance (Appendix 1; Figure 5). The wetlands in this region have large numbers of Anseriformes, occur in close proximity to many commercial poultry operations and are also important sites for Charadriiformes.
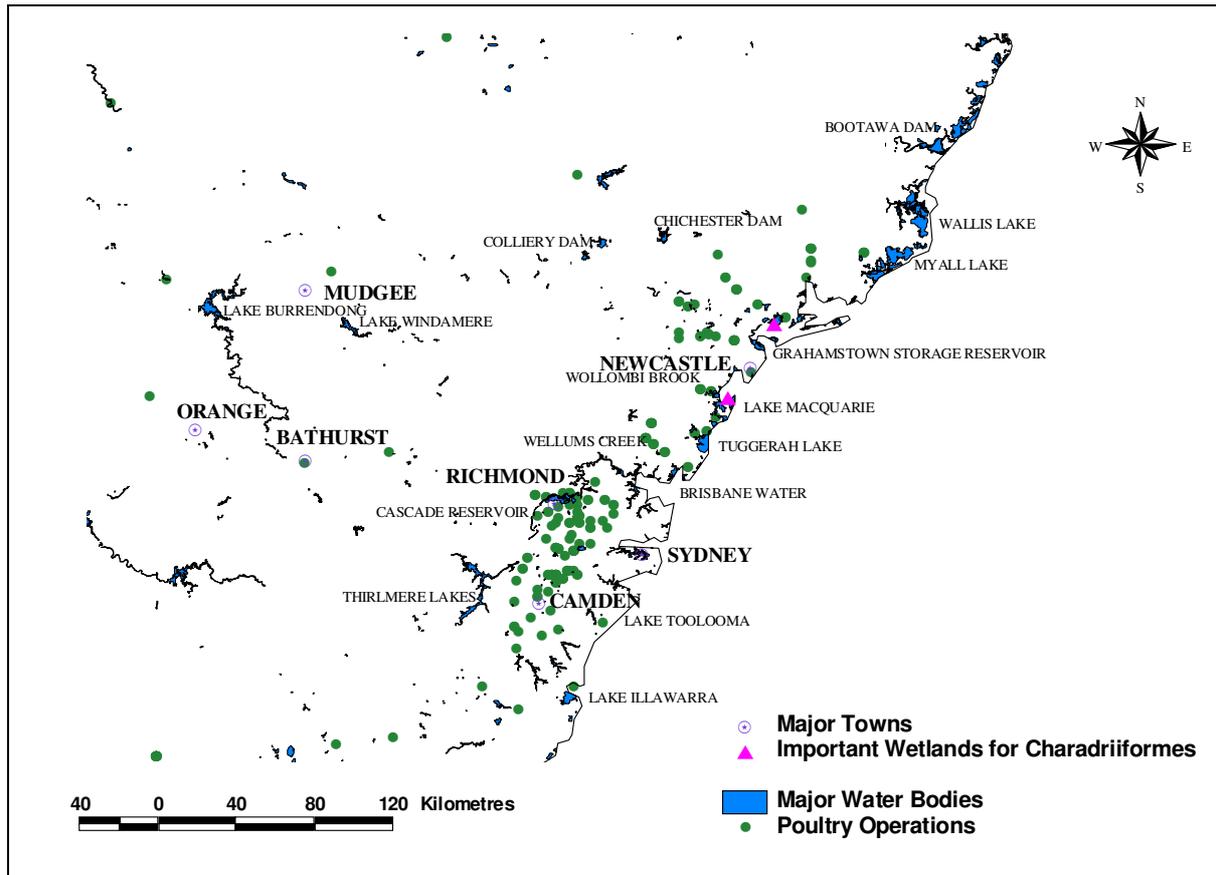
Figure 5: Major wetlands and commercial poultry operations on the Central Coast NSW

Wetland locations supplied by Geoscience Australia

3.3 FIELD SAMPLING PROTOCOLS

Three possible alternatives were identified for obtaining samples from wild birds: trapping, shooting for damage mitigation and targeted shooting.

3.3.1 Trapping

The proposed trap design has been in use for catching ducks in Australia for over 50 years (McNally and Falconer 1953). The design is a cage trap (approximately 1800 x 900 x 900 mm) with a funnel entrance (Appendix 2). Free-feeding with cereals (esp wheat) is recommended for at least 4 days prior to setting the traps. With trapping commencing after the majority (or all) of the free-feed material is consumed from inside the traps. Trapping should not be operated during rain. Traps are activated at dusk and checked early the following morning. Trapping should be targeted during peak feeding time for anatids, which varies for species, seasons and climatic conditions. Typically they are most active between first light until about 8:30am, hence trapping should be targeted during this time. Specialist duck trappers may assist during the early phase of the project to improve handling procedures, optimise trap placement and timing. Welfare guidelines for trapping (Sharp and Saunders 2004b) should be considered including:
- Approaching captured birds carefully and quietly to reduce panic, further stress and risk of injury.

- Operators should be competent in bird handling and restraint techniques. This will help to minimise harm to the birds.
- To minimise the animal welfare implications of leaving dependent nestlings and chicks to die from starvation it is preferable not to undertake trapping during the nesting season. If trapping must occur during nesting, reasonable efforts should be made to find nests containing young birds so they can be killed quickly and humanely.
- Birds caught in traps must be visually inspected for injuries and signs of illness or distress before release. Stressed birds will close their eyes and may also hunch-up their necks and maintain a stiff and unusual looking posture. A rapid heart rate, loss of feathers, change in body temperature, trembling or shaking may also be observed.
- Birds which are suffering from thermal stress should receive appropriate attention. A bird suffering from thermal stress can initially be placed in a suitable quiet holding area which provides warmth or shade to allow recovery before release.
- Birds that are unable to fly may be suffering from a slight strain to the wings. Place them on a perch in good cover and they will usually recover rapidly.
- Birds that have injuries which are untreatable or which would compromise their survival in the wild should be euthanased.
- Euthanasia of injured birds using one of the following methods:
  - *Cervical dislocation*. This involves separation of the skull and the brain from the spinal cord by pressure applied posterior to the base of the skull. The brain stem - which controls respiration and heart activity – is consequently damaged, stopping breathing and reducing blood flow to the brain, leading to death. Studies in rats have shown that electrical activity in the brain persists for around 13 seconds following cervical dislocation. This may represent a period of remaining consciousness.
  - *Inhalation of carbon dioxide*. When animals are placed into a chamber containing up to 70% CO_2 they lose consciousness very quickly due to the narcotic effect of the high intake of CO_2 on the brain without causing hypoxia. Death is caused by direct depression of CNS, respiratory and cardiac functions. One hundred percent CO_2 can cause severe dyspnoea (difficulty in breathing) and distress in conscious animals but this higher concentration is recommended for young chicks as they are more tolerant of CO_2.
  - *Injection of Barbiturate*. Act by depression of the central nervous system resulting in cardiac and respiratory arrest. Causes rapid euthanasia with minimal discomfort. The intravenous route causes the quickest death.

3.3.2 Shooting for Damage Mitigation

Shooting solely for recreation is prohibited in New South Wales. However, damage mitigation permits are provided to rice farmers who suffer damage from anatids. The NSW National Parks and Wildlife Service administers the required permits. The following animal welfare guidelines should be considered (Sharp and Saunders 2004a):
- Shooting must be conducted in a manner which maximises its effect thus causing rapid death. This requires the use of appropriate firearms and ammunition.
- Appropriate firearms and shot sizes should be used at an appropriate distance. For anatids a 12 gauge shotgun with 4's to 6's at an optimum range of 30-40 metres.
- Shooters should not shoot at a bird unless it is clearly visible and they are confident of killing it with a single shot.

- The shooter should aim to have the bird in the centre of the pattern at the point of impact.
- Only one bird should be targeted at a time. Shooting with a shotgun at a group of birds flying overhead often results in welfare problems as the birds aligned with the central cluster of pellets will usually be fatally injured, but those at the perimeter of the volley may only be hit be one or two pellets and stand a good chance of surviving. These birds are likely to experience suffering.
- Wounded birds must be located and killed as quickly and humanely as possible with either a second shot preferably directed to the head or in restrained or immobile birds, a blow to the rear of the skull to destroy the brain. If left, wounded birds can suffer from the disabling effects of the injury, from sickness due to infection of the wound, from pain created by the wound or from thirst or starvation if unable to drink or eat. Wing fractures, which increase the likelihood of being taken by a predator, are common in wounded birds.

3.3.3 Targeted Shooting

Many of the locations identified in Section 2 are outside rice growing areas where ducks are shot for damage mitigation. Trapping may also be inefficient at collecting samples from large numbers of birds. Therefore shooting by researchers or contract shooters may be required in priority areas. The same animal welfare guidelines outlined in Section 3.2 should be followed.

3.3.4 Sampling

Cloacal swabs will be taken from live captured or recently shot birds and placed in 2mls of viral transport media. For consistency the procedures for sampling in New South Wales will be the same as used by the Primary Industries Research Victoria (Appendix 3). Cloacal swabs have been found to be just as effective at detecting and isolating the virus as tracheal swabs (Simone Warner pers. comm. 2004). However, recent study of H5N1 found that these viruses replicated to higher levels in the trachea than the cloaca, and suggested that the digestive tract is not the main site of H5N1 replication in anatids (Sturm-Ramirez *et al.* 2005).

4. CONCLUSIONS AND FUTURE RESEARCH

An understanding of the ecology of the viruses within the wild bird population is essential in assessing the risks to human health and production industries. However, broad-scale surveillance is logistically difficult and cost prohibitive due to the natural low prevalence of the virus (MacKenzie *et al.* 1984; 1985; Stallknecht and Shane 1988; Peroulis and O'Riley 2004). Targeted sampling according to the species that are most likely to carry the virus, their relative abundance, seasonal fluctuations in virus prevalence, and at locations where these species have the greatest risk of interacting with poultry is likely to increase the probability of detecting avian influenza in wild birds, and also allows concentration of sampling effort where there is the greatest risk to poultry industries.

Surveillance for wildlife disease requires careful planning to ensure the desired outcomes are achieved. The aims of this study are to improve surveillance to (1) assess the risk of endemic low pathogenic avian influenza viruses in wild birds becoming highly pathogenic through interactions with poultry; and to (2) assess the risk of wild birds introducing foreign subtypes of avian influenza.

The probability of detecting avian influenza in wild birds is likely to be greatest in Anseriformes (Section 2.2, 3.1.2). Species of this order are common throughout NSW, including wetlands in close proximity to commercial poultry. The major centres for commercial poultry are Newcastle and the Sydney basin, followed by Griffith, Tamworth, Lismore, Young and Canberra.

For identifying the risks of the transfer of low pathogenic avian influenza to poultry surveillance of Anseriformes is recommended within buffer zones identified in Figure 2, with priority given to the major wetlands in Central Coast and Sydney basin. Based on information of breeding behaviour in Australia and seasonal patterns of virus prevalence overseas, sampling between December and February may have a greater probability of detecting the virus.

For identifying the risks of wild birds introducing foreign subtypes of avian influenza surveillance of Anseriformes is recommended on the Central and South Coast regions (Figure 5), and in other wetlands of importance for Charadriiformes (Figure 3). For this purpose sampling between August and October is recommended to coincide with the arrival of Charadriiformes in Australia.

Information on the ecology and epidemiology of avian influenza in wild birds in Australia is insufficient for assessing the risks for poultry industries and human health. Future priorities for research to address these deficiencies include:

- Field sampling of Anseriformes in priority areas for detecting endemic and foreign subtypes of avian influenza.

- An investigation of field sampling techniques for Anseriformes to obtain appropriate sample sizes.

- More detailed information on poultry operations, including biosecurity risk, size and type of operation (e.g. pasture, free-range shed, or fully enclosed) and an improved understanding of how these influence the transfer and persistence of avian influenza.

- An improved understanding of the relationship between waterbird occurrence and abundance and how this relates to the persistence of avian influenza in wild birds and their potential transfer to poultry. Using the number of species to assign priorities for surveillance assumes that there is increased risk with the number of species present, which may or may not be correlated with their density.

- An improved knowledge of waterbird ecology in Australia, specifically the extent of movements and the predictors of fluctuating densities. Predictions of when and where large changes in anatid populations occur are important when identifying priority areas for surveillance.

- More accurate information on the movements of nomadic waterbirds between Asia and Australia, particularly Anatidae and Ardeidae. This would aid our understanding of the importance of wild birds in introducing foreign subtypes of avian influenza as well as their potential to transmit other viruses, including West Nile virus, Japanese encephalitis and Newcastle disease.

- Further information on the subtypes of avian influenza present in other species. Note the danger of focussing surveillance on Anseriformes rather that other species is that we may be unable to detect distinct subtypes that are important in terms of risks to production industries. A significant study in North America found that Charadriiformes manifested a broader range of suptypes and suggested that these species are the leading source of some viruses including H5, which are isolated less frequently from Anseriformes (Krauss *et al.* 2004).

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# APPENDIX 1: Wetlands in NSW and priorities for surveillance

Priority rankings are based on the proximity (within 15km buffer) of major wetlands to commercial poultry operations, and the occurrence of Anseriformes, where *RR* is the reporting rate for Anseriformes (average number of Anseriformes observed per survey); *n* is the number of surveys within 15km of a commercial poultry operator; and Rank 1=RR >1.25; Rank 2=RR 1 – 1.25; Rank 3=RR 0.5-1; Rank 4=RR<0.5. Asterisk indicates the wetlands of importance to Charadriiformes.

Priority Ranking	Major Wetlands	Latitude	Longitude	n	RR
1	THISTLE SWAMP	146.97529	-35.608135	48	2.291667
1	DUDAL COMER SWAMP	146.979895	-35.53326	48	2.291667
1	GRUBBEN SWAMP	147.02736	-35.3892	48	2.291667
1	LAKE	153.051455	-28.972235	238	2.243697
1	LAKE WYANGAN	146.020105	-34.225235	149	1.765101
1	BOMBAH BROADWATER	152.265329	-32.528925	6766	1.3658
1	KARUAH RIVER	151.966176	-32.544259	6766	1.3658
1	GRAHAMSTOWN STORAGE RESERVOIR*	151.80665	-32.73811	6766	1.3658
1	MOSSMAN SWAMP	151.762215	-32.67847	6766	1.3658
1	MCCLEMENT SWAMP	151.672935	-32.694235	6766	1.3658
1	LAKE MUNMORAH	151.567416	-33.207602	6766	1.3658
1	LAKE MACQUARIE*	151.61463	-32.965762	6766	1.3658
1	TUGGERAH LAKE*	151.467471	-33.31513	6766	1.3658
1	COLLIERY DAM	151.505764	-32.866875	6766	1.3658
1	LOSTOCK DAM	151.450898	-32.333605	6766	1.3658
1	BRISBANE WATER	151.375197	-33.485006	6766	1.3658
1	MULLET CREEK	151.257784	-33.49264	6766	1.3658
1	ELLALONG LAGOON	151.289731	-32.91435	6766	1.3658
1	HUNTER RIVER	151.257986	-32.6037	6766	1.3658
1	MOONEY MOONEY CREEK	151.247296	-33.36883	6766	1.3658
1	MANGROVE CREEK	151.166013	-33.44024	6766	1.3658
1	MANGROVE CREEK DAM	151.122957	-33.191065	6766	1.3658
1	SAWYERS SWAMP CREEK DAM	150.107655	-33.38702	225	1.28
1	WALLERAWANG RESERVOIR	150.078275	-33.41649	225	1.28
2	DEEPWATER CREEK	153.057	-29.677335	377	1.164456
2	SALTWATER LAKE	152.931256	-31.32256	387	1.217054
2	CLARENCE RIVER	152.905841	-29.63955	377	1.164456
2	HASTINGS RIVER	152.788957	-31.432005	387	1.217054
2	MARIA RIVER	152.841772	-31.325545	387	1.217054
2	WILSON RIVER	152.809768	-31.32249	387	1.217054
2	ORARA RIVER	152.80288	-29.719575	377	1.164456
2	MACLEAY RIVER	152.658448	-31.017405	117	1.17094
2	LAKE	148.250915	-34.39818	121	1.016529
2	MURRAY RIVER	146.413421	-35.977898	4	1.25
2	NULLA NULLA SWAMP	146.367135	-35.95651	4	1.25
2	WALMA SWAMP	146.419375	-35.861445	4	1.25
2	CARSONS SWAMP	146.390095	-35.9088	4	1.25

Appendix 1 Cont

Priority Ranking	Major Water bodies	Latitude	Longitude	n	RR
3	LAKE AINSWORTH	153.59109	-28.783685	347	0.70317
3	CUMBEBIN SWAMP	153.603385	-28.651245	347	0.70317
3	BEROWRA CREEK	151.13355	-33.582155	7339	0.961166
3	LAKE TOOLOOMA	150.98537	-34.13882	7339	0.961166
3	LAKE GLENBAWN	150.986705	-32.06138	42	0.666667
3	LAKE WORONORA	150.946092	-34.145275	7339	0.961166
3	PROSPECT RESERVOIR	150.89085	-33.822815	7339	0.961166
3	HAWKESBURY RIVER	150.895965	-33.48821	7339	0.961166
3	COLO RIVER	150.877514	-33.436255	7339	0.961166
3	LAKE CATARACT	150.816129	-34.30783	7339	0.961166
3	NEPEAN RIVER	150.673002	-33.63857	7339	0.961166
3	LAKE NEPEAN	150.592533	-34.36335	7339	0.961166
3	FITZROY FALLS RESERVOIR	150.49663	-34.64438	7339	0.961166
3	WINGECARRIBEE RESERVOIR	150.49467	-34.560985	7339	0.961166
3	THIRLMERE LAKES	150.53913	-34.22513	7339	0.961166
3	TIMOR DAM	149.146915	-31.27841	73	0.945205
3	FOUR CORNERS LAGOON	145.827195	-35.420385	15	0.733333
3	MAIRJIMMY LAGOON	145.798945	-35.424545	15	0.733333
4	LAKE ILLAWARRA	150.832665	-34.53266	132	0.007576
4	LAKE CORDEAUX	150.781215	-34.37	132	0.007576
4	NAMOI RIVER	150.77145	-30.654645	11	0.454545
4	MANILLA RIVER	150.67801	-30.638915	11	0.454545
4	SWAMP	150.28897	-31.29766	29	0.37931
4	RESERVOIR	150.00153	-34.751565	58	0.172414
4	LAKE SOOLEY	149.67866	-34.708895	50	0.28
4	BELL RIVER	148.936355	-32.600295	41	0.487805
4	MACQUARIE RIVER	148.921378	-32.522185	41	0.487805

APPENDIX 2: TRAP DESIGN FOR ANATIDS
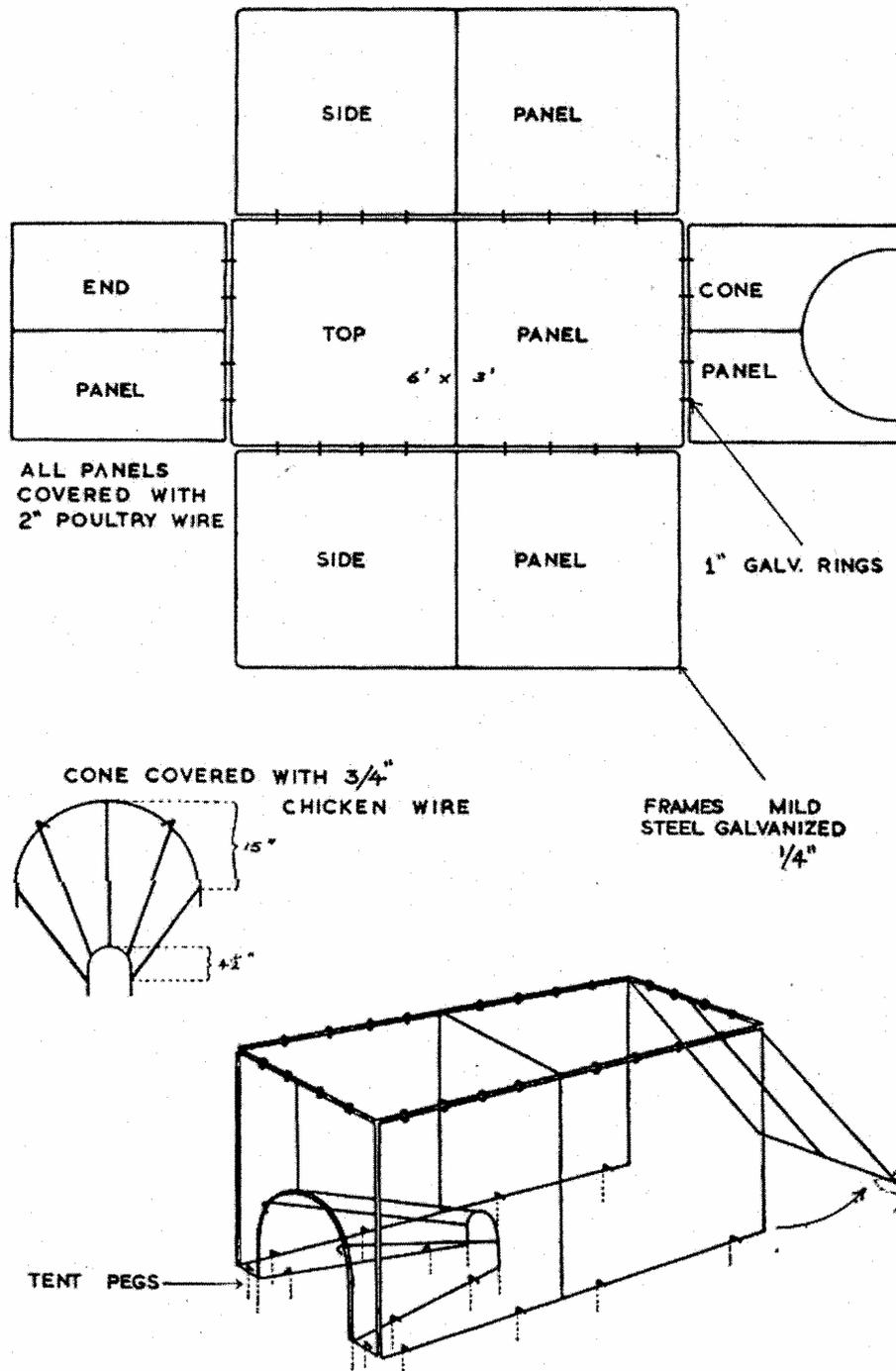
Source: McNally, J. and Falconer, D.D. (1953) Trapping and banding operations Lara Lake, 1952. *Emu* **53**: 51-70.

SAMPLING PROCEDURE FOR DUCKS

step 1 — Check contents of kit

- The relevant wildlife Research Permit is: #S11521
- Booklet with veterinary Laboratory Specimen Advice form with data tables,
- Pencil, erasor, scissors.
- 60 labelled vials with media. All in foam tray,
- 60 labelled vials for tissue samples,
- 60 sterile wooden swab sticks,
- 10 spare swabs,
- 3 pairs of surgical gloves,
- 2 press-seal plastic bags,
- 1 larger vial of ethanol for sterilizing scissors,
- Foam esky and freezer brick,
- Shreaded paper for padding vials,
- Courier voucher,
- Postage box and TNT courier bag,
- Small roll of packing tape.

VIALS WITH MEDIA (cloacal) MUST BE KEPT FROZEN UNTIL TAKEN INTO THE FIELD, BUT MEDIA WITH SAMPLES MUST NOT BE FROZEN, JUST CHILLED IN FRIDGE OR ESKY.

step 2 — Sampling Procedure

- Wear gloves during sampling.
- Start collecting from first numbered vials (e.g. A1) and sample each individual in order.
- Remove **one** swab from the sterile packaging and avoid touching cotton end.
- **Cloacal (Anus)** samples, wet the swab by dipping cotton end into an appropriately labelled tube of liquid transport medium (marked *Cloacal*).
- Insert the cotton swab until the cotton is no longer visible in the anus region of the bird, twirl and pull out.
- Place the cotton component of the swab in the appropriately labelled yellow capped tube and snap the stick so that the tube lid can be replaced (please fill data sheet as you go).
- For **Tissue** samples, cut a piece (~1cm sq.) of tissue (**foot webbing**), place in the numbered tube for the same individual, marked "*tissue*". (be sure to push tissue down into ethanol)
- Wash the scissors between each tissue cutting in the tube of pure ethanol.
- Fill in the relevant information for each **location** and each sample in the booklet provided with data tables.
- Store all collected samples at 4Deg.C. (chilled in esky with freezer brick or in fridge **NOT FREEZER**) until they reach the laboratory. Store unused collection tubes in the same manner.
- **Please ensure that the freezer brick is frozen prior to transport to the laboratory using the pre-paid mail. This will ensure that the samples remain cool during transport.**

step 3 — Fill in the Specimen Advice Form

Before sending the samples, fill in the *Veterinary Laboratory Specimen Advice* form. Please write in the spaces provided:

- The sample collector's name and Phone #
- Sign and date.
- Fill in **LOCATION TABLE** (for each different location). Property Address or GPS co-ordinates or Locality (e.g. the nearest large/small town/location name), we at least need locality
- Please write the vials used for each location,
- Fill in data sheet, circle age (if known, juvenile or adult) sex (if known) and sample type.

IMPORTANT NOTE:

- **Write the vials used for each new location in location table.**

step 4 — Packing the samples for dispatch

IMPORTANT: before packing, ensure samples are sent Monday Tuesday or Wednesday, they need to be stored in a fridge until then, and freezer brick frozen.

- Ensure tubes are placed in order in the provided foam trays.
- Place trays (**tissue vials first**) in foam esky.
- Place a freezer brick on top of cloacal samples and add shredded paper for padding (between and around).
- Enclose the *data booklet* in a seperate press-sealed plastic bag and place inside esky.
- Place used gloves in labelled press sealed plastic bag, put in esky.(also enclose scissors, instructions etc... in esky)
- The esky should then be placed in the cardboard box and secured with tape, then placed in bag.
- Follow Courier Voucher instructions and attach to bag and complete the details on the sender portion of the voucher.(TNT call 131150)

step 5 — Dispatch samples

Samples **must** be sent express within 2-3 days of sampling to (Call TNT for pickup or nearest depot):

Orange Agricultural Institute

Recievals (ATTN: Franz Zikesch VPRU)

1447, Forest Rd, Orange, NSW, 2800

NOTE: Please send Monday Tuesday or Wednesday (no later), samples can be held in fridge until then, but freezer brick must stay frozen untii day sent.
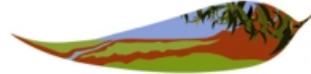
step 6 — Problems

If after reading the instructions you are unsure of any points, please contact Franz Zikesch on (02) 63913953 or Brent Waldron on: 0409 222 470 for clarification.

NSW DEPARTMENT OF PRIMARY INDUSTRIES

GAME COUNCIL
New South Wales
CONSERVATION HUNTING

Monitoring the health and movements of wild ducks in NSW

Recreational hunting and shooting for reducing crop damage provides us with an opportunity to improve our understanding of the health and behavioural ecology of wild duck populations. Samples can be easily obtained from ducks that are already shot for damage mitigation using the kits supplied. Information and all necessary equipment for sampling, postage and packaging will be provided to interested shooters.

This monitoring program is supported by the Wildlife and Exotic Disease Preparedness Program, NSW Department of Primary Industries, Department of Primary Industries Victoria and the NSW Game Council. Your contributions to this project will improve our understanding of the movements of wild ducks in Australia using genetics (DNA), and will allow us to assess the role of wild birds in introducing or maintaining viruses of potential importance to production industries. Tissue samples will be analysed using DNA extraction to identify genetic differences between sub-populations, which will provide information on the extent of movements between anatid species. Cloacal samples will be screened for detecting naturally circulating avian viruses.

Your assistance is essential for the success of this project.

For further information on this project or to obtain sampling kits please contact:

Franz Zikesch
Technical Officer (Scientific)

Brent Waldron
Game Manager

Vertebrate Pest Research Unit
Orange Agricultural Institute
1447 Forest Rd, Orange NSW 2800
Tel: 02 63913953
Fax: 02 63913972
Email: franz.zikesch@dpi.nsw.gov.au

Game Council NSW
241 Murray St.
Finley, NSW, 2713
Tel: 03 5883 1644 , Mob: 0409 222 470
Fax: 03 5883 1570
Email: gmmr@gamecouncil.nsw.gov.au