CSIRO PUBLISHING

Wildlife Research, 2010, **37**, 134–144 www.publish.csiro.au/journals/wr

Risk-based surveillance of avian influenza in Australia's wild birds

John P. Tracey

Invasive Animals Cooperative Research Centre, Vertebrate Pest Research Unit, Industry & Investment NSW, Forest Road, Orange, New South Wales 2800, Australia. Email: john.tracey@industry.nsw.gov.au

Abstract

Context. The epidemiology of avian influenza and the ecology of wild birds are inextricably linked. An understanding of both is essential in assessing and managing the risks of highly pathogenic avian influenza (HPAI).

Aims. This project investigates the abundance, movements and breeding ecology of Australia's Anseriformes in relation to the prevalence of low-pathogenicity avian influenza (LPAI) and provides risk profiles to improve the efficiency and relevance of wild-bird surveillance.

Methods. Generalised linear models and analysis of variance were used to examine the determinants of Anseriformes abundance and movements in Australia, and the observed prevalence of LPAI in Australia ($n = 33\,139$) and overseas ($n = 93\,344$). Risk profiles were developed using poultry density, estimated LPAI prevalence, the abundance of Anseriformes, and the probability of Anseriformes moving from areas of HPAI epizootics.

Key results. Analysis of Australian wild-bird surveillance data strongly supports other studies that have found the prevalence of LPAI in wild birds to be much lower (1%) in Australia than that in other countries (4.7%). LPAI prevalence was highly variable among sampling periods and locations and significantly higher in dabbling ducks than in other functional groups. Trends in Anseriformes movements, abundance and breeding are also variable, and correlated with rainfall, which could explain low prevalence and the failure to detect seasonal differences in LPAI in wild birds. Virus prevalence of faecal samples was significantly lower, whereas collecting faecal samples was 3–5 times less expensive and logistically simpler, than that of cloacal samples. Overall priority areas for on-going surveillance are provided for Australia.

Conclusions. Previous surveillance has occurred in high-priority areas, with the exception of Mareeba (North Queensland), Brisbane and Darwin, and has provided valuable information on the role of wild birds in maintaining avian influenza viruses. However, several practical considerations need to be addressed for future surveillance.

Implications. Long-term surveillance studies in wild birds in priority areas are required, which incorporate information on bird abundance, age, behaviour, breeding and movements, particularly for dabbling ducks. This is important to validate trends of LPAI prevalence, in understanding the main determinants for virus spread and persistence, and in predicting and managing future epizootics of HPAI in Australia.

Introduction

Highly pathogenic avian influenza (HPAI) has caused international concern during the past decade, particularly HPAI H5N1, because of its ability to infect and cause death in humans, its ability to cause significant mortality in wild birds (Liu *et al*. 2005), the longevity of HPAI circulation, the failure to isolate closely related precursor strains of low-pathogenicity avian influenza (LPAI) in wild birds (Mukhtar *et al*. 2007), and subsequent evolving viruses remaining highly pathogenic for poultry (Sims *et al*. 2005; Sims and Narrod 2009).

Between 1997 and 2004, mutations in HPAI H5N1 were progressively becoming more lethal to birds and mammals and hardier in the environment (Chen *et al*. 2004). Although there is still debate as to whether an independent cycle of infection of HPAI H5N1 is present in wild birds (Feare 2007; Wang *et al*. 2008), some evidence following wild-bird deaths suggests that wild birds, particularly anatids, can excrete virus without becoming ill (Hulse-Post *et al*. 2005; Gaidet *et al*. 2008; Keawcharoen *et al*. 2008) and transmission appears to occur even if the virus is difficult to detect (Stallknecht and Brown 2008).

HPAI H5N1 has now been circulating in close proximity to Australia for over 11 years and the likelihood of achieving eradication is considered low (Sims and Narrod 2009). With the exception of Antarctica, Australia remains the only continent that has not had a reported occurrence of HPAI since 1997 (Sims and Turner 2008). There has been several possible explanations for this (McCallum *et al*. 2008) including (1) enhanced biosecurity for the trade of live wild and domestic birds (2) low prevalence of LPAI H5 or H7 in Australia's wild birds (Haynes *et al*. 2009), (3) limited interchange of anatids between Australia and Asia (Tracey *et al*. 2004; McCallum *et al*. 2008), (4) few major waterbird breeding events and low waterbird abundance (Nebel *et al*. 2008), (5) low poultry-farm density (Westbury 1998; cf. Hamilton *et al*. 2009), and (6) high biosecurity of the commercial poultry industry.

An understanding of avian influenza epidemiology in wild birds is important in assessing and managing the risks of HPAI of any type. Many H and N subtypes of LPAI have been reported in Australia's wild birds, including H5 and H7 (Downie and Laver 1973; Downie *et al.* 1977; Mackenzie *et al.* 1984, 1985; Nestorowicz *et al.* 1987; Röhm *et al*. 1996; Peroulis and O'Riley

10.1071/WR09152 1035-3712/10/020134

2004; Hurt *et al*. 2006; Haynes *et al*. 2009). Anseriformes are the primary reservoir of LPAI (Stallknecht and Brown 2008; Haynes *et al*. 2009), with high prevalence associated with foraging behaviour (*Anas* species), age, breeding and movements (Hinshaw *et al*. 1985; Olsen *et al*. 2006; Haynes *et al*. 2009; Munster and Fouchier 2009). Charadriiformes are also considered potentially important (Hurt *et al*. 2006) and regularly travel through infected areas (Tracey *et al*. 2004), although unique lineages of influenza viruses in Australia compared with viruses in Europe and the Americas (Banks and Alexander 1997) suggest limited virus interchange via these species.

Although the importance of wild birds in avian influenza epidemiology is now widely accepted by the international animal health community (OIE, FAO), broad-scale surveillance is logistically difficult and costly because of the natural low prevalence. Initial surveillance in Australia followed a targeted approach to improve sampling efficiency (Tracey 2005; Warner *et al*. 2006). East *et al*. (2008*a*, 2008*b*) and Hamilton *et al*. (2009) have also applied useful approaches to classify risks of avian influenza in Australia. Since initial surveillance, there have been significant advancements in avian influenza epidemiology, particularly for HPAI H5N1; improved information on the abundance and movements of Australian waterfowl, although many gaps in knowledge remain (McCallum *et al*. 2008); and a substantial increase in wild-bird surveillance for avian influenza in Australia and internationally. This information can be used to maximise the efficiency and relevance of avian influenza wild-bird surveillance in Australia. The present project investigates trends of avian influenza in wild birds and waterfowl abundance and movements, identifies high-risk areas for avian influenza in Australia and provides recommendations for surveillance.

Materials and methods

Analysis of Australian surveillance data

Published sources of avian influenza wild-bird surveillance data were collated for Australia ($n = 33\,139$ wild birds: Downie and Laver 1973; Downie *et al*. 1977; Mackenzie *et al*. 1984, 1985; Peroulis and O'Riley 2004; Hurt *et al*. 2006; Haynes *et al*. 2009) and overseas ($n = 93\,344$ after Olsen *et al*. (2006)). Generalised linear models and analysis of variance were conducted using Asreml in R (Gilmour *et al*. 2002) to investigate the effects of the functional group (after Roshier *et al*. 2002), the availability of permanent water (km^2 per 1/4° grid from Geoscience Australia and National Water Commission data on rivers, dams and inland water), season and seasonal rainfall classification (a Bureau of Meteorology classification, identifying summer-dominant, summer, uniform, winter, winter-dominant, or arid rainfall) on Anseriformes abundance (log-transformed to remove heterogeneity of variance), movements (using bird banding data) and LPAI prevalence. Differences between terms for discrete variables are presented with 95% confidence intervals.

Comparison of sample methods: field trial

The estimates of prevalence of LPAI from cloacal, oropharyngeal and faecal samples were compared with quantitative real-time reverse transcriptase PCR (qRT–PCR) in a field trial in New South Wales where all three samples were collected from the same species at the same locations and sampling periods ($n = 3242$ samples from 2683 wild birds). These data are part of a larger surveillance dataset for eastern Australia (P. Hansbro *et al*., unpubl. data), which were not included in the overall analysis of Australian surveillance data described above, but were used only to compare the three methods of sample collection. Transport media, storage, transport, operators, testing preparation and testing procedures were identical for all samples.

Details of the data-collection methods, sampling techniques and testing procedures are described elsewhere (Tracey 2005; Kirkland and Tracey 2006; see also Rose *et al*. 2006). Briefly, swabs were taken from live-captured or recently shot birds by inserting a swab deeply into the vent (cloacal) or oropharynx and swabbing the mucosa. The tip of the plastic-shafted swab was placed into a vial containing phosphate-buffered gelatin saline (PBGS) transport media (8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, 0.24 g of KH_2PO_4 dissolved in 800 mL of distilled H_2O). The viral transport medium was stored frozen, or at 4°C before use. Samples were maintained cold (4°C) throughout the transport process, and transported to the laboratory within 48 h of collection. Samples were either tested on delivery, or if not able to be completed within 48 h, were stored in a −80°C freezer (or −20°C for serum samples). Testing was conducted at Elizabeth McArthur Agricultural Institute with qRT–PCR (cloacal, oropharyngeal and faecal) and the Influenza A group reactive competitive enzyme-linked immunosorbent assay (cELISA, serum), based on the method and reagents supplied by the Australian Animal Health Laboratory, Geelong (www.csiro.au/places/AAHL.html).

For faecal sampling, only freshly deposited moist samples were collected, the species or group of species were identified wherever possible, and a score given for the level of confidence in determining the species or group, as follows: Highly likely (sample collected immediately after a bird was observed defaecating), Likely (bird observed in the area immediately before collecting samples), Possible (bird observed in the area within 1 h of sampling), Unknown (birds known to occur in the area). The abundance of birds was estimated with point counts (Bibby *et al*. 2000) each morning before collecting samples at each site, which aided species identification. Size and shape of the faeces was distinguishable for different groups of species (ducks, large waders, small waders). The swab was lightly coated with faeces. Only samples where the species was identified as Likely or Highly likely were included when comparing sample techniques.

Costs of collection methods were estimated and included labour ($\$15\,h^{-1}$), costs of consumables (feed for traps, ammunition), and the average number of samples collected per hour. To allow for direct comparison of collection methods, cost of travel (vehicle, fuel, labour) to sites was not included.

Risk profiles

Ecological and epidemiological information has been used to assign risks of exposure by wild-bird species and location according to a range of variables to achieve the following two main aims:

(1) to assess the risk of endemic LPAI viruses in wild birds becoming highly pathogenic through interactions with poultry (Surveillance Aim 1) and
(2) to assess the risk of wild birds introducing foreign subtypes of avian influenza (Surveillance Aim 2).

Risk profiles are consistent with 'exposure assessment' under the OIE risk analysis framework (Murray 2002) and were developed for Surveillance Aim 1 by using (in order of importance): (a) log of poultry density (Robinson *et al*. 2007), (b) the estimated prevalence of LPAI, and (c) the abundance of Anseriformes. Risk profiles developed for Surveillance Aim 2 used (in order of importance): (a) the probability of moving from areas where HPAI epizootics have occurred in 2003–09 (FAO 2009; OIE 2009; WHO 2009), (b) the abundance of Anseriformes known to move into South-east Asia and (c) the estimated prevalence of LPAI.

To classify the risks to poultry, the risk of incursion was assumed to be dependent on poultry density (Robinson *et al*. 2007), using the natural log of the number of birds (Snow *et al*. 2007). The abundance of Anseriformes was estimated by using reporting rate and bird-count data from Birds Australia (Barrett *et al*. 2003). Atlas data were collected during the 'New Altas of Australian Birds' project 1998–2002 from 279 000 bird surveys by 7000 observers. Australian Bird Count Data were collected during 79 000 surveys involving repeated counts of birds by 952 observers at 1681 sites between 1989 and 1995. Surveys for both datasets followed the standard methods for Birds Australia's 20-min, 2-ha search (Barrett *et al*. 2003), with the Australian Bird Count Data including complete counts of all individual birds observed, as well as the number of species observed. The relationship between the number of birds and number of species per observation was examined to test the use of reporting rate (number of surveys a bird species was present divided by the total number of surveys for each 1/4° map grid) as an index of abundance. For each 1/4° grid cell, abundance and prevalence was estimated separately for functional groups, which was found to be important in predicting LPAI prevalence (see Results).

Distances moved and movement probabilities for Anseriformes were estimated using banding data from the Australian Bird and Bat Banding Scheme (www.environment.gov.au/biodiversity/science/abbbs/, accessed 1 January 2009). Spatial analyses were conducted in Arcview 3.2 (ESRI, Redlands, CA) and Manifold® (Carson City, NV). In addressing Surveillance Aim 2, movement probabilities were estimated for species identified as conducting regular or occasion movements in South-east Asia (after Tracey *et al*. 2004; Delaney and Scott 2006; Table 1), using a movement probability model (see Results; $y = 22\,928\ x^{-2.2541}$, where x is the distance to the current distribution of HPAI epizootics).

Prevalence of LPAI for each grid cell (p_{total}) was estimated by

$$p_{\text{total}} = \frac{\sum(p_f \times a_f)}{\sum a_f} \quad (1)$$

where p_f is the prevalence of LPAI according to the functional group, using Australian surveillance data (Table 1) and a_f is the abundance index for Anseriformes in each functional group in each 1/4° grid cell.

The final scores were calculated with a normalised weight (w_i), by using a rank sum (2) (Malczewski 1999),

$$w_i = \frac{n - r_j + 1}{\sum(n - r_k + 1)} \quad (2)$$

where w_i was the normalised weight for the jth criterion, n was the number of criteria under consideration ($k = 1, 2, \ldots, n$), and r_j was the rank position of the criterion. Each criterion was weighted ($n - r_j + 1$) and then normalised by the sum of all weights, i.e. $\sum(n - r_k + 1)$.

The value for each criterion for each grid cell was normalised by using (3) before applying weights, as follows:

$$\delta = \frac{d - d^{\min}}{d^{\max} - d^{\min}} \quad (3)$$

where δ is the normalised value and d is the original value.

Results

Analysis of Anseriformes movements and abundance

The number of species recorded during Australian Bird Counts was found to be sufficient in predicting the log of the number of birds per observation ($y = 0.6913x + 1.4456$, $P < 0.001$). Movement probabilities were estimated for Anseriformes by using distance moved from recapture data ($n = 8095$), with a power model showing a good fit to the data ($r^2 = 0.89$, $y = 22\,928x^{-2.2541}$). From banding and recovery data, within 14 days of capture, 75% of birds remained within 5 km of capture, 80% within 10 km, 90% within 35 km, and 95% within 100 km; the maximum distance moved from the capture location was 2305 km ($n = 1314$). Greater movements of Anseriformes occurred in winter (mean = 247 km ± 79, $n = 200$) than in other seasons (summer: mean = 92 km ± 12, $n = 1013$; autumn: mean = 63 km ± 10, $n = 643$; spring: mean = 39 km ± 13, $n = 440$).

The abundance of Anseriformes was correlated with the availability of permanent water ($P = 0.00025$, $y = 28.248x + 0.448$, $r^2 = 0.814$) and the seasonal rainfall zone ($P < 0.0001$), as follows (in a decreasing order of abundance – mean reporting rate): uniform (0.57 ± 0.05, $n = 1\,291$), summer (0.47 ± 0.03, $n = 1\,840$), winter-dominant (0.46 ± 0.05, $n = 496$), winter (0.42 ± 0.02, $n = 2\,631$), summer-dominant (0.29 ± 0.03, $n = 2625$) and arid (0.19 ± 0.02, $n = 4\,548$) rainfall zone.

Analysis of Australian surveillance data

LPAI prevalence was significantly greater in dabbling ducks than in all other functional groups for Australia and overseas (Fig. 1). Prevalence of LPAI was significantly lower in Australia (1.04% ± 0.06, $n = 29\,167$) than that in other countries (4.67% ± 0.02, $n = 95\,441$), with the prevalence 2.6–4 times less for all functional groups, with the exception of seabirds, where there was no significant difference between Australia and overseas, and small migratory waders, where the prevalence was 11 times less in Australia than in overseas (Fig. 1). However, sampling is unlikely to be representative across all species and locations, particularly for dabbling ducks in North America where many samples are regularly taken in areas of previous high virus activity. In Australia and overseas, LPAI was either not detected

Table 1. Prevalence of low-pathogenicity avian influenza (LPAI) of Australian Anseriformes

Common name[A]	Scientific name	Functional group	Prevalence of LPAI[B] (%)	Movements into South-east Asia[C]
Anseranatidae				
Magpie Goose	*Anseranas semipalmata*	Dabbling ducks	3.12	Regular
	Anatidae			
Anatinae (dabbling ducks)				
Australian wood duck[U]	*Chenonetta jubata*	Grazing waterfowl	0.71	Unknown
Cotton pygmy-goose[U]	*Nettapus coromandelianus*	Dabbling ducks	3.12	Occasional
Green pygmy-goose[U]	*Nettapus pulchellus*	Dabbling ducks	3.12	Occasional
Garganey	*Anas querquedula*	Dabbling ducks	3.12	Rare
Australasian shoveler	*Anas rhynchotis*	Dabbling ducks	3.12	Unknown
Northern shoveler[V]	*Anas clypeate*	Dabbling ducks	3.12	Rare
Grey Teal	*Anas gracilis*	Dabbling ducks	3.12	Occasional
Chestnut teal	*Anas castanea*	Dabbling ducks	3.12	Unknown
Northern pintail[V]	*Anas acuta*	Dabbling ducks	3.12	Rare
Kerguelen pintail[AAT/V]	*Anas eatoni*	Dabbling ducks	3.12	Unknown
Mallard[I]	*Anas platyrhynchos*	Dabbling ducks	3.12	Unknown
Pacific black duck	*Anas superciliosa*	Dabbling ducks	3.12	Occasional
Anserinae (swans and geese)				
Cape barren goose[U]	*Cereopsis novaehollandiae*	Grazing waterfowl	0.71	Unknown
Black swan	*Cygnus atratus*	Deep-water foragers	0.94	Unknown
Mute swan[I]	*Cygnus olor*	Deep-water foragers	0.94	Unknown
Canada goose[V/I]	*Branta canadensis*	Grazing waterfowl	0.71	Unknown
Aythyinae (diving ducks)				
Hardhead	*Aythya australis*	Deep-water foragers	0.94	Unknown
Dendrocygninae (whistling ducks)				
Spotted whistling-duck	*Dendrocygna guttata*	Grazing waterfowl	0.71	Rare
Plumed whistling-duck	*Dendrocygna eytoni*	Grazing waterfowl	0.71	Rare
Wandering whistling-duck	*Dendrocygna arcuata*	Dabbling ducks	1	Regular
Oxyurinae (stiff-tailed ducks)				
Musk duck	*Biziura lobata*	Deep-water foragers	0.94	Unknown
Blue-billed duck	*Oxyura australis*	Deep-water foragers	0.94	Unknown
Stictonettinae (freckled duck)				
Freckled duck	*Stictonetta naevosa*	Dabbling ducks	3.12	Unknown
Tadorninae (shelducks)				
Radjah shelduck	*Tadorna radjah*	Dabbling ducks	3.12	Rare
Australian shelduck	*Tadorna tadornoides*	Grazing waterfowl	0.71	Unknown
Paradise shelduck[LH/V]	*Tadorna variegata*	Grazing waterfowl	0.71	Unknown
Pink-eared duck	*Malacorhynchus membranaceus*	Dabbling ducks	3.12	Unknown

[A]Superscripts (after Christidis and Boles 2008): V = vagrant to Australia (fewer than 10 records); I = introduced to Australia; AAT = Australian Antarctic Territory; LH = Lord Howe Island; U = subfamily unresolved, based on Livezey (1986), Sraml *et al*. (1996), Johnson and Sorenson (1999).
[B]Estimated for functional groups (after Roshier *et al*. 2002).
[C]After Tracey *et al*. (2004), Delaney and Scott (2006).

or was of low prevalence for other functional groups (quail and wild Galliformes: 0/27 (Australia), 4/899 (overseas); large waders: 0/58 (Australia), 0/87 (overseas); small resident waders: 0/260 (Australia), 1/58 (overseas); birds of prey: 0/6 (Australia), 2/192 (overseas); pigeons and doves: 0/1 (Australia), 1/166 (overseas); or bush birds: 0/34 (Australia), 0/92 (overseas)).

Comparison of sample methods: field trial

The cost of collecting faecal samples ($1.95 per sample) was less than the cost of collecting samples by shooting ($6.12 per bird) and trapping ($9.10 per bird). When compared on the same populations during the same time periods, detection of antibodies with cELISA from serum (18.45% ± 4.38, 95% confidence interval, $n = 374$) was much more likely than detection of the virus (1.07% ± 2.16, $n = 2\ 868$). Also, the prevalence of LPAI viruses detected with qRT–PCR was similar for cloacal (2.27% ± 0.97, $n = 948$) and oropharyngeal (2.17% ± 2.3, $n = 185$) samples, whereas it was significantly lower from faecal samples (0.29% ± 0.28, $n = 1\ 735$).

Risk profiles

On the basis of risk profiles, highest priorities to assess risks of endemic viruses becoming highly pathogenic are in the region of state capitals, i.e. Melbourne, Sydney, Brisbane, Adelaide, Perth,

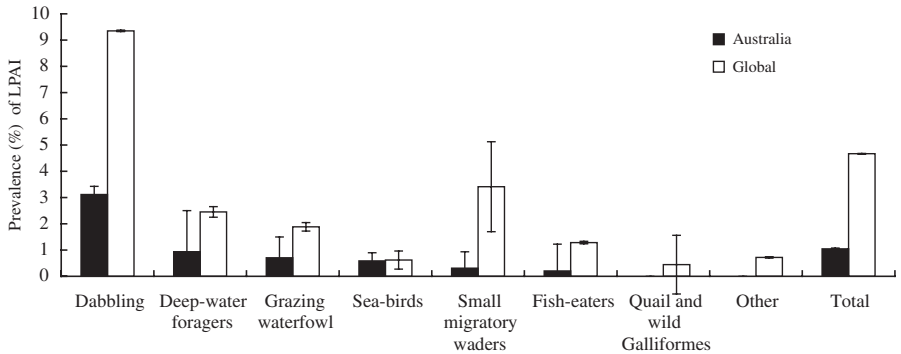


Fig. 1. Prevalence (% with s.e.) of low-pathogenicity avian influenza (LPAI) in wild birds by functional group from Australian (black) and global (white) surveillance data. Australia: $n = 29\,167$; Global: $n = 93\,344$ (after Olsen *et al*. 2006).

Darwin and Hobart, and in the Mareeba area near Cairns, Queensland (Fig. 2*a*). Current poultry densities for areas where previous HPAI epizootics occurred are 266 km^{-2} for Keysborough, Victoria (1976, Turner 1976), 464 km^{-2} for Bendigo, Victoria (1985, Barr *et al*. 1986; 1992, Selleck *et al*. 1997), 222 km^{-2} for Lowood, Queensland (1994, Westbury 1998), and 900 km^{-2} for Tamworth, New South Wales (1997, Selleck *et al*. 2003). Highest priorities to assess risks of wild birds introducing foreign viruses are the regions of north-western Australia from Broome through to Arnhem Land, particularly in the Kimberley, Western Australia (Fig. 2*b*). Combined ranks to address both surveillance aims include all these high-priority locations (Fig. 2*c*). Australian surveillance (1971–2007) has generally occurred in these priority areas, with the exception of Brisbane, Darwin and Mareeba, where surveillance is currently underway.

Discussion

Anseriformes and avian influenza in Australia

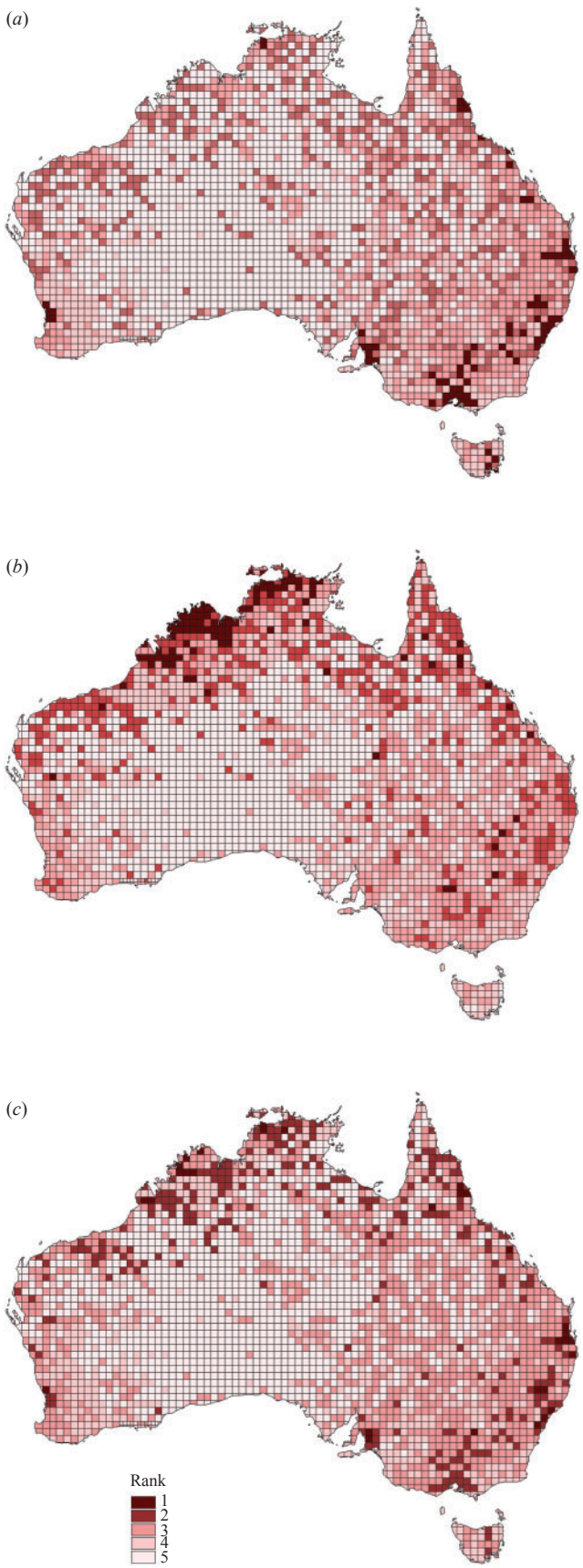
Abundance and movement patterns for Anseriformes are found to be irregular, varying with the availability of permanent water and seasonal rainfall, which is commonly reported for Australian anatids, with flood events and temporary rainfall particularly important (Roshier *et al*. 2001). Anatids are often more dispersive in arid areas, and more sedentary and abundant on permanent water (Frith 1982; Woodall 1985). The greater Anseriformes movements being evident in winter is consistent with historical studies in southern Australia, where anatids were previously thought to be more concentrated (high local abundance) in summer and dispersive in winter (low local abundance) (Ford 1958). However, cues for and patterns of anatid movements are complex, determined by individual behavioural strategies (Roshier *et al*. 2008) and surface water over large spatial scales (Roshier *et al*. 2001), rather than season. Anatid movements and abundance in Northern Australia, however, may fluctuate seasonally, where large numbers can congregate during the dry (May–October, includes southern winter) period and disperse very widely during the wet (November–April) period (Morton *et al*. 1990).

The abundance and movements of waterbirds have implications for understanding persistence of LPAI in natural reservoirs and for managing HPAI epizootics. In Australia, LPAI would be expected to peak in Anseriformes during 'boom' breeding periods where thousands of birds congregate on major wetlands or floodplains. This occurs infrequently, with the largest breeding events occurring perhaps once every 10 years. In tropical Australia, LPAI may be more seasonal with peaks expected in winter, and greater potential for dispersal of LPAI in summer. This is consistent with HPAI H5N1 in tropical South-east Asia, where prevalence was significantly higher in winter, when large flocks of Anseriformes congregate during the winter (= dry season) (Siengsanan *et al*. 2009).

The lower prevalence of AI found in Australia than overseas (Olsen *et al*. 2006; Haynes *et al*. 2009) is likely to be a result of differences in the behaviour and movements of Australian Anseriformes from those overseas (Tracey *et al*. 2004; McCallum *et al*. 2008). Australia is dry with irregular rainfall and as a consequence breeding and movements of waterbirds are irregular. During the past 20 years, breeding has been infrequent and waterbird abundance has declined markedly in some areas (Kingsford and Porter 2006), by up to 80% for some species (Nebel *et al*. 2008). Loss of wetlands because of dams, water extractions and levee banks, particularly in south-eastern Australia, is likely to have contributed to these declines (Kingsford 2000; Nebel *et al*. 2008).

The persistence of avian influenza viruses is likely to be affected by the regularity of breeding, as well as movement patterns, both being correlated with water availability (Frith 1982). Hence, breeding occurs in southern Australia in spring and in northern Australia at the end of the wet season (April–May, southern autumn). During severe drought, most Australian anatids do not breed (Frith 1982), which is likely to limit LPAI prevalence. Increased virus prevalence following breeding is often observed or assumed for animal pathogens, including avian influenza virus (Hinshaw *et al*. 1985; Alfonso *et al*. 1995), as a result of the boost in immunologically naïve individuals (juveniles) (Clark and Hall 2006; Munster and Fouchier 2009).

LPAI in Australian wild birds was highly variable among sampling periods and locations and no seasonal trends were apparent. This is likely to be a consequence of a lack of long-term studies (low sample sizes over time) coupled with a high variability in rainfall and Anseriformes movements and



abundance between climatic zones (northern: wet season – summer-dominant rainfall *v.* southern: uniform or winter-dominant rainfall).

Functional group was clearly important in predicting LPAI prevalence, with dabbling ducks identified as the main reservoir for Australia and overseas. The propensity of dabbling ducks to skim surface water is a likely explanation (Olsen *et al*. 2006). Avian influenza viruses are known to persist in water (Webster *et al*. 1978; Stallknecht *et al*. 1990*a*, 1990*b*; Brown *et al*. 2009; Roche *et al*. 2009) and high levels of faecal material may occur on the surface (Lang *et al*. 2008).

The substantial difference in prevalence (11 times) for small migratory waders between Australia and overseas may suggest that these species are unlikely to be responsible for transferring viruses into Australia. Rather, these species may act as a sentinel for endemic viruses maintained by dabbling ducks. In contrast, seabirds, whereas also conducting regular global travel, have similar prevalence between Australia and overseas. This may support the view that seabirds maintain viruses that are unique from viruses on mainland Australia, which is consistent with their behaviour and movements and phylogenetic differences among virus groups (Munster and Fouchier 2009). Future investigations of genetic differences between Australian and Eurasian and American subtypes may confirm these trends.

Practical considerations of sample-collection methods and testing procedures are important to consider when interpreting results of surveillance (Munster *et al*. 2009) and in preparation for future HPAI epizootics. Although variable, faecal sampling was three and five times less expensive than sampling involving shooting and trapping respectively. However, the significantly lower prevalence from faecal samples than from cloacal samples highlights the need for reporting results separately. Possible reasons include degradation of samples (low volumes of RNA), or contamination as a result of excess faecal material or other substances from the environment. In comparison, Pannwitz *et al*. (2009) reported similar recovery rates from faecal and cloacal samples for some species (geese and swan, but not ducks). Pannwitz *et al*. (2009), however, compared recovery rates from different locations and time periods, which is problematic because of low prevalence and considerable variation in prevalence commonly reported between locations and over time. Improved collection procedures for faecal samples may increase the rate of detection, for example, by minimising the amount of faecal material, or collecting samples from hardened or

Fig. 2. Priorities for the surveillance of avian influenza in Australia's wild birds: (*a*) to assess the risk of endemic low-pathogenicity avian influenza viruses in wild birds becoming highly pathogenic through interactions with poultry (Surveillance Aim 1), (*b*) to assess the risk of wild birds introducing foreign subtypes of avian influenza (Surveillance Aim 2), and (*c*) a combined classification to address Surveillance Aims 1 and 2. Priorities (Rank 1 (highest) to 5 (lowest)) are based on risk profiles developed using the log of poultry density (Robinson *et al*. 2007), the estimated prevalence of low-pathogenicity avian influenza by functional group using Australian surveillance data (Downie and Laver 1973; Downie *et al*. 1977; Mackenzie *et al*. 1984, 1985; Peroulis and O'Riley 2004; Hurt *et al*. 2006; Haynes *et al*. 2009), the abundance of Anseriformes (source: Birds Australia), and the probability of Anseriformes moving from areas where HPAI epizootics have occurred in 2003–09 (FAO 2009; OIE 2009; WHO 2009; source: Australian Bird and Bat Banding Scheme).

more sterile surfaces (e.g. concrete, bitumen, compacted soil or gravel, sand and decks). The prevalence from cloacal swabs was not significantly different from that from oropharyngeal swabs, which is consistent with Peroulis and O'Riley (2004). However, Ellström *et al*. (2008) and Munster *et al*. (2009) reported significantly higher LPAI prevalence from cloacal samples. In comparison, for HPAI H5N1, virus recovery was significantly higher from the respiratory tract than from the cloaca (Sturm-Ramirez *et al*. 2005; Keawcharoen *et al*. 2008). For on-going surveillance, faecal (environmental) samples may be collected as a rapid and cost-effective means of investigating virus presence. However, to verify virus prevalence, the collection of oropharyngeal and cloacal samples from hunted or captured birds is recommended.

Risk profiles

There is some uncertainty as to the role of poultry density in initiating HPAI in Australia. Although a shift in pathogenicity for avian influenza can occur rapidly (Brugh and Beck 1992, one or two passages; Arzey 2005), population size or density is likely to be important in determining the levels of prevalence, transmissibility and mutation rates for many viruses (e.g. rabbit haemorrhagic disease virus in rabbits (Calvete and Estrada 2000; Henzell *et al*. 2002), brucellosis in bison (Dobson and Meagher 1996), *Mycoplasma gallisepticum* in house sparrows (Hochachka and Dhondt 2000)), including avian influenza (Bunn 2004; Turner 2004; Pfeiffer *et al*. 2007; Snow *et al*. 2007). Westbury (1998) suggested that poultry-farm density was low in the first four HPAI epizootics in Australia. However, the current study indicates that both poultry-farm density and poultry density are highest in the areas where previous epizootics occurred; areas where all five HPAI epizootics took place are ranked highest by using poultry population per 1/4° grid. Hamilton *et al*. (2009) identified the density of poultry farms as a risk factor for HPAI in Australia, listing five regions (the Sydney region, Central Coast NSW, Tamworth, Mornington Peninsula and Bendigo) that had poultry-farm density equal to or greater than regions of Canada and Italy affected by large epizootics of HPAI (>0.05 farms km^{-2}), which is consistent with the current study. Hamilton *et al*. (2009) also emphasised the importance of biosecurity measures to prevent the spread of the virus from infected farms in the event of an epizootic, resulting from service providers regularly contacting multiple farms.

Although currently unavailable, future risk profiles could incorporate additional variables, including housing (caged, floor, free range, barn, deep litter; Pfeiffer 2006; Fossum *et al*. 2009) and the type of operation (pullets, breeders, broilers, layers; Snow *et al*. 2007). Poultry-farm density may also be more appropriate than poultry density in predicting spread, once an epizootic occurs (Truscott *et al*. 2007; Hamilton *et al*. 2009).

There has been considerable debate on the ability of wild birds to spread HPAI virus over large distances while infectious (up to 14 days, Kida *et al*. 1980), with several recent studies suggesting that this is likely to have occurred (Sabirovic *et al*. 2006; Stallknecht and Brown 2008). However, the persistence of virus in the environment, the connectivity of the landscape relevant to wild birds (particularly dabbling ducks, Roshier *et al*. 2001) and the frequency of movements for multiple species (McCallum *et al*. 2008) are likely to be more important than individual bird movements within short periods. Bird populations can maintain avian influenza viruses despite low prevalence (Stallknecht and Brown 2008) and viruses can remain infective in freshwater lakes for 4 days at 22°C, more than 30 days at 0°C (Webster *et al*. 1978), or up to 200 days at 17°C, when virus concentrations are higher (Stallknecht *et al*. 1990*b*).

When estimating the distance moved from banding data, there are several biases that should be considered when interpreting risk profiles. In particular, individual ducks are more likely to be recaptured at the same location than elsewhere when consecutive trapping periods occur at the same location. This would create an underestimate of HPAI risk for these criteria. Recoveries may also be more likely where damage mitigation permits are issued to protect rice. Satellite transmitters have demonstrated that large movements of grey teal can occur within hours (up to 345 km) (Roshier *et al*. 2006), and within days (up to 1268 km) (Roshier *et al*. 2008), with some birds returning to their point of origin. These individual movements would have been difficult to detect with banding studies. However, movement probabilities estimated in the current study ($y = 22\,928x^{-2.2541}$, see Results) are consistent with overall patterns of movement reported using satellite transmitters (Roshier *et al*. 2006, 2008). For example, Roshier *et al*. (2006) found that 78% and 83% of grey teal movements occurred within 5 km in the Riverina and Lake Eyre Basin respectively.

To develop risk profiles for avian influenza in wild birds, a range of simple seasonal and climatic variables have been explored to explain the abundance of anatids and the likelihood of their movement over a large area. However, these ignore the finer-scale processes of wetland quality, the temporary availability of wetlands and flood events. These are known to be important in predicting anatid movements and abundance, particularly in arid Australia; however, they are difficult to incorporate when presenting spatial data that can be interpreted over time.

Although highest priorities have been assigned to dabbling ducks, because they represent the major reservoir of LPAI in Australia, surveillance of other species should not be excluded. Migratory and resident Charadriiformes, seabirds (including pelagic gulls and terns and Procellariiformes), quail, ratites and other functional groups may also play a role in maintaining avian influenza viruses, including those with unique lineages (e.g. gulls and terns, Munster and Fouchier 2009).

There are many uncertainties that affect the risks of an incursion of HPAI. Risk profiles developed here are not for predicting future epizootics, but rather, are a tool to maximise the efficiency and relevance of wild-bird surveillance, and to provide insights into patterns of LPAI occurrence. Hence, poultry producers should continue to maintain high biosecurity (including limiting contact with wild birds, regular treatment of water, rapid reporting of unusual mortalities), regardless of whether they are located in high- or low-priority areas. The major risks for poultry operations are likely to be Anseriformes in the vicinity, a failure in biosecurity (e.g. water quality or entry of contaminated personnel) and confined poultry of sufficient density to allow development and dissemination of a pathogenic virus (Bunn 2004). However, there are other potential sources of LPAI, including live-bird markets and movements of domestic

birds (poultry, turkeys, ducks, emus, quails) (Arzey 2004), and more important modes of transmission following outbreaks of HPAI (Sims *et al*. 2005; Feare 2007; Gilbert *et al*. 2008; Hamilton *et al*. 2009). In Australia, service providers regularly contact multiple farms (Hamilton *et al*. 2009) and are a direct potential source of secondary spread.

Previous surveillance for avian influenza in Australia has generally occurred in areas identified as highest priority, with the exception of Mareeba (northern Queensland), Brisbane and Darwin, with the current Avian Influenza Wild Bird Surveillance Program addressing these gaps. This surveillance has provided valuable information on the role of wild birds in maintaining LPAI viruses, and provides the basis for future insights into global patterns of avian influenza, in particular in the investigation of genetic similarities of subtypes between continents. However, surveillance has been sporadic, with a limited number of samples collected (35 000 samples in 1970–2007 in Australia *v.* 300 000 samples per year in other countries, Munster and Fouchier 2009) and with information on bird abundance, age, behaviour, breeding and movements rarely being collected during surveillance activities. This limits our ability to offer explanations for the spatial and temporal variability of virus prevalence. Enhanced surveillance in priority areas that incorporates ecological information over a longer time frame is important to validate trends of LPAI prevalence, in understanding the main determinants for virus spread and persistence, and in predicting and managing future epizootics of HPAI in Australia.

Acknowledgements

I am indebted to Glen Saunders, Peter Fleming (Industry and Investment NSW), Rupert Woods, Leesa Haynes, Tiggy Grillo (Australian Wildlife Health Network), Chris Bunn (Department of Agriculture Fisheries and Forestry), Tony Peacock (IACRC) and Brian Boyle (Game Council NSW) for their on-going support and for collaborating on aspects of this project, and Camilla Myers for suggesting this paper. Thanks also go to Brian Lukins, Franz Zikesch, Megan Moppett, Brent Waldron, Shannon Slade and Game Council NSW for assistance with bird sampling; Peter Kirkland and Edla Arzey for testing samples; Remy van de Ven and Steven McLeod for valuable advice on data analysis; the staff of the Vertebrate Pest Research Unit for advice and technical support, and Simone Warner, David Roshier, Phil Hansbro and the Avian Influenza Wild Bird Steering Group for useful discussions. Thank you to David Drynan and Andrew Silcocks and many participants of Birds Australia Atlas, the Australian Bird Count Data and the Australian Bird and Bat Banding Scheme for collecting and collating data on anatids, in particular Greg Hocking, Clive Minton, Roz Jessop, Victorian Wader Study Group, Australasian Wader Study Group, Raoul Mulder and Patrick-Jean Guay. Thank you to Iain East and Graeme Garner (Department of Agriculture Fisheries and Forestry) for advice on Australian commercial poultry operations. Funding was provided by the Wildlife and Exotic Disease Preparedness Program, Industry and Investment NSW and the Invasive Animals Cooperative Research Centre.

References

Alfonso, C. P., Cowen, B. S., and van Campen, H. (1995). Influenza A viruses isolated from waterfowl in two wildlife management areas of Pennsylvania. *Journal of Wildlife Diseases* **31**, 179–185.

Arzey, G. (2004). The role of wild aquatic birds in the epidemiology of avian influenza in Australia. *Australian Veterinary Journal* **82**, 377–378. doi:10.1111/j.1751-0813.2004.tb11110.x

Arzey, G. (2005). The role of wild waterfowl in the epidemiology of AI in Australia. *Australian Veterinary Journal* **83**, 445. doi:10.1111/j.1751-0813.2005.tb13090.x

Banks, J., and Alexander, D. J. (1997). Molecular epidemiology of the H5 and H7 avian influenza viruses submitted to the international reference laboratory, Weybridge. In 'Fourth International Symposium on Avian Influenza'. (Eds D. E. Swayne and R. D. Slemons.) pp. 105–109. (United States Animal Health Association: Athens, GA.)

Barr, D. A., Kelly, A. P., Badman, R. T., and Campey, A. R. (1986). Avian influenza on a multi-age chicken farm. *Australian Veterinary Journal* **63**, 195–196.

Barrett, G., Silcocks, A., Barry, S., Cunningham, R., and Poulter, R. (2003). 'New Atlas of Australian Birds.' (CSIRO: Canberra.)

Bibby, C. J., Burgess, N. D., Hill, D. A., and Mustoe, S. H. (2000). 'Bird Census Techniques.' (Academic Press: San Diego, CA.)

Brown, J. D., Goekjian, G., Poulson, R., Valeika, S., and Stallknecht, D. E. (2009). Avian influenza virus in water: infectivity is dependent on pH, salinity and temperature. *Veterinary Microbiology* **136**, 20–26.

Brugh, M., and Beck, J. R. (1992). Recovery of minority subpopulation of highly pathogenic avian influenza virus. In 'Proceedings of the Third International Symposium on Avian Influenza'. (Ed. B. C. Easterday.) pp. 166–174. (University of Wisconsin: Madison, WI.)

Bunn, C. (2004). Correspondence regarding the role of wild aquatic birds in the epidemiology of avian influenza in Australia. *Australian Veterinary Journal* **82**, 4. doi:10.1111/j.1751-0813.2004.tb12619.x

Calvete, C., and Estrada, R. (2000). 'Epidemiología de Enfermedad Hemorrágica (VHD) y Mixomatosis en el conejo silvestre en el Valle medio del Ebro – Herramientas de gestión.' (Consejo de Protección de la Naturaleza: Zaragoza, Spain.)

Chen, H., Deng, G., Li, Z., Tian, G., Li, Y., Jiao, P., Zhang, L., Liu, Z., Webster, R. G., and Yu, K. (2004). The evolution of H5N1 influenza viruses in ducks in southern China. *Proceedings of the National Academy of Sciences, USA* **101**, 10 452–10 457. doi:10.1073/pnas.0403212101

Christidis, L., and Boles, W. E. (2008). 'Systematics and Taxonomy of Australian Birds.' (CSIRO Publishing: Melbourne.)

Clark, L., and Hall, J. (2006). Avian influenza in wild birds: status as reservoirs, and risks to humans and agriculture. *Ornithological Monographs* **60**, 3–29. doi:10.1642/0078-6594(2006)60[3:AIIWBS]2.0.CO;2

Delaney, S., and Scott, D. (2006). 'Waterbird Population Estimates.' 4th edn. (Wetlands International: Wageningen, The Netherlands.)

Dobson, A., and Meagher, M. (1996). The population dynamics of Brucellosis in the Yellowstone National Park. *Ecology* **77**, 1026–1036.

Downie, J. C., and Laver, W. G. (1973). Isolation of type A influenza virus from an Australian pelagic bird. *Virology* **51**, 259–269. doi:10.1016/0042-6822(73)90426-1

Downie, J. C., Hinshaw, V. S., and Laver, W. G. (1977). The ecology of influenza. Isolation of type A influenza viruses from Australian pelagic birds. *Australian Journal of Experimental Biology and Medical Science* **55**, 635–643. doi:10.1038/icb.1977.62

East, I. J., Hamilton, S. A., and Garner, G. (2008*a*). Identifying areas of Australia at risk of H5N1 avian influenza infection from exposure to migratory birds: a spatial analysis. *Geospatial Health* **2**, 203–213.

East, I. J., Hamilton, S. A., Sharp, L. A., and Garner, G. (2008*b*). Identifying areas of Australia at risk for H5N1 avian influenza infection from exposure to nomadic waterfowl moving throughout the Australo-Papuan region. *Geospatial Health* **3**, 17–27.

Ellström, P., Latorre-Margalef, N., Griekspoor, P., Waldenström, J., Olofsson, J., Wahlgren, J., and Olsen, B. (2008). Sampling for low-pathogenic avian influenza A virus in wild Mallard ducks: oropharyngeal versus cloacal swabbing. *Vaccine* **26**, 4414–4416. doi:10.1016/j.accine.2008.06.027

Feare, C. (2007). The spread of avian influenza. *Ibis* **149**, 424–425. doi:10.1111/j.1474-919X.2007.00711.x

Food and Agriculture Organization of the United Nations (FAO) (2009). 'Highly Pathogenic Avian Influenza Confirmed Outbreaks.' Available at http://www.fao.org/avianflu/en/maps.html [verified November 2009].

Ford, J. R. (1958). Seasonal variation in populations of Anatidae at the Bibra Lake District, Western Australia. *Emu* **58**, 31–41.

Fossum, O., Jansson, D., Etterlin, P., and Vagsholm, I. (2009). Causes of mortality in laying hens in different housing systems in 2001 to 2004. *Acta Veterinaria Scandinavica* **51**, 3. doi:10.1186/1751-0147-51-3

Frith, H. J. (1982). 'Waterfowl in Australia.' Revised edn. (Angus and Robertson: Sydney.)

Gaidet, N., Cattoli, G., Hammoumi, S., Newman, S. H., Hagemeijer, W. *et al*. (2008). Evidence of infection by H5N2 highly pathogenic avian influenza viruses in healthy wild waterfowl. *PLoS Pathogens* **4**, e1 000 127. doi:10.1371/journal.ppat.1000127

Gilbert, M., Xiao, X., Pfeiffer, D. U., Epprecht, M., Boles, S. *et al*. (2008). Mapping H5N1 highly pathogenic avian influenza risk in Southeast Asia. *Proceedings of the National Academy of Sciences, USA* **105**, 4769–4774. doi:10.1073/pnas.0710581105

Gilmour, A. R., Gogel, B. J., Cullis, B. R., Welham, S. J., and Thompson, R. (2002). 'ASReml User Guide Release 1.' (VSN International Ltd: Hemel Hempstead, UK.)

Hamilton, S. A., East, I. J., Toribio, J.-A., and Garner, M. G. (2009). Are the Australian poultry industries vulnerable to large outbreaks of highly pathogenic avian influenza? *Australian Veterinary Journal* **87**, 165–174. doi:10.1111/j.1751-0813.2009.00423.x

Haynes, L., Arzey, E., Bell, C., Buchanan, N., Burgess, G. *et al*. (2009). Australian surveillance for avian influenza viruses in wild birds (July 2005 to June 2007). *Australian Veterinary Journal* **87**, 266–272. doi:10.1111/j.1751-0813.2009.00446.x

Henzell, R. P., Cunningham, R. B., and Neave, H. M. (2002). Factors affecting the survival of Australian wild rabbits exposed to rabbit haemorrhagic disease. *Wildlife Research* **29**, 523–542. doi:10.1071/WR00083

Hinshaw, V. S., Wood, J. M., Webster, R. G., Deible, R., and Turner, B. (1985). Circulation of influenza viruses and paramyxoviruses in waterfowl originating from two different areas of North America. *Bulletin of the World Health Organization* **63**, 711–719.

Hochachka, W. M., and Dhondt, A. A. (2000). Density-dependent decline of host abundance resulting from a new infectious disease. *Proceedings of the National Academy of Sciences, USA* **97**, 5303–5306. doi:10.1073/pnas.080551197

Hulse-Post, D. J., Sturm-Ramirez, K. M., Humberd, J., Seiler, P., Govorkova, E. A. *et al*. (2005). Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. *Proceedings of the National Academy of Sciences, USA* **102**, 10 682–10 687. doi:10.1073/pnas.0504662102

Hurt, A. C., Hansbro, P., Selleck, P., Olsen, B., Minton, C., Hampson, A. W., and Barr, I. G. (2006). Isolation of avian influenza viruses from two different transhemispheric migratory shorebird species in Australia. *Archives of Virology* **151**, 2301–2309. doi:10.1007/s00705-006-0784-1

Johnson, K. P., and Sorenson, M. D. (1999). Phylogeny and biogeography of dabbling ducks (genus Anas): a comparison of molecular and morphological evidence. *The Auk* **116**, 792–805.

Keawcharoen, J., van Riel, D., van Amerongen, G., Bestebroer, T., Beyer, W. E., van Lavieren, R., Osterhaus, A., Fouchier, R. A. M., and Kuiken, T. (2008). Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). *Emerging Infectious Diseases* **14**, 600–607. doi:10.3201/eid1404.071016

Kida, H., Yanagawa, R., and Matsuoka, Y. (1980). Duck influenza lacking evidence of disease signs and immune response. *Infection and Immunity* **30**, 547–553.

Kingsford, R. T. (2000). Ecological impacts of dams, water diversions and river management on floodplain wetlands in Australia. *Austral Ecology* **25**, 109–127.

Kingsford, R. T., and Porter, J. L. (2006). Waterbirds and wetlands across Eastern Australia. Technical report. Department of the Environment and Heritage, Canberra.

Kirkland, P. D., and Tracey, J. P. (2006). Detecting avian influenza in wild birds in New South Wales. Final report to the Department of Agriculture, Fisheries and Forestry, Wildlife and Exotic Disease Preparedness Program. NSW Department of Primary Industries, Orange, NSW.

Lang, A. S., Kelly, A., and Runstadler, J. A. (2008). Prevalence and diversity of avian influenza viruses in environmental reservoirs. *Journal of General Virology* **89**, 509–519. doi:10.1099/vir.0.83369-0

Liu, J., Xiao, H., Lei, F., Zhu, Q., Qin, K. *et al*. (2005). Highly pathogenic H5N1 influenza virus infection in migratory birds. *Science* **309**, 1206. doi:10.1126/science.1115273

Livezey, B. C. (1986). A phylogenetic analysis of recent anseriform genera using morphological characters. *The Auk* **103**, 737–754.

McCallum, H., Roshier, D., Tracey, J. P., Joseph, L., and Heinsohn, R. (2008). Will Wallace's line save Australia from Avian Influenza? *Ecology and Society* **13**, 41–56.

Mackenzie, J. S., Edwards, E. C., Holmes, R. M., and Hinshaw, V. S. (1984). Isolation of ortho- and paramyxoviruses from wild birds in Western Australia and the characterisation of novel influenza A viruses. *Australian Journal of Experimental Biology and Medical Science* **62**, 89–99. doi:10.1038/icb.1984.9

Mackenzie, J. S., Britten, D., Hinshaw, V. S., and Wood, J. (1985). Isolation of avian influenza and paramyxoviruses from wild birds in Western Australia. In 'Veterinary Viral Diseases: Their Significance in South-east Asia and the Western Pacific'. (Ed. A. J. Della-Porta.) pp. 336–339. (Academic Press: Sydney.)

Malczewski, J. (1999). 'GIS and Multicriteria Decision Analysis.' (John Wiley and Sons: New York.)

Morton, S. R., Brennan, K. G., and Armstrong, M. D. (1990). Distribution and abundance of ducks in the Alligator Rivers region, Northern Territory. *Wildlife Research* **17**, 573–590. doi:10.1071/WR9900573

Mukhtar, M. M., Rasool, S. T., Song, D., Zhu, C., Hao, Q., Zhu, Y., and Wu, J. (2007). Origin of highly pathogenic H5N1 avian influenza virus in China and genetic characterization of donor and recipient viruses. *Journal of General Virology* **88**, 3094–3099. doi:10.1099/vir.0.83129-0

Munster, V. J., and Fouchier, R. A. M. (2009). Avian influenza virus: of virus and bird ecology. *Vaccine* **27**, 6340–6344. doi:10.1016/j.vaccine.2009.02.082

Munster, V. J., Baas, C., Lexmond, P., Bestebroer, T. M., Guldemeester, J. *et al*. (2009). Practical considerations for high-throughput influenza A virus surveillance studies of wild birds by use of molecular diagnostic tests. *Journal of Clinical Microbiology* **47**, 666–673. doi:10.1128/JCM.01625-08

Murray, N. (2002). 'Import Risk Analysis: Animals and Animal Products.' (Ministry of Agriculture and Forestry: Wellington, New Zealand.)

Nebel, S., Porter, J. L., and Kingsford, R. T. (2008). Long-term trends of shorebird populations in eastern Australia and impacts of freshwater extraction. *Biological Conservation* **141**, 971–980. doi:10.1016/j.biocon.2008.01.017

Nestorowicz, A., Kawaoka, Y., Bean, W. J., and Webster, R. G. (1987). Molecular analysis of the hemagglutinin genes of Australian H7N7 influenza viruses: role of passerine birds in maintenance or transmission. *Virology* **160**, 411–418. doi:10.1016/0042-6822(87)90012-2

Office International Epizooties (OIE) (2009). Update on highly pathogenic avian influenza in animals (Type H5 and H7). Available at http://www.oie.int/downld/AVIAN%20INFLUENZA/A_AI-Asia.htm [verified November 2009].

Olsen, B., Munster, V. J., Wallensten, A., Waldernstrom, J., Osterhaus, A. D. M. E., and Fouchier, R. A. M. (2006). Global patterns of influenza A virus in wild birds. *Science* **312**, 384–388. doi:10.1126/science.1122438

Pannwitz, G., Wolf, C., and Harder, T. (2009). Active surveillance for avian influenza virus infection in wild birds by analysis of avian fecal samples from the environment. *Journal of Wildlife Diseases* **45**, 512–518.

Peroulis, I., and O'Riley, K. (2004). Detection of avian paramyxoviruses and influenza viruses amongst wild bird populations in Victoria. *Australian Veterinary Journal* **82**, 79–82. doi:10.1111/j.1751-0813.2004.tb14650.x

Pfeiffer, D. U. (2006). Assessment of H5N1 risk and the importance of wild birds. *Journal of Wildlife Diseases* **43**, S47–S50.

Pfeiffer, D. U., Minh, P. Q., Martin, V., Epprecht, M., and Otte, M. J. (2007). An analysis of the spatial and temporal patterns of highly pathogenic avian influenza occurrence in Vietnam using national surveillance data. *Veterinary Journal* **174**, 302–309. doi:10.1016/j.tvjl.2007.05.010

Robinson, T. P., Franceschini, G., and Wint, W. (2007). The Food and Agriculture Organisation's gridded livestock of the world. *Veterinaria Italiana* **43**, 745–751.

Roche, B., Lebarbenchon, C., Gauthier-Clerc, M., Chang, C.-M., Thomas, F., Renaud, F., van der Werf, S., and Guégan, J.-F. (2009). Water-borne transmission drives avian influenza dynamics in wild birds: the case of the 2005–2006 epidemics in the Camargue area. *Infection, Genetics and Evolution* **9**, 800–805. doi:10.1016/j.meegid.2009.04.009

Röhm, C., Zhou, N., Suss, J., Mackenzie, J., and Webster, R. G. (1996). Characterization of a novel influenza hemagglutinin, H15: criteria for determination of influenza A subtypes. *Virology* **217**, 508–516. doi:10.1006/viro.1996.0145

Rose, K., Newman, S., Uhart, M., and Lubroth, J. (2006). 'Wild Bird HPAI Surveillance: Sample Collection from Healthy, Sick and Dead Birds.' (FAO: Rome.)

Roshier, D. A., Robertson, A. I., Kingsford, R. T., and Green, D. G. (2001). Continental-scale interactions with temporary resources may explain the paradox of large populations of desert waterbirds in Australia. *Landscape Ecology* **16**, 547–556. doi:10.1023/A:1013184512541

Roshier, D. A., Robertson, A. I., and Kingsford, R. T. (2002). Responses of waterbirds to flooding in an arid region of Australia and implications for conservation. *Biological Conservation* **106**, 399–411. doi:10.1016/S0006-3207(01)00268-3

Roshier, D. A., Klomp, N. I., and Asmus, M. (2006). Movements of nomadic waterfowl, grey teal *Anas gracilis*, across inland Australia – results from satellite telemetry spanning fifteen months. *Ardea* **94**, 461–475.

Roshier, D., Asmus, M., and Klaassen, M. (2008). What drives long-distance movements in the nomadic grey teal *Anas gracilis* in Australia? *Ibis* **150**, 474–484. doi:10.1111/j.1474-919X.2008.00806.x

Sabirovic, M., Wilesmith, J., Hall, S., Coulson, N., and Landeg, F. (2006). 'Outbreaks of HPAI H5N1 Virus in Europe during 2005/2006.' (International Animal Health Division: London.)

Selleck, P. W., Gleeson, L. J., Hooper, P. T., Westbury, H. A., and Hansson, E. (1997). Identification and characterisation of an H7N3 influenza A virus from an outbreak of virulent avian influenza in Victoria. *Australian Veterinary Journal* **75**, 289.

Selleck, P. W., Arzey, G., Kirkland, P. D., Reece, R. L., Gould, A. R., Daniels, P. W., and Westbury, H. A. (2003). An outbreak of highly pathogenic avian influenza in Australia in 1997 caused by an H7N4 virus. *Avian Disease* **47**, 806–811.

Siengsanan, J., Chaichoune, K., Phonaknguen, R., Sariya, L., Prompiram, P. *et al*. (2009). Comparison of outbreaks of H5N1 highly pathogenic avian influenza in wild birds and poultry in Thailand. *Journal of Wildlife Diseases* **45**, 740–747.

Sims, L. D., and Narrod, C. (2009). 'Understanding Avian Influenza.' (Food and Agriculture Organization of the United Nations: Rome.)

Sims, L. D., and Turner, A. J. (2008). Avian Influenza in Australia. In 'Avian Influenza'. (Ed. D. E. Swayne.) pp. 239–250. (Blackwell Publishing Ltd: Ames, IA.)

Sims, L. D., DomèNech, J., Benigno, C., Kahn, S., Kamata, A., Lubroth, J., Martin, V., and Roeder, P. (2005). Origin and evolution of highly pathogenic H5N1 avian influenza in Asia. *The Veterinary Record* **157**, 159–164.

Snow, L. C., Newson, S. E., Musgrove, A. J., Cranswick, P. A., Crick, H. Q. P., and Wilesmith, J. W. (2007). Risk-based surveillance for H5N1 avian influenza virus in wild birds in Great Britain. *The Veterinary Record* **161**, 775–781.

Sraml, M., Christidis, L., Easteal, S., Horn, P., and Collet, C. (1996). Molecular relationships within Australasian waterfowl (Anseriformes). *Australian Journal of Zoology* **44**, 47–58. doi:10.1071/ZO9960047

Stallknecht, D. E., and Brown, J. D. (2008). Ecology of avian influenza in wild birds. In 'Avian Influenza'. (Ed. D. E. Swayne.) pp. 43–58. (Blackwell Publishing Ltd: Ames, IA.)

Stallknecht, D. E., Kearney, M. T., Shane, S. M., and Zwank, P. J. (1990*a*). Effects of pH, temperature, and salinity on persistence of avian influenza viruses in water. *Avian Diseases* **34**, 412–418. doi:10.2307/1591429

Stallknecht, D. E., Shane, S. M., Kearney, M. T., and Zwank, P. J. (1990*b*). Persistence of avian influenza viruses in water. *Avian Diseases* **34**, 406–411. doi:10.2307/1591428

Sturm-Ramirez, K. M., Hulse-Post, D. J., Govorkova, E. A., Humberd, J., Seiler, P. *et al*. (2005). Are ducks contributing to the endemicity of highly pathogenic H5N1 influenza virus in Asia? *Journal of Virology* **79**, 11 269–11 279. doi:10.1128/JVI.79.17.11269-11279.2005

Tracey, J. P. (2005). Targeting surveillance for avian influenza in wild birds: a pilot investigation in New South Wales. Final report to the Department of Agriculture, Fisheries and Forestry, Wildlife and Exotic Disease Preparedness Program. NSW Department of Primary Industries, Orange, NSW.

Tracey, J. P., Woods, R., Roshier, D., West, P., and Saunders, G. R. (2004). The role of wild birds in the transmission of avian influenza for Australia: an ecological perspective. *Emu* **104**, 109–124. doi:10.1071/MU04017

Truscott, J., Garske, T., Chis-Ster, I., Guitian, J., Pfeiffer, D., Snow, L., Wilesmith, J., Ferguson, N. M., and Ghani, A. C. (2007). Control of a highly pathogenic H5N1 avian influenza outbreak in the GB poultry flock. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **274**, 2287–2295. doi:10.1098/rspb.2007.0542

Turner, A. J. (1976). The isolation of fowl plague virus in Victoria. *Australian Veterinary Journal* **52**, 384.

Turner, A. J. (2004). The role of wild aquatic birds in the epidemiology of avian influenza in Australia. *Australian Veterinary Journal* **82**, 713. doi:10.1111/j.1751-0813.2004.tb12168.x

Wang, J., Vijaykrishna, D., Duan, L., Bahl, J., Zhang, J. X., Webster, R. G., Peiris, J. S. M., Chen, H., Smith, G. J. D., and Guan, Y. (2008). Identification of the progenitors of Indonesia 1 and Vietnam avian influenza A (H5N1) viruses from southern China. *Journal of Virology*. doi:10.1128/JVI.02468-07

Warner, S., Welch, A., Ainsworth, C., Tracey, J. P., Zikesch, F., Saunders, G. R., and Lukins, B. (2006). Application of rapid diagnostic tests in the targeted surveillance of avian influenza virus within Victorian wild bird populations. Final Report to the Wildlife and Exotic Disease Preparedness Program. Primary Industries Research Victoria, Melbourne. Available at www.daff.gov.au/__data/assets/pdf_file/0009/581886/ai-wildbirds-vic-august06.pdf [verified November 2009].

Webster, R. G., Yakhno, M., Hinshaw, V. S., Bean, W. J., and Murti, K. G. (1978). Intestinal influenza: Replication and characterization of influenza virus in ducks. *Virology* **84**, 268–278. doi:10.1016/0042-6822(78)90247-7

Westbury, H. A. (1998). History of high pathogenic avian influenza in Australia and the H7N3 outbreak (1995). In 'Proceedings of the Fourth International Symposium on Avian Influenza, May 29–31 1997'. (Eds D. E. Swayne and R. D. Slemons.) pp. 23–30. (United States Animal Health Association: Athens, GA.)

Woodall, P. F. (1985). Waterbird populations in the Brisbane region, 1972–83, and correlates with rainfall and water heights. *Australian Wildlife Research* **12**, 495. doi:10.1071/WR9850495

World Health Organization (WHO) (2009). 'Areas Reporting Confirmed Occurrence of H5N1 Avian Influenza in Poultry and Wild Birds since 2003. Available at http://gamapserver.who.int/mapLibrary/Files/Maps/Global_SubNat_H5N1inAnimalConfirmedCUMULATIVE_200802021.png [verified November 2009].

Manuscript received 4 November 2009, accepted 8 February 2010