Australian VETERINARY JOURNAL
THE JOURNAL OF THE AUSTRALIAN VETERINARY ASSOCIATION LTD
AVIAN

Avian influenza in Australia: a summary of 5 years of wild bird surveillance

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Background Avian influenza viruses (AIVs) are found worldwide in numerous bird species, causing significant disease in gallinaceous poultry and occasionally other species. Surveillance of wild bird reservoirs provides an opportunity to add to the understanding of the epidemiology of AIVs.

Methods This study examined key findings from the National Avian Influenza Wild Bird Surveillance Program over a 5-year period (July 2007–June 2012), the main source of information on AIVs circulating in Australia.

Results The overall proportion of birds that tested positive for influenza A via PCR was 1.9 ± 0.1%, with evidence of widespread exposure of Australian wild birds to most low pathogenic avian influenza (LPAI) subtypes (H1–13, H16). LPAI H5 subtypes were found to be dominant and widespread during this 5-year period.

Conclusion Given Australia's isolation, both geographically and ecologically, it is important for Australia not to assume that the epidemiology of AIV from other geographic regions applies here. Despite all previous highly pathogenic avian influenza outbreaks in Australian poultry being attributed to H7 subtypes, widespread detection of H5 subtypes in wild birds may represent an ongoing risk to the Australian poultry industry.

Keywords Australia; avian influenza; biosecurity; surveillance; wild birds

Abbreviations AIV, avian influenza virus; LPAI, low pathogenic avian influenza; HA, haemagglutinin; HPAI, highly pathogenic avian influenza; NA, neuraminidase; rRT-PCR, real-time reverse transcription polymerase chain reaction; The Program, National Avian Influenza Wild Bird Surveillance Program

Aust Vet J 2015;93;387–393 doi: 10.1111/avj.12379

Avian influenza viruses (AIVs) are found worldwide in numerous bird species and can cause significant disease in gallinaceous poultry and occasionally infect a range of other species, including humans.[1] Classification of AIVs is based on the serological subtypes of the viral surface glycoproteins, with 16 haemagglutinin (HA; H1–16) and 9 neuraminidase (NA; N1–9) subtypes recognised in birds.[1] Of global concern is the capacity of AIV subtypes H5 and H7 to mutate from low pathogenic (LPAI) into the highly pathogenic (HPAI) forms that have caused substantial losses in poultry[2] and wildlife.[3] Furthermore, serious public health implications have arisen from avian-to-human transmission of specific AIV subtypes (i.e. H5, H7, H9 and H10), mainly from domestic birds.[4] These concerns are highlighted by regular HPAI H5N1 outbreaks since 2003 in Asia that spread to other continents, affecting humans and domestic and wild birds.[2] Furthermore, recent LPAI H7N9- and LPAI H10N8-associated human mortalities have occurred in China.[4] The role of wild bird reservoirs in the epidemiology of AIVs continues to gain the attention of public health, agricultural and wildlife agencies globally, with surveillance targeting the main natural host reservoirs of the avian orders Anseriformes and Charadriiformes.[3,5,6]

The majority of Anseriformes in Australia are non-migratory, unlike in the northern hemisphere, but instead are nomadic within the Australo-Papuan region, with movements largely determined by the presence of flooding and ephemeral wetlands.[7,8] In contrast, 3 million Charadriiformes make annual trans-hemispheric migrations via HPAI H5N1-endemic south-east Asian countries to spend their non-breeding season in Australia.[8] Therefore, many of the findings regarding AIV ecology, molecular phylogenetics and spread in Asia, Europe or North America may not be relevant to Australia.[9,10] For this reason, Australian surveillance of wild bird reservoirs, both migratory (Charadriiformes) and nomadic (Anseriformes), is necessary to further our understanding of AIVs on the island continent and to assess and manage the risk to both animals and humans.

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Prior to 2005, Australian wild bird AIV studies had detected a range of LPAI subtypes (H1–6, H11, H12, H15);[11–15] however, geographic and temporal coverage was limited and sampling was not well coordinated. In 2006, Australia established the National Avian Influenza Wild Bird Surveillance Program (The Program) to facilitate collaboration between a range of government and non-government organisations to promote coordination of information to inform the national picture. The Program includes: (i) pathogen-specific, risk-based surveillance via convenience sampling of apparently healthy, live and hunter-killed wild birds; and (ii) enhanced passive surveillance via investigation of significant, unexplained morbidity and mortality events in wild birds. Here we provide some key findings from the national wild bird AIV aggregated data generated from pathogen-specific, risk-based surveillance over a 5-year period (July 2007–June 2012).

Materials and methods

Between 2007 and 2012, timing and locations targeted for surveillance were determined by participating agencies (see author affiliations) in seven states and territories (Figure 1) and were chosen based on: (i) close interaction between migratory Charadriiformes and resident Anserifomes species; (ii) populations with previous evidence of AIVs; and (iii) close proximity to poultry farms and/or human populations[8,16,17] to maximise efficiency and relevance. Surveillance activities were pathogen-specific and risk-based as defined by Hoinville et al.[18] Samples were collected specifically for the purpose of testing for AIVs. To further maximise surveillance efficiency, sampling focused on waterbird populations, which, based on their ecology and possibly physiology, are particularly prone to AIV infection.[5,6]

For virological analysis, oropharyngeal, cloacal or fresh faecal environmental swabs were collected from individual wild birds. Swabs were tested using pan influenza A real-time reverse transcription polymerase chain reaction (rRT-PCR) assays targeting the matrix gene.[19] All influenza A-positive samples were further tested using specific rRT-PCRs for influenza A H5 and H7 viruses.[19] Whenever possible, positive samples were subjected to virus culture in embryonated chicken eggs and further molecular analysis (e.g. subtyping PCR and DNA sequencing, and/or microarray subtyping), with all H5- and H7-positive samples characterised and confirmed at the CSIRO Australian Animal Health Laboratory.[17,19] Serum samples were collected from wild birds and tested for antibodies to the influenza A virus nucleoprotein using a blocking ELISA (b-ELISA).[19,20] All sampling of wild birds was approved by the relevant institutional animal ethics committees in each state/territory (details available on request).

Statistical analysis

The proportion of birds positive for AIV was defined as the number of positives for influenza A genome or antibody, respectively, divided by the total number of birds from which swabs or sera were collected. One bird is equal to one swab or serum sample. If an individual bird was sampled using both cloacal and oropharyngeal

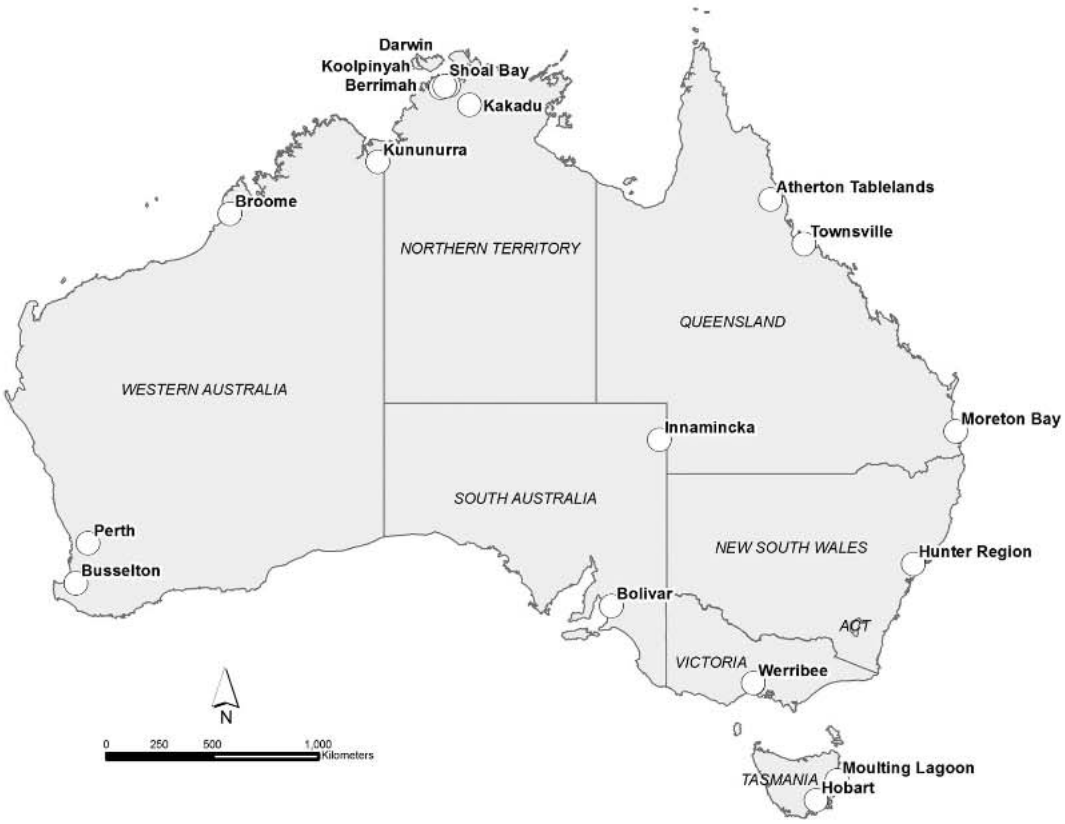

Figure 1. Key locations for targeted wild bird influenza A surveillance in each state/territory.

swabs (≈7% of samples), these were considered as one bird/swab sample for calculations. If swabs were pooled for analysis, a positive pool was taken as most likely to represent one positive. Confidence intervals were provided to show variability within the subpopulation studied, rather than for the total population of wild birds, and calculated as if a random sample had been taken from the subpopulation.

Binomial generalised linear models were fitted in Program R[21] to test the effects of year (2007–2012), state/territory (n = 7) and interactions on the proportion of birds rRT-PCR-positive for influenza A. The same approach was used to test the effects of taxonomic order (Anseriformes, Charadriiformes, other) and year, and taxonomic order and sample type (cloacal/oropharyngeal and faecal environmental). Stepwise regression was performed to remove non-significant terms ($P > 0.05$) sequentially. Analyses were conducted separately for the proportion of birds positive for influenza A genome and antibodies.

Results

Between July 2007 and June 2012, 50,684 swabs and 8387 serum samples were collected (Figure 2, Table S1). Approximately 73% of swabs (71% of sera) were collected from Anseriformes, 26% (21% of sera) from Charadriiformes and 1–2% (8% of sera) from other bird orders (Table 1).

The overall proportion of birds that tested positive for influenza A virus was 1.9 ± 0.1% (n = 50,684), with a total of 988 birds rRT-PCR-positive for influenza A virus (Table 2). The overall proportion of birds that tested positive for influenza A antibodies was 22.4 ± 1.0% (n = 8387), with 1881 bird sera b-ELISA positive for influenza A (Table 3). No differences were found between the proportion of birds rRT-PCR-positive for influenza A or the virus isolation rate in cloacal/oropharyngeal compared with faecal environmental samples (Table 1).

The proportion of birds that were rRT-PCR-positive for influenza A varied significantly with year ($z = 2.967$, $P = 0.003$), state/territory ($z = -4.856$, $P < 0.001$) and their interaction ($z = -2.391$, $P < 0.001$). The proportion of birds that were rRT-PCR-positive for influenza A was greater in 2010–2011 (3.0 ± 0.4%, n = 8621) and 2011–2012 (3.1 ± 0.4%, n = 9636) than in other years (1.3 ± 0.1%, n = 32,427). The proportion of birds that were rRT-PCR-positive for influenza A was greater in Victoria (3.1 ± 0.3%, n = 11,377) and New South Wales (2.7 ± 0.3%, n = 12,962) than in the other states/territories (1.1 ± 0.1%, n = 26,345). The proportion of birds that were rRT-PCR-positive for influenza A was significantly greater in Anseriformes (2.5 ± 0.2%, n = 36,885; $P < 0.001$, $z = -45.8$) than in Charadriiformes (0.6 ± 0.1%, n = 12,988) and other species (0.5 ± 0.5%, n = 811).

Further molecular analysis was attempted on 20 AIV isolates and 85% (n = 834) of rRT-PCR positives. Subtyping was successful for 53% (n = 442) of positive samples and all HA subtypes were identified, except H14 and H15 (Fig. 3). HPAI H5 or H7 viruses were not detected in any sample. A total of 94 LPAI H5 subtypes were detected in five states (New South Wales, South Australia, Tasmania, Victoria and Western Australia) and 17 LPAI H7 subtypes were detected in three states (New South Wales, South Australia, Victoria) (Figure 3).

Discussion

The surveillance of Australian wild birds through The Program continues to contribute to the understanding of AIV epidemiology, provides valuable information concerning circulating AIV subtypes in wild birds and maintains sampling and diagnostic capability. Specifically, Australian wild bird AIV surveillance provides the principle

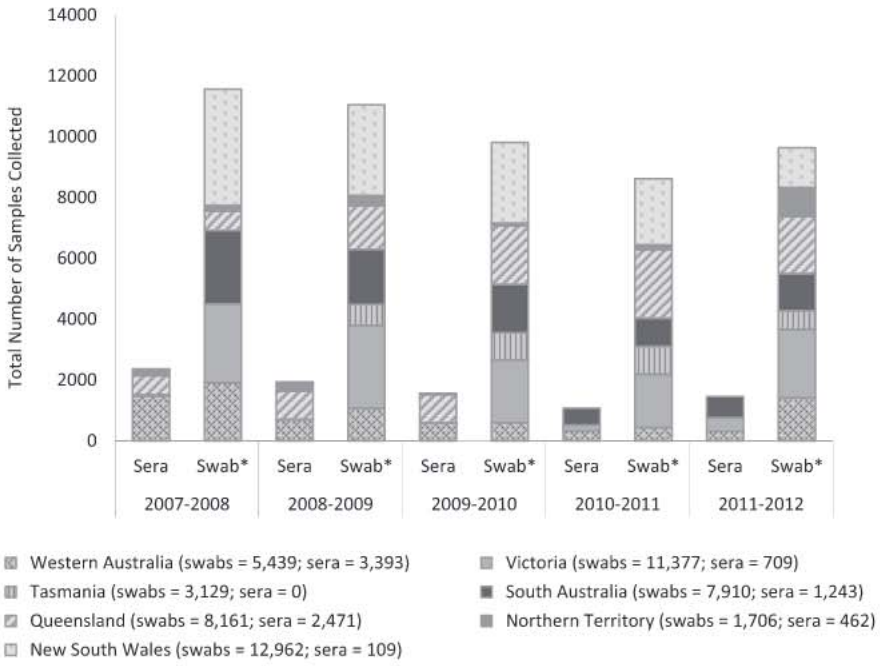

Figure 2. Number of swabs and serum samples collected from Australian wild birds for avian influenza surveillance (July 2007–June 2012). One bird is equal to one swab or serum sample. If an individual bird was sampled using both cloacal and oropharyngeal swabs (approximately 7% of samples), these were considered as one bird/swab sample for calculations (see Table S1).

Table 1. Comparison of the proportion of wild birds positive for influenza A virus[a] by sample type and Order

Sample type	Anseriformes	Charadriiformes	Other[b]	All bird orders
Faecal environmental[c]	2.3 ± 0.2%	0.4 ± 0.2%	4[d] ± 4.4%	2.0 ± 1.5%
	(n = 28,253)	(n = 6299)	(n = 100)	(n = 34,652)
Cloacal/oropharyngeal	3.0 ± 0.4%	0.7 ± 0.2%	0 ± 0.3%	1.9 ± 0.2%
	(n = 8632)	(n = 6689)	(n = 711)	(n = 16,032)
Total	2.5 ± 0.2%	0.6 ± 0.1%	0.5 ± 0.5%	1.9 ± 0.1%
	(n = 36,885)	(n = 12,988)	(n = 811)	(n = 50,684)

[a]Calculated as the number of birds positive for influenza A virus on PCR and/or virus isolation divided by the total number of birds sampled ± 95% confidence intervals. n, number of swabs collected from wild birds or the environment. One bird is equal to one swab or serum sample. If an individual bird was sampled using both cloacal and oropharyngeal swabs (approximately 7% of samples), these were considered as one bird/swab sample for calculations. If swabs were pooled for analysis, a positive pool is taken as most likely to represent one positive.
[b]Includes Ciconiiformes, Gruiformes, Pelecaniformes and Procellariiformes.
[c]Faecal environmental swabs collected from Anseriformes/Charadriiformes at locations where small numbers of other bird species may have been present.
[d]Ciconiiformes were identified as the most likely source of all four influenza A-positive faecal environmental samples.

Table 2. Proportion of wild birds positive for influenza A virus in Australia (July 2007–June 2012)[a]

State/Territory	2007–08[b]	2008–09[b]	2009–10[b]	2010–11[b]	2011–12[b]	Overall (2007–12)
NSW	2.1 ± 0.5%	3.2 ± 0.6%	1.3 ± 0.4%	2.6 ± 0.7%	6.4 ± 1.4%	2.7 ± 0.3%
NT	0 ± 1.1%	0 ± 0.6%	0 ± 2.1%	0.7 ± 1.8%	0.5 ± 0.5%	0.4 ± 0.3%
QLD	1.3 ± 0.9%	0.9 ± 0.5%	0.77 ± 0.4%	0.5 ± 0.3%	2.1 ± 0.7%	1.1 ± 0.2%
SA	0.4 ± 0.3%	0.7 ± 0.4%	0.6 ± 0.4%	1.9 ± 0.9%	3.9 ± 1.1%	1.2 ± 0.2%
TAS	NA	0.3 ± 0.5%	0 ± 0.2%	1.3 ± 0.8%	1.3 ± 1.0%	0.7 ± 0.3%
VIC	1.3 ± 0.4%	0.9 ± 0.4%	3.0 ± 0.7%	9.1 ± 1.4%	3.6 ± 0.8%	3.1 ± 0.3%
WA	0.1 ± 0.2%	2.3 ± 0.9%	0.5 ± 0.7%	0.2 ± 0.6%	2.7 ± 0.9%	1.3 ± 0.3%
Overall	1.1 ± 0.2%	1.6 ± 0.2%	1.2 ± 0.2%	3.0 ± 0.4%	3.1 ± 0.4%	1.9 ± 0.1%

[a]Calculated as the number of birds positive for influenza A virus on PCR and/or virus isolation divided by the total number of birds sampled ± 95% confidence intervals. One bird is equal to one swab or serum sample. If an individual bird was sampled using both cloacal and oropharyngeal swabs (approximately 7% of samples), these were considered as one bird sample for calculations. If swabs were pooled for analysis, a positive pool is taken as most likely to represent one positive.
[b]A year runs from 1 July until 30 June in the next year.
NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia; NA, not applicable.

source of AIV detections and sequence data to allow monitoring of HA and NA gene primer target sequence variability. This monitoring reduces the possibility of AIV detection failure, which could result from tests based solely on non-Australian AIV strains,[22,23] and provides confidence that the tests in use will detect contemporary strains of AIVs,[24,25] especially H5/H7, in Australia. These outputs provide valuable support for contingency planning and preparedness for response and management.

Although the present results showed widespread exposure of Australian wild birds, with the overall proportion of birds that were rRT-PCR-positive for influenza A being 1.9 ± 0.1%, longitudinal surveillance shows that AIV detections fluctuate temporally and geographically and justifies future analyses to explore in more detail the spatial and temporal trends, as well as environmental and species-specific variables.

The variability of AIVs detected between years and state to state in this study aligns with results of previous Australian studies.[16,17,26] Although analysis of the proportion of birds positive for influenza A based on an aggregated dataset is useful for overall trends, there are inherent biases (e.g. specific locations and time periods were not taken into account; datasets were not balanced; and pathogen-specific, risk-based surveillance can increase the likelihood of AIV detection[5]) and therefore our ability to interpret these trends is limited. Further analysis could explore differences between species, functional groups and seasons as well as climatic and rainfall zones.

Overall, no differences were found between the proportion of birds that were rRT-PCR-positive for influenza A or the virus isolation rate in cloacal/oropharyngeal compared with faecal environmental samples. This is in contrast to previous studies, which found higher

Table 3. Proportion of wild birds positive for influenza A antibodies (July 2007–June 2012)[a]

State/Territory	2007–08[b]	2008–09[b]	2009–10[b]	2010–11[b]	2011–12[b]	Overall (2007–12)
NSW	26 ± 14.5%	0 ± 3.3%	NA	NA	NA	11.9 ± 6.7%
NT	37.3 ± 9.0%	1.2 ± 1.6%	2.5 ± 6.9%	NA	NA	15.2 ± 3.6%
QLD	10.6 ± 2.6%	12.7 ± 2.3%	19.7 ± 2.9%	NA	NA	14.7 ± 1.5%
SA	NA	NA	NA	6.6 ± 2.2%	17.4 ± 3.1%	12.6 ± 2.0%
TAS	NA	NA	NA	NA	NA	NA
VIC	0 ± 5.6%	NA	0 ± 17.9%	39.2 ± 8.6%	44.9 ± 6.1%	40.3 ± 4.7%
WA	33.5 ± 3.0%	23.4 ± 3.6%	27.8 ± 4.3%	23.3 ± 5.3%	30.7 ± 6.2%	29.2 ± 1.8%
Overall	27.0 ± 2.1%	14.7 ± 1.7%	22.2 ± 2.3%	17.8 ± 2.5%	28.8 ± 2.8%	22.4 ± 1.0%

[a]Calculated as the number of birds positive for influenza A antibodies on blocking ELISA divided by the total number of sera collected ± 95% confidence intervals. One bird is equal to one swab or serum sample.
[b]A year runs from 1 July until 30 June in the next year.
NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia; NA, not applicable.

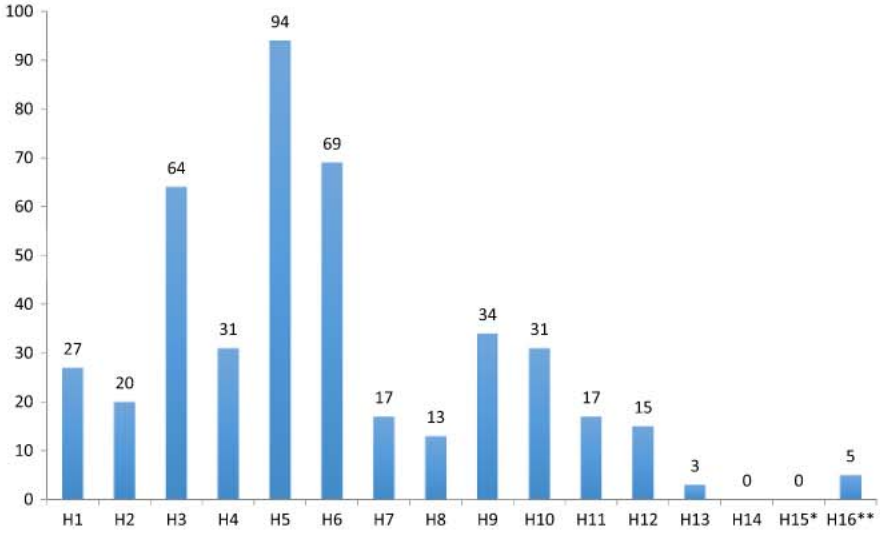

Figure 3. Number of Australian wild bird avian influenza virus (AIV) subtype detections (July 2007–June 2012). For three real-time reverse transcription polymerase chain (rRT-PCR)-positives, two subtypes were detected and confirmed via sequencing and/or successful virus isolation (combination detected were: H5/H7 (pooled sample), H5/H10 (pooled sample), H6/H9 (individual cloacal sample) with both subtype detections included in each subtype count in the histogram). For five rRT-PCR-positives, two presumptive subtypes were detected; however, further testing could not confirm a final subtype (combinations detected were: H1/H4; H3/H5; H3/H6; H6/H16; H8/H12). The subtypes from these five rRT-PCR positives were not included in the subtype count in the histogram. *H15 was detected in Australian wild birds prior to 2005.[14] **All samples positive for H16 were faecal environmental swabs collected from Anseriformes at locations where small numbers of other bird species may have been present.

prevalence in cloacal/oropharyngeal samples.[16,27] The ability to further subtype rRT-PCR-positive samples also did not differ with sample type (≈47% of rRT-PCR-positives from cloacal/oropharyngeal samples subtyped vs approximately 56% of rRT-PCR-positive from faecal environmental samples subtyped). Given faecal environmental samples are less expensive and logistically easier to collect,[16] our study supports their continued use for wild bird AIV surveillance in Australia where broad-scale wild bird surveillance is logistically challenging,[8] while continuing to take into account the limitations associated with using this sample type (e.g. species identification).

Successful subtyping in this study showed exposure of Australian wild birds to most LPAI subtypes (H1–13, H16). Although not detected in this study, H15 was detected in Australian wild birds prior to 2005.[14] Prior to 2007, LPAI H5 had been detected in Queensland[15] and LPAI H7 in Tasmania.[17] Our new data show that LPAI H5 subtypes are a predominant and widespread subtype, and may therefore represent a risk to the poultry industry,[28] despite all previous Australian HPAI incidents in poultry being attributed to H7.[29–31]

Phylogenetic analysis of the HA genes show that Australian AIVs typically form separate sub-clades of the Eurasian AIV lineage.[26] However, recent analysis of H10 AIVs detected in Australia since 2010 show that their HA genes are derived from North American-lineage AIVs,[24] which adds to the limited evidence of virus introductions from North America.[11] This molecular epidemiology and phylogenetic analysis highlights the value of Australian wild bird surveillance in enhancing the global understanding of AIV distribution and dispersal.[24,25]

Although HPAI H5N1 remains endemic in many neighbouring Asian countries, risk analyses indicate a low likelihood of AIVs being introduced into Australia via migratory birds.[32,33] Our data show a low frequency of AIVs in these birds soon after their arrival.[26] However, recent wild bird population genetic studies show New Guinea to be the source of Anseriformes populations in northern Australia[34]

and the recent introduction and establishment of an H10 AIV reassortant virus with a North American HA gene emphasises the need to remain vigilant to the further incursion of exotic AIVs into Australia.

Acknowledgments

The Program depends on the participation, expertise and logistic support from a large number of people from a range of organisations and properties, including the Australian Department of Environment; Birdlife Australia; Gidgealpa Station; Innamincka Station; Melbourne Water; Parks South Australia; Rural Industries Research and Development Corporation; Santos; state and territory bird banding groups and wildlife/shorebird groups (South Australian, Queensland and Victorian Wader Study Groups); hunter groups and Wetlands International. We would like to specifically acknowledge Sue Martin, Marianne Douglass, Teresa Wilson, Department of Primary Industries, Parks, Water and Environment, Tasmania; Kerri Bruce, Susie Daglas, Kelly Davies, Debbie Eagles, Wendy Ha, Peter Mohr, Songhua Shan, Paul Selleck, Vicky Stevens, Stacey Valdeter, Som Walker, James Watson, Frank Wong, Diagnosis Surveillance and Response team at the Australian Animal Health Laboratory; Melinda Frost, New South Wales Department of Primary Industries; and the Biosecurity Science Laboratory Team, Biosecurity Queensland, Department of Agriculture and Fisheries for their valuable work on the analysis and characterisation of avian influenza samples. We would also like to thank to Tim Kerlin, Chris Rodwell, Josef Schmidt and Cassandra Wittwer, Australian Government Department of Agriculture, and Fred van Gessel, the University of Newcastle for wild bird sample collection. Thank you to Chris Barker and John Worsley for assistance with the final images. A special thanks to Chris Bunn, Australian Government Department of Agriculture; Leesa Haynes; and Keren Cox-Witton and Karen Magee, Wildlife Health Australia. The Program was supported with funding from the Australian Government Department of Agriculture; the Wildlife Exotic Disease Preparedness Program; National Health & Medical Research Council; Australian Biosecurity Cooperative Research Centre; National Institute of Health (NIH contract NIAIDHHSN266200700010C) and the Rural Industries Research and Development Corporation (RIRDC Project no. PRJ-008337; 2011–2012). The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health. Significant in-kind support is provided by participating agencies, without which The Program would not be possible. The authors would like to thank the reviewers for their valuable comments.

Authors' contributions

VLG, KEA, PMH, ACH, SW drafted and revised the manuscript for important intellectual content. Additional important editorial content changes were provided by JB, GB, BC, TH, MK, DR, JPT, RW, LP. VLG, KEA, PMH, ACH, SW, JB, GB, BC, CJD, TH, RBJ, MK, NYK, DR, JPT, RW, LP contributed to the analysis and interpreted the data. Conception and surveillance design for The Program was undertaken by KEA, PMH, SW, GB, BC, CJD, MF, TH, MDAH, RBJ, MK, PDK, NYK, DR, LFS, JPT, RW, LP. KEA, PMH, ACH, SW, JB, GB, BC, CJD, MF, TH, MDAH, RBJ, MK, PDK, NYK, SL, KO, DR, JPT coordinated and undertook acquisition of wild bird samples. VLG, KEA, SW, GB, BC, CJD, MF, TH, MDAH, RBJ, MK, PDK, NYK, DR, JPT, RW coordinated and collated data for the nationally aggregated dataset. Laboratory and sequence analysis of samples was conducted by KEA, PMH, ACH, SW, JB, GB, MF, MDAH, PDK, SL, MO, KO and XW.

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Supporting Information

Additional supporting information can be found in the online version of this article at the publisher's website: http://onlinelibrary.wiley.com/doi/10.1111/avj.12379/suppinfo.

Table S1. Number of swabs and serum samples collected from Australian wild birds for avian influenza surveillance (July 2007–June 2012).

(Accepted for publication 25 May 2015)