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Contribution to the 2012 Avian Influenza in Wild Birds Surveillance Program



March 2015

RIRDC Publication No. 15/016



Australian Government

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Development Corporation**

Contribution to the 2012 Avian Influenza in Wild Birds Surveillance Program

by Tiggy Grillo

March 2015

RIRDC Publication No 15/016
RIRDC Project No PRJ-008337

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ISBN 978-1-74254-760-2
ISSN 1440-6845

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Electronically published by RIRDC in March 2015
Print-on-demand by Union Offset Printing, Canberra at www.rirdc.gov.au
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Foreword

Avian influenza (AI) is an infectious disease of birds caused by influenza virus type A strains and is a significant problem around the world due to the devastating effects of highly pathogenic avian influenza (HPAI) virus subtypes in poultry.

In 2006 Australia further strengthened national surveillance for AI in poultry and wild birds and established the National Avian Influenza Wild Bird (NAIWB) Steering Group to ensure national coordination and collaboration for wild bird AI surveillance under the NAIWB Surveillance Program (the Program). The Steering Group comprises members from Australia's governments, industry, universities and zoos. There is also representation from others actively undertaking AI surveillance activities in wild birds in Australia, or those who have a need for outputs of the program to inform both policy and decision making.

The objective of the Program is to ensure that Australia can be 95% confident that AI infection would be detected in at least one wild bird if it was present at a prevalence of at least 1% in Australia's wild bird population. Other objectives include: excluding H5 (including H5N1) or H7 as a cause of wild bird mortality events; detection and reporting of circulating AI viruses (AIVs) (including low pathogenic avian influenza (LPAI) and HPAI H5 and H7); maintaining laboratory capability and capacity for influenza virus detection, and; gaining an understanding of the influenza viruses circulating in wild birds in Australia.

The Program activities are conducted Australia-wide, with national funding provided by the Australian Government (Department of Agriculture - DoA) and, in the year covered by this survey, the Rural Industries Research and Development Corporation – RIRDC. In-kind support is provided by the jurisdictional agencies, researchers and representative's institutions.

The Program continues to find evidence of a wide range of subtypes of LPAI AIVs, including LPAI H7 and H5 subtypes in wild birds in Australia. The Program contributes to the understanding of the risk of AIVs from wild birds, provides valuable information concerning circulating AIV subtypes as well as maintaining AIV sampling and diagnostic capability and capacity. Outcomes continue to inform policy for prevention and management of AI outbreaks in Australian poultry flocks. These findings support the need for continuing surveillance activities in wild birds and reiterate the need for poultry producers to remain alert and ensure that appropriate biosecurity arrangements and effective risk reduction measures for AI are in place at their premises.

While the results presented in this report cover survey activities between July 2011 and June 2012, the National Avian Influenza Wild Bird Surveillance Program is ongoing. For further information go to <https://www.wildlifehealthaustralia.com.au/ProgramsProjects/AvianInfluenzaWildBirdSurveillance.aspx>, or for an update on more recent results contact the National Coordinator (researcher contact details provided above).

This report is an addition to RIRDC's diverse range of over 2000 research publications and it forms part of our Chicken Meat R&D program, which aims to stimulate and promote R&D that will deliver a productive and sustainable Australian chicken meat industry that provides quality wholesome food to the nation.

Most of RIRDC's publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
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About the Author

Tiggy Grillo is the National Coordinator for Wildlife Health Australia (WHA; formerly Australian Wildlife Health Network). WHA is a not-for-profit, incorporated association that comprises a network of government and private stakeholders across Australia. Its mission is to promote and facilitate collaborative links in the investigation and management of wildlife health in support of human and animal health, biodiversity and trade. Core funding is provided by the Australian Department of Agriculture (DoA).

An important business activity for WHA is the provision of administrative support to WHA stakeholders and funding bodies to assist them with running and managing national wildlife health projects and programs. One of these projects is the National Avian Influenza Wild Bird Surveillance Program (the NAIWB Surveillance Program; the Program), which coordinates national surveillance activities for avian influenza viruses (AIVs) in wild birds in Australia.

Acknowledgments

The NAIWB Surveillance Program depends upon the participation of a large number of people from a range of organisations and agencies. The Program would not have been possible without the valuable help of many people including those assisting in the collection and analysis of samples and those coordinating and reporting results.

The author would like to acknowledge the support of all contributors including Edla Arzey, Jemma Bergfeld, Stewart Blackhall, Kim Bonner, Graham Burgess, Tom Mary Carr, Beth Cookson, Keren Cox-Witton, Kim Critchley, Celia Dickason, Hume Field, Phil Hansbro, Tom Hollingsworth, Aeron Hurt, Bruce Jackson, Tim Kerlin, Marcel Klaassen, Peter Kirkland, Nina Kung, Sue Martin, Helen McCracken, James O'Connor, Mark O'Dea, Paul O'Neill, Kim O'Riley, Annie Philips, Lyndel Post, Stephen Pycroft, Chris Rodwell, David Roshier, Melissa Scarlett, Paul Selleck, Margaret Sexton, Elena Virtue, Dragon Wang, Simone Warner, Doug Watkins, James Watson, Teresa Wilson, Frank Wong and Vivien Kite.

Agencies and organisations which have contributed to this program include the Australian Department of Agriculture (DoA); Australian Department of Agriculture's Northern Australia Quarantine Strategy (NAQS) program; Australian Department of Environment (DoE); Australian Registry of Wildlife Health; CSIRO Australian Animal Health Laboratory (AAHL); Birdlife Australia; bird banding groups and wildlife/shorebird groups (Victorian Waders Study Group); Department of Agriculture and Food Western Australia (DAFWA); Department of Primary Industries, Parks, Water and Environment Tasmania (DPIPWE); Department of Economic Development, Jobs, Transport and Resources, Victoria (DEDJTR, Vic); Department of Sustainability and Environment, Victoria; hunter groups; New South Wales Department of Primary Industries (NSW DPI); Northern Territory Government, Department of Resources; Primary Industries and Resources, South Australia (PIRSA); Department of Agriculture, Fisheries and Forestry Queensland (DAFF – Queensland); University research groups (Deakin University, James Cook University, University of Newcastle and Victoria University); Wetlands International; World Health Organisation Collaborating Centre for Reference and Research on Influenza, Melbourne (WHO); wildlife hospitals at the major Australian zoological parks (Adelaide, Australia, Currumbin, Healesville Sanctuary, Melbourne, Perth, Taronga); and the Rural Industries Research and Development Corporation (RIRDC).

Abbreviations

AAHL	CSIRO Australian Animal Health Laboratory
AI	Avian Influenza
AIV	Avian Influenza Virus
ARWH	Australian Registry of Wildlife Health
AWHN	Australian Wildlife Health Network
DoA	Australian Department of Agriculture
DAFF (Queensland)	Department of Agriculture, Fisheries and Forestry Queensland
DAFWA	Department of Agriculture and Food Western Australia
DPIPWE	Department of Primary Industries, Parks, Water and Environment Tasmania
DEDJTR Vic	Department of Economic Development, Jobs, Transport and Resources, Victoria
DoE	Australian Department of Environment
LPAI	Low pathogenic avian influenza
HA	Haemagglutinin
HPAI	Highly pathogenic avian influenza
NA	Neuraminidase
NAIWB	National Avian Influenza Wild Bird
NAQS	Australian Department of Agriculture, Fisheries and Forestry's Northern Australia Quarantine Strategy program
PCR	Polymerase chain reaction
PIRSA	Primary Industries and Resources, South Australia
NSW DPI	New South Wales Department of Primary Industries
RIRDC	Rural Industries Research and Development Corporation
The Program	The NAIWB Surveillance Program
WHA	Wildlife Health Australia
WHO	World Health Organisation Collaborating Centre for Reference and Research on Influenza, Melbourne

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Executive Summary

What the report is about

This report outlines results from the sampling of wild birds as part of the National Avian Influenza Wild Bird Surveillance Program (the Program) between July 2011 and June 2012.

The Program provides a good understanding of the avian influenza viruses (AIVs) circulating in wild birds in Australia, contributes to better decision-making and knowledge of risk factors, and provides an early warning system. Wild bird surveillance is part of the broad national approach to identifying and managing the risks from avian influenza (AI).

Who is the report targeted at?

National wild bird surveillance information contributes to Australia's international reporting responsibilities and underpins trade negotiations and certification. The information gathered by this project ensures that Australian governments and the poultry industry are informed about potential risks from AIVs carried by wild birds in Australia.

Where are the relevant industries located in Australia?

The whole of the poultry industry in Australia can benefit from the results of this program. As home and hobby farms increase and industries diversify to organic and free-range systems, there will be a greater need to monitor the risk of transmission of AIVs from wild birds to these flocks. The majority of commercial poultry activities reside in the south-eastern parts of Australia extending from the eastern coastline of Queensland, through New South Wales, Victoria and Tasmania. Poultry activities are also located in South Australia, focused around south-east corner of the state, and Western Australia, predominately near Perth and stemming slightly into the south-west corner of the state (OCVO, 2010).

Background

AI is an infectious disease of birds caused by influenza virus type A strains and is a significant problem around the world due to the devastating effects of highly pathogenic avian influenza (HPAI) virus subtypes in poultry. Wild birds, predominantly from the Order Anseriformes and Charadriiformes form the natural reservoir for AIVs and usually show no clinical signs of disease (Stallknecht *et al.*, 2007; Olsen *et al.*, 2006). Globally, wild bird AI surveillance continues to further our knowledge and understanding, but there is still much to be understood about the ecology of these viruses in their natural reservoir hosts (Feare 2010; Klaassen *et al.*, 2011; Munster *et al.*, 2007).

In 2006 Australia further strengthened national surveillance for AI in poultry and wild birds and established the National Avian Influenza Wild Bird Steering Group (the Steering Group) to ensure national coordination and collaboration for AI wild bird surveillance under NAIWB Surveillance Program. The Steering Group comprises members from Australia's governments, industry, universities and zoos. There is also representation from others actively undertaking AI surveillance activities in wild birds in Australia, or those who have a need for outputs of the program to inform both policy and decision making.

The Program conducts activities Australia-wide, with national funding provided by DoA, including contributions from specific programs such as the Northern Australian Quarantine Strategy (NAQS) program and the Wildlife Exotic Disease Preparedness Program (WEDPP) and the Rural Industries Research and Development Corporation (RIRDC). In-kind support is provided by state jurisdictional agencies, researchers and their institutions.

Aims/objectives

The objective of the Program is to ensure that Australia can be 95% confident that AI infection would be detected in at least one wild bird if it was present at a prevalence of at least 1% in Australia's wild bird population. Other objectives include: excluding H5 (including H5N1) or H7 as a cause of wild bird mortality events; detection and reporting of circulating AI viruses (AIVs) (including low pathogenic avian influenza [LPAI] and highly pathogenic avian influenza [HPAI] H5 and H7); maintaining laboratory capability and capacity for influenza virus detection, and; gaining an understanding of the influenza viruses circulating in wild birds in Australia.

Methods used

The Program is made up of two sampling components; targeted surveillance via sampling of 'apparently' healthy, live and hunter-killed wild birds and general surveillance via investigation of significant, unexplained morbidity and mortality events in wild birds, including captive and wild birds within zoo grounds (with a focus on H5 and H7 exclusion testing). For targeted surveillance, the assumption was made that cohorts of wild birds sampled at each location over a 12 month period are from the same very large Australian wild bird population and that the polymerase chain reaction (PCR) tests used, for influenza A, have a perfect sensitivity and specificity.

Cloacal, oropharyngeal and faecal environmental swabs collected from wild birds were tested using PCR for the influenza A matrix gene. All H5 or H7 samples are forwarded to CSIRO Australian Animal Health Laboratory (AAHL), for further molecular analysis. Where possible, positives for influenza A via PCR underwent further subtyping and virus culture.

Results/key findings

A total of 8,244 swabs were collected and analysed for AIVs from 8,244 wild birds in Australia between July 2011 and June 2012. Anseriformes were primarily targeted with a number of Charadriiformes also sampled. No HPAI viruses were identified. However, 262 PCR tests were positive for influenza A matrix gene, 111 of which were identified to subtype¹. LPAI H5 was present in wild birds in New South Wales, Western Australia and Victoria. LPAI H7 was present in wild birds in Victoria and New South Wales. Positive PCR samples which underwent further subtyping, identified H1-H12 virus subtypes. In addition, between July 2011 and June 2012, 424 wild bird mortality and morbidity events were investigated. AI was excluded by PCR in 191 events. AI exclusion testing was not warranted in the remaining 233 events based on clinical signs, history, prevailing environmental conditions or other diagnoses.

Results of the Program suggest widespread exposure of Australian wild birds to LPAI virus subtypes with an apparent prevalence of 3.2 %¹. Whilst phylogenetic analysis suggests that many of these AIVs are probably endemic strains, there remains a very low likelihood of AIVs being introduced via migratory birds such as red-necked stints, eastern curlews and bar-tailed godwits. Importantly, the data suggest the presence of endemic LPAI H5 and H7 in Australian wild birds which could spill-over to poultry and mutate into HPAI.

The findings support the need for continuing Australia's surveillance activities for AI in wild birds to provide further epidemiological information about circulating AIV subtypes. There is the suggestion that environmental conditions may be an important risk factor in AIV spill-over from wild birds to poultry. If this risk factor is to be better understood then further surveillance and analysis of one or more longitudinal datasets is necessary. This will aid in determining what factors are contributing to disease emergence and could be used to better identify and manage risk to the poultry industry.

¹ A number of wild bird samples, positive for influenza for which H5 and H7 have been excluded, are pending subtype analysis.

Implications for relevant stakeholders:

The findings support the need for continuing surveillance activities in wild birds and reiterate the need for poultry producers to remain alert and ensure that appropriate biosecurity arrangements and effective risk reduction measures for AI are in place at their premises. Information generated via the Program can assist those directly involved in poultry activities to make more meaningful assessment of risk exposure in their area.

A multi-agency and cross-jurisdictional approach that includes industry is necessary to manage AIV risks to Australia.

Recommendations

- Evidence of a wide range of subtypes of low pathogenic avian influenza viruses, including LPAI H5 and LPAI H7 subtypes, is found in wild birds in Australia. Surveillance should be continued.
- Findings from the Program should be used by policy makers to inform management of the risk of AIV from wild birds, including the information gathered concerning circulating AIV subtypes.
- The Program is required to provide positive samples necessary to maintain diagnostic capability and capacity and to improve effective AIV sampling techniques.
- Program outcomes should continue to inform policy development for prevention and management of avian influenza outbreaks in Australian poultry flocks.
- The collaborative national approach that includes industry should be further fostered under the Program and continue to support surveillance activities in wild birds.
- Poultry producers should be alert to the need to have appropriate biosecurity arrangements and effective risk reduction measures in place at their premises.

Introduction

Avian influenza (AI) is an infectious disease of birds caused by influenza virus type A strains and is a significant problem around the world due to devastating effects of highly pathogenic avian influenza (HPAI) virus subtypes in poultry.

Wild birds form the natural reservoir for all influenza A viruses and usually show no clinical signs of disease (Stallknecht *et al.*, 2007, Webster *et al.*, 1992). The Anseriformes (ducks, swans, geese) and Charadriiformes (gulls, terns and shorebirds) represent the major component of the natural reservoir (Olsen *et al.*, 2006).

Influenza A viruses are RNA viruses belonging to the family Orthomyxoviridae. Influenza A viruses are classified according to the antigenic properties of their surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA) (Stallknecht *et al.*, 2007). There are 16 HA (H1–16) and 9 NA (N1–9) subtypes recognised; they are found in different combinations (Stallknecht *et al.*, 2007). At least 103 of the possible 144 influenza A subtype combinations have been found in wild birds (Alexander 2007; Dugan *et al.*, 2008; Munster *et al.*, 2007). Avian influenza viruses (AIVs) have the potential to mutate from low pathogenic forms (low pathogenic AI - LPAI) into the much deadlier highly pathogenic forms (high pathogenic AI - HPAI).

Up to the time period covered by this survey, Australia had experienced five HPAI outbreaks in commercial poultry. All were due to H7N7, H7N3 or H7N4 subtypes (Barr *et al.*, 1986; Selleck *et al.*, 1997, 2003; Turner, 1976; Westbury, 1997). These subtypes have not been isolated from Australian wild birds (Arzey, 2004). Since then, a further two HPAI outbreaks have occurred in commercial layer flocks – in Maitland in November 2012, and in Young (in two epidemiologically related properties), in October 2013. The outbreak viruses in these cases were H7N7 and H7N2 subtypes respectively (OIE, 2015).

In 2006 Australia further strengthened national surveillance for AI in both poultry and wild birds and established the National Avian Influenza Wild Bird Steering Group (the Steering Group) to ensure national coordination and collaboration for wild bird surveillance under the NAIWB Surveillance Program (the Program).

The objective of the Program is to ensure that Australia can be 95% confident that AI infection would be detected in at least one wild bird if it was present at a prevalence of at least 1% in Australia's wild bird population. Other objectives include: excluding H5 (including H5N1) or H7 as a cause of wild bird mortality events; detection and reporting of circulating AI viruses (AIVs) (including LPAI and HPAI H5 and H7); maintaining laboratory capability and capacity for influenza virus detection, and; gaining an understanding of the influenza viruses circulating in wild birds in Australia.

The Steering Group is responsible for development and implementation of a yearly operating plan and surveillance activities for AI in wild birds in Australian states and territories. The NAIWB Steering Group comprises representation from:

- Australian Department of Agriculture (DoA)
- Australian Department of Environment (DoE)
- CSIRO Australian Animal Health Laboratory (AAHL)
- Australian Department of Agriculture's Northern Australia Quarantine Strategy (NAQS) program
- State and Territory government animal health departments in NSW, NT, QLD, SA, TAS, WA and VIC

- World Health Organisation Collaborating Centre for Reference and Research on Influenza, Melbourne (WHO)
- University research groups from the University of Newcastle, James Cook University and Deakin University
- Wildlife hospitals at the major Australian zoological parks (Adelaide, Australia, Currumbin, Healesville Sanctuary, Melbourne, Perth, Taronga)
- The Australian Registry of Wildlife Health
- Hunter groups, wildlife/wader groups and bird banding groups
- Wetlands International and Birdlife Australia

National wild bird surveillance projects have been conducted Australia-wide each year since 2006. This report outlines results from the sampling of wild bird between July 2011 and June 2012.²

The Program is ongoing, and more information is available from <https://www.wildlifehealthaustralia.com.au/ProgramsProjects/AvianInfluenzaWildBirdSurveillance.aspx>. For an update on survey results since, contact Tiggy Grillo, National Coordinator, Wildlife Health Australia (formerly Australian Wildlife Health Network) at tgrillo@wildlifehealthaustralia.com.au.

² Information regarding AIV in wild birds in Australia since 2005 has been summarised previously in Hansbro *et al.*, 2010; Haynes *et al.*, 2009 and OCVO 2010.

Objectives

At the time that this research was undertaken, there were six program objectives.

1. Targeted risk based³ active (waterbirds) and general (wild bird mortality events) surveillance by continuing to
 - a) develop, review and undertake targeted surveillance ('apparently' healthy, live and hunter-killed wild birds)
 - b) detect and report virulent AI viruses by investigating significant, unexplained morbidity and mortality events in wild bird deaths (with a focus on H5 and H7 exclusion testing).
2. Focus on detection and reporting of H5 and H7 (both LPAI and HPAI).
3. Maintain national laboratory AI testing capacity and capability to detect subtypes of AIV including H5 and H7.
4. Provide data on occurrence of AI viruses in wild birds in Australia.
5. Use the data for risk analysis management and communication to industry and other stakeholders
6. Contribute to knowledge of the ecology of AI viruses in Australia by investigation of AI virus genotypes circulating in wild birds in Australia and better describe their ecology and epidemiology over time and space.

Note: Since 2012, the objectives of the Program have been updated. For details of the current objectives of the National Avian Influenza Wild Bird Surveillance Program see <https://www.wildlifehealthaustralia.com.au/ProgramsProjects/AvianInfluenzaWildBirdSurveillance.aspx>

³ Priority high-risk areas for AI to the Australian poultry industry.

Methodology

General

Both targeted and general risk-based convenience sampling approaches were utilised. Samples were collected by state and territory government agencies, university research projects and the DoA's NAQS program, from wild birds in NSW, NT, QLD, SA, TAS, WA and VIC. A combination of healthy, live and hunter-killed wild birds (targeted surveillance) and sick/dead wild birds (general surveillance) was targeted. Surveillance results were received and collated nationally by WHA.

Targeted Avian Influenza Wild Bird Surveillance

Targeted AI surveillance focused on collection of samples from Anseriformes (eg. ducks, magpie geese, swans), with a smaller number from other species such as shorebirds, shearwaters and gulls. The objective of the Program was to ensure that Australia can be 95% confident that AI infection would be detected in at least one wild bird if it was present at a prevalence of at least 1% in Australia's wild bird population. For targeted surveillance, the assumption was made that cohorts of wild birds sampled at each location over a 12 month period are from the same very large Australian wild bird population and that the polymerase chain reaction (PCR) tests used, for influenza A, have a perfect sensitivity and specificity. To achieve this objective a minimum total population of $n = 300$ birds was collected from each state and territory over the 12 month period.

Where possible, sampling aimed to be longitudinal, targeting locations sampled in previous 12 month period. Location criteria included:

- breeding areas for wild waterfowl, areas in which waterfowl and shorebirds congregate together
- areas located in close proximity to poultry activities and/or human populations
- consideration of bird migration and movement patterns
- large aggregations of waterfowl.

During 2011-2012, targeted wild bird surveillance occurred at sites in all of the States and Territories. The main types of sample collected were cloacal, oropharyngeal or faecal environmental swabs. Each collaborator chose the type of samples collected based on funding, available staff and/or laboratory capacity and targeted a minimum of one or two locations within each state and territory.

Healthy, live wild birds were captured using either walk-in traps or cannon nets, and sampled by cloacal and/or oropharyngeal swabbing. Fresh faecal environmental samples (i.e. wet faeces) were collected from roosting or feeding waterbirds. Hunter-killed birds were sampled (cloacal and/or oropharyngeal swabbing) during duck hunting.

All sampling of wild birds was approved by the relevant institutional animal ethics committee in each state and territory.

Wild Bird General Surveillance

General surveillance for AIVs occurred via investigation of significant, unexplained mortality and morbidity events in wild birds. General wild bird disease surveillance included submissions of samples (cloacal and/or oropharyngeal swabs) collected from mortality and morbidity events from members of the public, private practitioners, universities, zoos and sanctuaries. All wild bird mortality and morbidity events were captured in the national wildlife health information system (eWHIS). General surveillance included birds from a number of different Orders.

Testing

Cloacal, oropharyngeal and faecal environmental swabs were tested at government or university laboratories using real-time PCR for the AI type A matrix gene, the most highly conserved genome segment of influenza A viruses (Fouchier *et al.*, 2000; Heine *et al.*, 2005; Spackman *et al.*, 2002). All H5 or H7 positive samples were forwarded to CSIRO AAHL, for further molecular analysis and viral culture in embryonated hen eggs. Where possible, testing of samples found to be positive by PCR for influenza A for which H5 and H7 has been excluded, underwent further subtyping of the HA and NA genes at the CSIRO AAHL and/or the World Health Organization Collaborating Centre for Reference and Research on Influenza (Melbourne; WHO).

Influenza A apparent prevalence is defined as the number of positives for influenza A (via PCR and / or viral isolation) divided by the total number of swabs collected. Swabs include all three sample types; faecal environmental, cloacal and oropharyngeal and unless otherwise stated apparent prevalence was determined based on data combining numbers from all three swab types. Where swabs were collected and pooled in the field (mainly faecal environmental swabs and on a few occasions' cloacal swabs) prior to laboratory analysis, apparent prevalence was calculated with the following assumptions: one faecal environmental swab equals one bird sampled and one positive pooled sample equals one positive swab. The latter assumption is made as one influenza A positive pooled sample via PCR is most likely to represent one positive swab out of the number pooled. For example, where one positive pooled faecal environmental sample consists of three swabs and is analysed as one sample in the laboratory, the pooled sample is most likely to contain one positive swab out of three, rather than two or three positives per three swabs.

Reporting

A standard pro forma was utilised to facilitate data capture. The pro forma was completed each month and returned to WHA for collation, moderation, analysis and reporting.

Results

Targeted Avian Influenza Wild Bird Surveillance

Between July 2011 and June 2012, a total of 8,244 swabs (1,790 cloacal swabs, 43 oropharyngeal swabs and 6,411 faecal environmental swabs) were collected from 8,244 wild birds in Australia (see Table 1).

Sampling occurred at sites in NSW, NT, QLD, SA, TAS, WA and VIC (table 1 and figure 1). The majority of samples were collected from Anseriformes (eg. ducks, magpie geese, swans), with a smaller number from other species such as shorebirds, shearwaters and gulls.

Table 1. Locations from which targeted AI wild bird surveillance samples were collected (July 2011 to June 2012)

State/Territory	Location	No. Birds Sampled
NSW	Newcastle	1,305
NT	Kakadu	500
	Koolpinyah	458
QLD	Billabong Sanctuary	480
	Moreton Bay	849
	Atherton Tablelands	552
SA	Bolivar Lagoon	507
	Salt Creek	54
	Langhorne Creek	10
TAS	Hobart Area (Kingston, Kingston Beach, Old Beach, Richmond and Tea Tree)	389
	North – North East (Launceston, Gladstone)	46
	Central (Campbell Town, Ross, Verwood, Macquarie River, Longford)	41
	East Coast (Little Swanport, Moulting Lagoon)	132
VIC	Werribee	1,041
	Various (Swan Hill, Horsham, Reedy Lake, Bairnsdale, Ballarat, Lake Nillahcootie and Wodonga)	460
WA	Busselton	498
	Herdman's Lake/Perth Area	350
	Kununurra	397
	Broome	175
TOTAL		8,244



Figure 1. Map of locations from which targeted AI wild bird surveillance samples were collected (July 2011 to June 2012)

The positive results came from a variety of waterbird species, mainly from the Order Anseriforme. No HPAI viruses were identified. However, 262 PCR tests were positive for the influenza A matrix gene.

Influenza A virus was detected by RT-PCR and / or viral isolation, from a total 262 swabs collected between July 2011 and June 2012 with an overall apparent prevalence of 3.2% (Table 2). Apparent prevalence ranged from 0.5% in NT to 6.4% in NSW.

From a total of 262, 111 influenza A positives were successfully subtyped⁴. Positive PCR samples which underwent further subtyping identified H1-H12 subtypes.

LPAI H5s were detected in 27 out of 262 influenza A positive wild bird samples, with detections in New South Wales, Western Australia and Victoria. In addition, a total of six LPAI H7s were detected in wild birds sampled in Victoria and New South Wales. The remaining 229 influenza A positive samples were negative for H5 and H7.

⁴ A number of wild bird samples, positive for influenza for which H5 and H7 have been excluded, are pending subtype analysis.

Table 2. Total number of wild birds sampled and apparent prevalence for AIVs in Australian wild birds collected (July 2011 to June 2012)

State/Territory	No. Birds Sampled	No. of Swabs*	No. Positive Influenza A Virus	Apparent Prevalence (%)#
NSW	1,305	1,305	83	6.4%
NT	958	958	5	0.5%
QLD	1,881	1,881	39	2.1%
SA	571	571	33	5.8%
TAS	608	608	8	1.3%
VIC	1,501	1,501	55	3.7%
WA	1,420	1,420	39	2.8%
TOTAL	8,244	8,244	262	3.2%

*a number of environmental samples were analysed in pools of three; # apparent prevalence is based on the number of influenza A positives (by RT-PCR and / or viral isolation) divided by the number of swabs collected. A pooled swab sample is taken as most likely to represent 1 positive sample.

Wild Bird General Surveillance

Between July 2011 and June 2012, AI was excluded in the 424 wild bird mortality and morbidity events recorded for Australia in the national electronic wildlife health information system (ACT=1, NSW=53, NT=8, QLD= 107, SA=20, WA=41, VIC=159, TAS = 35). While all of these events were atypical of AI, specific AI testing was undertaken in 191 events and AI was excluded as the cause of death. The remaining 233 mortality events did not warrant AI testing based on the clinical signs, history, environmental conditions and/or other diagnosis.

The majority of wild bird mortality and morbidity events included birds from the Orders Psittaciformes (n=191); Columbiformes (n=81) and Passeriformes (n=45). The reported diagnosis in these events included; aspergillosis, avipox, beak and feather disease⁵, botulism/suspected botulism, chlamydiophilosis enteritis, megabacteriosis, paramyxovirus, poisoning/suspected poisoning, starvation, trauma and trichomoniasis.

There were two major events causing wild bird mortalities between July 2011 and May 2012. Avian paramyxovirus 1 (APMV-1) in pigeons was first detected in hobby pigeon in Victoria in August 2011, with the first confirmed report in free-ranging birds (the common introduced feral rock dove; *Columba livia*) in Melbourne, Victoria in October 2011. APMV-1 was also confirmed in two new, free-living species (one sick collared sparrowhawk – *Accipiter cirrocephalus* and one spotted turtle dove – *Streptopelia chinensis*). The second major event report was in March 2012, where necrotic enteritis, caused by *Clostridium perfringens*, is believed to be the cause mortality and morbidity in more than 300 free-living rainbow lorikeets (*Trichoglossus haematodus*) at 25 sites in the eastern and north-eastern suburbs of Melbourne, Victoria. At one site over 80 deaths were recorded. A number of these events occurred in locations where rainbow lorikeets were being fed by members of the public.

⁵ Events in eWHIS with a diagnosis of beak and feather disease include cases based on clinical presentation only.

Discussion

Targeted Avian Influenza Wild Bird Surveillance

Over recent years AI has continued to be in the spotlight internationally, with continued outbreaks of Asian-lineage HPAI H5N1 in parts of Asia and controversy over two recent H5N1 transmission studies (Herfst *et al.*, 2012, Imai *et al.*, 2012). Despite researchers continuing to publish thousands of studies on influenza (with over 4,000 articles between 2001 and 2011), major puzzles and knowledge gaps persist (WHO, 2012). In particular, further research into AI in wild birds including surveillance is required to better understand the role of these animals in AI outbreaks (specifically Asian-lineage HPAI H5N1). This was emphasised at two key meetings in the past year: the East Asian Australasian Flyway (EAAF) 6th Meeting of Partners Avian Influenza Working Group and the FAO Scientific Task Force on Avian Influenza and Wild Birds.

The nationwide coordinated surveillance of Australian wild birds through the Program continues to contribute to the understanding of the risk of AIV from wild birds in Australia. The Program provides valuable information concerning circulating AIV subtypes in wild birds and maintains AIV sampling and diagnostic capability and capacity in Australia. These outputs support preparedness, contingency planning and response, should there be an outbreak in poultry.

Targeted risk based active surveillance of ‘apparently’ healthy, live and hunter-killed wild birds (waterbirds) suggest widespread evidence of exposure to LPAI AIV subtypes, with an apparent prevalence of 3.2%⁶. This is higher than apparent prevalence estimates detected between July 2005 and July 2010 (between 1.05% – 1.49%) but is similar to apparent prevalence for 2010–2011 (2.69%). Whilst calculating a national apparent prevalence can be useful, it should be noted that the relevance is limited. More importantly detection of AI positives appears to fluctuate through the year and from location to location (see state and territory apparent prevalence in table 1).

In Australia, breeding of Anseriformes is known to vary with food availability which in turn is related to climatic conditions such as rainfall (Birdlife Australia, 2011). Movements of Anseriformes in Australia are less predictable than their counterparts in the northern hemisphere and many populations are nomadic with distribution largely determined by available water (Roshier *et al.*, 2002, 2008). Environmental conditions such as drought and flood therefore impact on waterbird abundance in local areas and increase serologically naïve juveniles entering the populations (Klaassen *et al.*, 2011; Roshier *et al.*, 2002, 2008; Tracey *et al.*, 2004). Therefore, it is likely that environmental conditions will affect Anseriformes abundance and thus apparent prevalence of AIVs.

There has been widespread increase in rainfall in eastern Australia (associated with flooding in many areas) over the past two years. In a number of locations, this has resulted in an increased breeding season success for many species of wild birds, increased mixing of wild birds on water bodies, the increased movement as birds follow food and move into or out of flooded areas. Flooding may also lead to increased opportunities for wild birds to come into closer contact with poultry. In contrast, periods of drought may lead to increased bird densities. All these factors add to the ecological complexity and may lead to an increased risk to poultry. Data from previous HPAI in poultry in south-eastern Australia suggests outbreaks occur during periods of drought following a wet period (Klaassen *et al.*, 2011). WHA has continued to communicate this increased risk to the poultry industry through quarterly reports from distributed on the Program by DoA.

Subtyping has successfully identified 111 subtypes from the 262 influenza A positive samples. Further subtype information is pending for a number of wild bird samples positive for influenza A, for which

⁶ Apparent prevalence is based on the number of influenza A positives (by RT-PCR and / or viral isolation) divided by the number of swabs collected. A pooled sample is taken as most likely to represent 1 positive sample.

H5 and H7 have been excluded. A high degree of capacity building in AI PCR assay design and development continues to occur through this program. Wild bird surveillance continues to detect LPAI H5, the most common subtype detected this year (n=27/262). LPAI H7 is detected less frequently in Australian wild birds, with only six LPAI H7s detected out of 262 influenza A positives; one in Victoria and five in New South Wales, all detected in May 2012. The last detection of H7 as part of this Program was in December 2009. This program continues to provide samples positive for AI, essential for maintaining and developing current and specific primers and probes to ensure confidence that the tests being used in Australia will detect any strains of H5 and H7 in the event of an HPAI H5 or H7 outbreak in poultry.

In late January 2012, LPAI H5 was detected from a commercial duck farm in Victoria at the DPI Attwood laboratories as a result of routine diagnostic investigations. Follow up work at CSIRO AAHL confirmed that the virus was LPAI H5N3. Further investigations were then undertaken by the WHO to compare the HA and NA sequence of the virus with H5 PCR positive wild bird samples collected over the last few years of the Program. Comparison of full and partial HA sequences showed that the LPAI H5N3 virus had greater genetic similarity to three Victorian H5 wild bird HA sequences than to any other H5 sequences available in Genbank (including other Australian H5 wild bird sequences identified prior to 2008). The next closest matches were HA sequences from H5 viruses detected at Newcastle, NSW in 2007 from wild birds. This result is consistent with the hypothesis that the recent commercial duck farm virus was transmitted from wild birds in Victoria, although it is important to note that recent (2010 or 2011) H5 sequences from elsewhere in Australia were not available for comparison. This highlights the value of continuing the wild bird surveillance program, the benefits of sequencing influenza positive samples and the need to share resulting data.

The generation of large number of field samples through this Program also ensures Australia's diagnostic laboratories can maintain capacity and develop capability for high throughput testing for AI. This was exploited during Australia's response to an outbreak of equine influenza in 2007. In addition, comparison of Australian wild bird AI virus sequence data with subtypes circulating regionally and in the northern hemisphere is possible. This helps elucidate the relative importance of any endemic versus exotic transmission pathway into Australian poultry flocks. The molecular and phylogenetic analysis is ongoing and contributes to knowledge of AI virus ecology, epidemiology and risk, both spatially and temporally.

Wild Bird General Surveillance

General surveillance is an important component of the AI surveillance program and has been shown to be valuable as an early warning mechanism in other countries (Breed *et al.*, 2009; European Commission 2008; Knight-Jones *et al.*, 2010). General surveillance for AIVs in wild bird mortality and morbidity events demonstrates that Australia has the capacity to investigate these events on a national scale.

WHA supported the Steering Group, provided recent scientific papers, media and other information to them and collating data from the surveillance activities. WHA provided a consolidated annual report and regular progress reports to DoA; these are used as the basis for regular reporting to the poultry industry and Australia's Animal Health Committee and to support our international animal health reporting obligations.

Implications

Globally, wild bird AI surveillance continues to further our knowledge and understanding, but there is still much to be understood about the ecology of these viruses in their natural reservoir hosts (Feare 2010; Klaassen *et al.*, 2011; Munster *et al.*, 2007). The nationwide coordinated surveillance of Australian wild birds during 2011–2012 has provided valuable information concerning circulating AIV subtypes. Based on results to date, evidence of exposure to LPAI virus subtypes is widespread. While many of the AIV subtypes are probably endemic strains (Hansbro *et al.*, 2010; Bulach *et al.*, 2010), there remains a very low likelihood of AIVs being introduced via migratory birds such as red-necked stints, eastern curlews and bar-tailed godwits (Haynes *et al.*, 2009; Hurt *et al.*, 2004). There is also a possibility of endemic LPAI H5 and H7 subtypes present in Australian wild birds mutating into HPAI if there is spill-over to poultry.

The nationwide coordinated surveillance of Australian wild birds through the Program continues to contribute to the understanding of the risk of AIV from wild birds in Australia, provides valuable information concerning circulating AIV subtypes in wild birds and maintains AIV sampling and diagnostic capability and capacity in Australia.

The Program has gained momentum over the last few years and has been adding to a valuable longitudinal dataset of wild bird AI in Australia (Haynes *et al.*, 2009). Though of lower priority, this is starting to provide a better picture of the AIVs circulating within the Australian wild bird population, including which subtypes seem to dominate each year and in which locations. For example, data collated to date has identified unique Australian lineages of AIV (Hansbro *et al.*, 2010). Continued AI wild bird surveillance will improve knowledge of these lineages, and may identify new and emerging strains, some of which may have enhanced pathogenicity.

Furthermore, the Program continues to detect LPAI H5 more frequently than other LPAI subtypes. Further work is required to determine the significance of this finding in terms of risk to the poultry industry. Surveillance conducted in both 2010–2011 and 2011–2012 has shown a dramatic change in the pattern and number of positive results nationally compared to the previous 5 years. Continued surveillance and analysis is necessary to better identify the factors that are contributing to these changes. Improved knowledge of the drivers for AIV emergence can be used to better identify and manage risks to the poultry industry.

The ecology of wild bird reservoirs in Australia is unique. This dataset is very important because it, and only it, can be used to assess risk. Assumptions about AIV risk based on findings from overseas are not likely to be applicable to the Australian situation.

Samples positive for influenza A provide the opportunity for laboratories to maintain currency of diagnostic tests to ensure that current circulating Australian AIVs can be detected and where available, compare Australian wild bird AI virus sequence data to subtypes circulating in the northern hemisphere. This improves understanding of the infection pathway for AIVs in wild birds. Specifically, the Program provides the opportunity for laboratories to monitor sequence variation in HA genes, and in some cases NA and matrix genes. Importantly, detection and sequence analysis are required to maintain and develop current and specific primers and probes. This ensures continued confidence that the tests being used in Australia are able to detect any strains of H5 and H7. This is particularly important in the event of an HPAI H5 or H7 outbreak in poultry. For example, if there are base changes within the primer or probe regions of the specific H5 and H7 Taqman assays and this cannot be detected; there could be a potential false negative result from a positive sample.

Further work is also necessary to fully develop and maintain new technologies already established (e.g. microarray sample testing technology in NSW). In-kind funds already invested would potentially be lost if further AIV positives samples are not available to determine the reliability, sensitivity and specificity of the microarray for Australian isolates.

Wild bird AI updates have assisted in maintaining communication pathways between government and industry nationally and within jurisdictions. The Program provides an alert and warning system to poultry producers when potentially pathogenic strains of AIVs are found. This system of reporting reinforces the need for poultry producers to ensure that they have appropriate on farm biosecurity measures in place.

Furthermore, the multi-agency and cross-jurisdictional approach of this project has fostered development of a collaborative “One Health” approach which will facilitate future collaborative efforts to manage national animal health issues.

These factors provide valuable support for contingency planning and preparedness for response, and management should there be a disease outbreak in poultry. Outcomes from national wild bird surveillance activities inform policy for prevention and management.

Recommendations

The NAIWB Surveillance Program continues to find evidence of a wide range of subtypes of LPAI viruses, including low pathogenic H7 and H5 subtypes in wild birds in Australia. No HPAI has been detected. These findings reiterate the need for poultry producers to remain alert and review biosecurity arrangements to ensure that effective risk reduction measures are in place.

There is the suggestion that environmental conditions may be an important risk factor in AIV spill-over from wild birds to poultry. It should be noted that Australia is unique in terms of AIVs and wild bird ecology. Evidence suggests that previous HPAI H7 outbreaks in Australia were due to endemic rather than exotic strains of virus. It is therefore important to be aware of the AIVs currently circulating within the Australian wild bird population, whilst continuing to be alert to introduced, exotic viruses. Relying upon knowledge of AIVs circulating in the Northern Hemisphere wild bird population will not be sufficient.

The findings of the Program support the need for continuing Australia's surveillance activities for AIVs in wild birds. If the relative importance of an endemic versus introduced transmission pathway, drivers of disease emergence and changes in risk to the poultry industry is required then further epidemiological information about circulating AIV subtypes and a more representative and longitudinal dataset is necessary. Results to date suggest that the Program is a good mechanism for generating this information.

Specific recommendations:

- Evidence of a wide range of subtypes of low pathogenic avian influenza viruses, including LPAI H5 and LPAI H7 subtypes is found in wild birds in Australia. Surveillance should be continued.
- Findings from the Program should be used by policy makers to inform management of the risk of AIV from wild birds, including the information gathered concerning circulating AIV subtypes.
- The Program is required to provide positive samples necessary to maintain diagnostic capability and capacity and to improve effective AIV sampling techniques.
- Program outcomes should continue to inform policy development for prevention and management of avian influenza outbreaks in Australian poultry flocks.
- The collaborative national approach that includes industry should be further fostered under the Program and continue to support surveillance activities in wild birds.
- Poultry producers should be alert to the need to have appropriate biosecurity arrangements and effective risk reduction measures in place at their premises.

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Contribution to the 2012 Avian Influenza in Wild Birds Surveillance Program

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March 2015

Pub. No. 15/016

Avian influenza (AI) is an infectious disease of birds caused by influenza virus type A strains and is a significant problem around the world due to the devastating effects of highly pathogenic avian influenza (HPAI) virus subtypes in poultry.

This report outlines results from the sampling of wild birds as part of the National Avian Influenza Wild Bird Surveillance Program (the Program) between July 2011 and June 2012. The Program provides a good understanding of the avian influenza viruses (AIVs) circulating in wild birds in Australia, contributes to better decision-making and knowledge of risk factors, and provides an early warning system.

Wild bird surveillance is part of the broad national approach to identifying and managing the risks from avian influenza (AI).



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