

NATIONAL AVIAN INFLUENZA WILD BIRD SURVEILLANCE PROGRAM (AUSTRALIA)

Tiggy Grillo
Australian Wildlife Health Network
PO Box 20
Mosman NSW 2088
National Avian Influenza Wild Bird Steering Committee

All Australian Jurisdictions require that all avian influenza virus infection is reported to the relevant Chief Veterinary Officer(CVO). The national notifiable diseases list does not specify strains of AI but includes avian influenza.

<http://www.daff.gov.au/animal-plant-health/pests-diseases-weeds/animal/notifiable>

INTRODUCTION

Avian influenza (AI) is a globally significant infectious disease of birds which can cause disease in range of other species including humans (Olsen et al., 2006). Its significance was highlighted in outbreaks of highly pathogenic avian influenza (HPAI) H₅N₁ throughout South-East (SE) Asia from 2003-2005. HPAI H₅N₁ continues to be a concern in South-East Asia, Northern Africa and Europe (Feare, 2010).

Avian Influenza is caused by influenza A viruses of the Orthomyxoviridae family. Further classification is based on the antigenic properties of their surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA). Sixteen HA (H1–H16) and 9 NA (N1–N9) surface glycoproteins are currently recognised and these are found in many different combinations or “subtypes” (Stallknecht et al., 2007). The subtypes are classified as either as high (HPAI) or low pathogenic (LPAI) based upon their experimental virulence in chickens or molecular sequencing. Similar HA and NA types may be either HPAI or LPAI dependent upon a number of other factors and AI subtypes H₅ and H₇ have the potential to mutate from LPAI forms into the HPAI forms with the potential to cause devastating losses in poultry flocks. HPAI H₅ has not been detected in Australia, however there have been five HPAI outbreaks due to HPAI H₇ subtypes, in commercial poultry operations in Victoria (1976 and 1985 - H₇N₇ and 1992 - H₇H₃), Queensland (1994 - H₇N₃) and the most recent in New South Wales (1997 - H₇N₄) (Barr et al., 1986; Selleck et al., 1997, 2003; Turner 1976; Westbury 1997).

Wild birds form the natural reservoir for AI viruses and rarely show signs of clinical disease. Based on long term surveillance studies around the world, wetland birds of the orders Anseriformes (ducks, geese, swans) or Charadriiformes (gulls, terns) appear to be the main natural reservoirs (Munster et al., 2007; Olsen et al., 2006; Wahlgren, 2011). Following the first reported mass mortality of wild birds due to HPAI H₅N₁ at Lake Qinghai, northern China, in spring 2005 (Newman et al., 2011), interest increased in the role of wild birds in the spread and maintenance of AI. 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 both poultry and wild birds. The

National Avian Influenza Wild Bird (NAIWB) Steering Group was established to ensure national coordination and collaboration of wild bird surveillance (NAIWB Surveillance Program). The NAIWB Steering Group oversees the operating plan and surveillance activities for AI in wild birds in Australian states and territories. The Australian Wildlife Health Network (AWHN) is responsible for coordination of national wildlife health surveillance program activities in Australia¹ and supports the NAIWB Steering Group.

National wild bird surveillance is conducted Australia-wide, with national funding together with in-kind support from jurisdictional agencies and representative institutes. Wild bird surveillance is part of a broad national approach to identifying and managing the risks from AI. The approach includes surveillance of domestic/production birds, communications, research and regional capacity building. National wild bird surveillance information contributes to Australia's international reporting and capacity building responsibilities.

The objective of the NAIWB program is to gain an understanding of the influenza viruses circulating in wild birds in Australia and, as a result, improve decision-making and understanding of risk factors, and provide an alert system for the poultry industry. The program aims to detect and report circulating AI viruses (including LPAI and HPAI H₅ and H₇) and to determine whether H₅ (including H₅ N1) or H₇ are responsible for wild bird mortality events in Australia. The detection of LPAI H₅ and LPAI H₇ is valuable given their potential to mutate into HPAI viruses when introduced into poultry hosts.

The NAIWB Steering Group comprises representation from:

- Australian Department of Agriculture, Fisheries and Forestry
- Australian Department of Sustainability, Environment, Water, Population and Communities
- CSIRO Livestock Industries, Australian Animal Health Laboratory (AAHL)
- Australian Quarantine and Inspection Service's Northern Australia Quarantine Strategy (NAQS) program
- State government animal health departments
- World Health Organisation Collaborating Centre for Reference and Research on Influenza, Melbourne
- University research groups from the University of Newcastle, James Cook University and Deakin University
- The major Australian Zoological Parks
- The Australian Registry of Wildlife Health
- Hunter groups, wildlife/wader groups and bird banding groups
- Wetlands International

The six objectives for the program are:

- Targeted risk based active² (waterbirds) and general (wild bird morbidity/mortality events) surveillance.
- Focus on detection and reporting of LPAI and HPAI H₅ and H₇
- Maintenance of national laboratory AI testing capacity and capability to detect strains of AIV including H₅ and H₇.
- Provide data on the occurrence of AI viruses in wild birds in Australia.

¹ <http://www.wildlifehealth.org.au/AWHN/home.aspx>

² Priority high-risk areas for avian influenza to the Australian Poultry Industry

- Use the data for risk analysis, management and communication to industry and other stakeholders.
- Contribute to knowledge of the ecology of AI viruses in Australia.

METHODS

This paper outlines the sampling effort from June 2005 through to June 2011 and represents the data collected as part of the NAIWB surveillance program. Surveillance activities target a combination of healthy, live and hunter-killed wild birds, various environmental samples (targeted surveillance) and sick or dead wild birds (general surveillance).

Wild bird samples were collected through various state and territory government agency programs, university research programs, the Victorian Waders Study Group and the DAFF Northern Australia Quarantine Strategy (NAQS) in New South Wales (NSW), Northern Territory (NT), Queensland (QLD), South Australia (SA), Tasmania (TAS), Western Australia (WA) and Victoria (VIC). Criteria for sampling locations included 1) breeding areas for wild waterfowl, areas in which waterfowl and shorebirds congregate together, 2) areas located in close proximity to poultry activities and or human populations with consideration of bird migration and movement patterns and 3) large aggregations of waterfowl. In addition, samples were collected to allow analysis of temporal patterns (year to year) and hence targeted locations previously sampled and where AI has been detected in wild birds.

Targeted surveillance samples include collection of swabs (cloacal, oropharyngeal, faecal environmental) and/or serum. General surveillance from sick wild birds included submissions from members of the public, private practitioners, department of conservation, universities, zoos and sanctuaries of samples (cloacal and/or oropharyngeal swabbing) collected from mortality / morbidity events as part of Australia's general wildlife disease surveillance program. All sampling of wild birds was approved by the relevant institutional animal ethics committee in each State / Territory.

Cloacal, oropharyngeal and fresh faecal environmental swabs collected in the absence of disease (targeted surveillance) were tested at a National Association of Testing Authorities (NATA) accredited facility or another appropriate laboratory with the approval of the State and Territory CVO or at AAHL (e.g. government or university laboratory). Influenza A was tested for using either a real-time (quantitative) reverse transcriptase PCR (qRT-PCR) or a nested PCR targeting the type A matrix gene, the most highly conserved genome segment of influenza A viruses (Fouchier et al., 2000; Heine et al., 2005, 2007; Spackman et al., 2002). Positive influenza A samples then testing positive by specific qRT-PCRs for influenza A H₅ or H₇ were forwarded to the CSIRO Australian Animal Health Laboratory for viral culture in embryonated hen eggs and/or the World Health Organization Collaborating Centre for Reference and Research on Influenza (Melbourne) for subtyping of the HA and NA genes and included H₅ / H₇ pathogenicity testing. Serological samples were also collected from WA & QLD and were analysed at regional government laboratories or at the CSIRO Australian Animal Health Laboratory for the presence of antibodies using a competitive enzyme linked immunosorbent assay (c-ELISA) for influenza A virus nucleoprotein (Hartley et al., 1990; Zhou et al., 1998). Influenza A prevalence is defined as the number of positives for Influenza A (via PCR and / or virus isolations) divided by the total number of swabs collected.

See Haynes et al., 2009 for further details.

Results from the NAIWB surveillance program are provided to Australian governments, industry and other stakeholders and are included in Australia's National Animal Health Information System (NAHIS) and Australia's international reports.

RESULTS

Since 2005, over 57 000 samples have been collected from wild bird in Australia. Almost all AI subtypes have been detected, including LPAI viruses of the subtypes H₅ and H₇. No HPAI viruses have been detected. In addition, over 700 wild bird mortality events have been investigated. The presentation will provide more specific findings from this program. A detailed analysis of the dataset is currently in progress. Results from this work to date have been published by Haynes et al. (2009) and Hansbro et al. (2010).

DISCUSSION

As there is naturally a low prevalence of AI virus, broad scale wild bird surveillance is logistically difficult and costly (Tracey et al., 2004). Despite these challenges, the NAIWB program to date has provided Australia with important outcomes and continues to contribute to the understanding of AIV in wild birds, and resultant risks to the poultry industry, as well as maintaining AIV sampling and diagnostic capability and capacity in Australia. Furthermore, these outcomes inform policy for prevention and management of avian influenza outbreaks in Australian poultry flocks.

Specifically, this program continues to be a key source of sample positives 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/H₇ in the event of an HPAI H₅ or H₇ outbreak in chickens. Influenza A positives, specifically those positive for H₅ or H₇, allow laboratories to monitor sequence variation in haemmagglutinin (HA) genes, and in some cases neuraminidase (NA) genes. Detection and sequence analysis are required to maintain and develop current and specific qRT-PCR primers and probes. Using influenza A isolate sequences from surveillance studies, Munster et al. (2009) found variability of primer target sequence of the HA gene to be considerably more variable than the matrix gene target sequence. This makes sense considering that the matrix gene is one of the most highly conserved genome segments of influenza A viruses. Whereas, the HA gene is under constant selection by the host immune system. Genetic variation in HA genes may show as mutations affecting primer and/or probe binding sequences and lead to possible detection failure (Arafa et al., 2010). This highlights the importance of continued monitoring of subtype specific RRT-PCR primers and probes to ensure that sensitivity and specificity are maintained and are essential to continue confidence that the tests being used in Australia will detect any strains of H₅/H₇ in the event of an HPAI H₅ or H₇ outbreak in chickens. Further to this, the program continues to ensure that laboratory capacity for high-throughput molecular testing is available in Australia and provides Australian laboratories with confidence that review and modification of testing regimes continue to be effective.

General surveillance for AIVs in wild bird mortality and morbidity events demonstrates that Australia has the capacity to investigate wild bird mortality and morbidity events on a national scale. General surveillance is an important component of avian influenza surveillance and has been shown to be valuable as an early warning mechanism in other countries. For example, almost all HPAI H₅ N1 detections in the U.K. occurred as part of general surveillance and investigation of dead or clinically affected birds (Breed et al., 2010). Knight-Jones et al., (2010) identified that passive surveillance (i.e. general) was the most sensitive method for the detection of HPAI, which is logical given that HPAI subtypes are most likely to be observed as mortalities or morbidities in infected birds. In order to increase the probability of detection of these potential incidents, passive surveillance of dead or diseased birds in Australia includes a variety of species and is not limited to high risk species. This strategy is supported in the literature (Herterberg et al., 2008).

The multi-agency and cross-jurisdictional approach of this project has led to an improved relationship between the participating parties and fostered development of a collaborative “One Health” approach; this will provide benefits to future collaborative efforts to manage national animal health issues. Wild bird samples collected as part of the AIV surveillance program can also be tested for other diseases. These valuable samples are costly to obtain and hence any additional meaningful testing that can be done further increases the efficiency and value of the program.

FURTHER INFORMATION

AUSVETPLAN

Avian Influenza current understanding and policy in Australia is outlined in the **AI AUSVETPLAN** - see:

http://www.animalhealthaustralia.com.au/wp-content/uploads/2011/04/AI3_4-06FINAL16Feb11.pdf

This includes agreed policy in Australia with respect to LPAI or HPAI detection in wild birds.

An additional Guidance document for the use of avian influenza (AI) vaccine in the event of an AI outbreak in Australia can be found at:

<http://www.animalhealthaustralia.com.au/wp-content/uploads/2011/04/Guidance-AI.pdf>

Australian Department of Agriculture Fisheries and Forestry

For further information on Avian Influenza Surveillance in Australia please follow the link to the National Avian Influenza Surveillance Dossier (OCVO, 2010). This is Australia’s first comprehensive document to collate, for strategic and analytic purposes, all available information about Australia’s poultry sectors, wild bird and domestic poultry surveillance, and poultry health management arrangements that relate to avian influenza and other diseases.

http://www.daff.gov.au/__data/assets/pdf_file/0007/1846015/national-avian-influenza-sept-2010.pdf
<http://www.daff.gov.au/animal-plant-health/animal>

- **Information on Avian Influenza or Bird Flu:**
<http://www.daff.gov.au/animal-plant-health/pests-diseases-weeds/animal/avian-influenza>
- **Information on Bird Biosecurity:**
http://www.daff.gov.au/animal-plant-health/pests-diseases-weeds/biosecurity/animal_biosecurity/bird-owners
- **National Farm Biosecurity Manual - Poultry Production:**
http://www.daff.gov.au/animal-plant-health/pests-diseases-weeds/biosecurity/animal_biosecurity/bird-owners/poultry_biosecurity_manual
- **National Water Biosecurity Manual – Poultry Production:**
http://www.daff.gov.au/animal-plant-health/pests-diseases-weeds/biosecurity/animal_biosecurity/bird-owners/water_biosecurity

Australian Department of Health and Ageing

- **Health Advice: Interim guidelines for persons working with poultry and other birds at risk of highly pathogenic avian influenza (CDNA, 2008)**
<http://www.health.gov.au/internet/main/publishing.nsf/Content/avian-influenza-poultry-guidelines.htm>

REFERENCES

- Arafa AM, Selim AA, Hassan MK and Aly MM. 2010. Genetic Characterization of Variant Strains of Highly Pathogenic Avian Influenza H5N1 That Escaped Detection by Real-Time Reverse Transcriptase-PCR Diagnostic Tests. *Avian Diseases* 54, 673-676.
- Barr DA, Kelly AP, Badman RT and Campey AR. 1986. Avian Influenza on a multi-age chicken farm. *Australian Veterinary Journal* 63, 195-196.
- Breed AC, Harris K, Hesterberg U, Gould G, Londt BZ, Brown IH, and Cook AJ. 2010. Surveillance for Avian Influenza in Wild Birds in the European Union in 2007. *Avian Diseases* 54, 399-404.
- Feare CJ. 2010. Role of wild birds in the spread of highly pathogenic Avian influenza Virus H5N1 and implications for global surveillance. *Avian Diseases* 54, 201-212.
- Fouchier RAM, Bestebroer TM, Herfst S, Van Der Kemp L, Rimmelzwaan GF and Osterhaus ADME. 2000. Detection of influenza A viruses from different species by PCR amplification of conserved sequences in the matrix gene. *Journal of Clinical Microbiology* 38, 4096-4101.
- Hartley VR, Hudson PJ, Coupar BEH, Selleck PW, Westbury H and Boyle DB. 1990. Vaccinia virus expression and sequence of an avian influenza nucleoprotein gene: its potential use in diagnosis. *Archives of Virology* 113, 133-141.
- Hansbro PM, Warner S, Tracey JP, Arzey KE, Selleck P, O'Riley K, Beckett EL, Bunn C, Kirkland PD, Vijaykrishna D, Olsen B, Hurt AC. 2010. Surveillance and Analysis of Avian Influenza Virus, Australia. *Emerging Infectious Diseases* 16, 1896-1904.
- Haynes L, Arzey E, Bell C, Buchanan N, Burgess G, Cronan V, Dickason C, Field H, Gibbs S, Hansbro PM, Hollingsworth T, Hurt AC, Kirkland P, McCracken H, O'Connor J, Tracey J, Wallner J, Warner S, Woods R, and Bunn C. 2009. Australian surveillance for avian influenza viruses in wild birds (July 2005 to June 2007). *Australian Veterinary Journal* 87, 266-272.
- Heine H, Trinidad L, Selleck P. 2005. Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease, Project 1.001R, Development of diagnostic capabilities for influenza H5N1 isolates technical report. Influenza virus type A and subtype H5-specific real-time reverse transcription (RRT)-PCR for detection of Asian H5N1 isolates. Melbourne (VIC, Australia): Commonwealth Scientific and Industrial Research Organisation Livestock Industries, Australian Animal Health Laboratory.
- Heine HG, Trinidad L, Selleck P, Lowther S. 2007. Rapid detection of highly pathogenic avian influenza H5N1 virus by Taqman reverse transcriptase-polymerase chain reaction. *Avian Dis* 51,370-2.
- Hesterberg U, Harris K, Moir R, Stroud D, Fabre C, Knight-Jones T, Londt B, Cook A, and Brown I (2008). Annual report on surveillance for avian influenza in wild birds in the EU during 2007 (SANCO/2181/2008 – Rev.1) DG-SANCO surveillance report. Available from:
http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/res_ai_surv_wildbirds_2007_en.pdf
- Klaassen M, Hoyer BJ, and DA Roshier. 2011. Identifying crucial gaps in our current knowledge of the life-history of Avian Influenza Viruses – an Australian perspective. *Emu* 111, 103-112.

- Knight-Jones TJD, Hauser R, Matthes D and Stärk KD. 2010. Evaluation of effectiveness and efficiency of wild bird surveillance for avian influenza. *Veterinary Research* 41, 50 (16 pages).
- Munster VJ, Baas C, Lexmond P, Waldenström J, Wallensten A, Fransson T, Rimmelzwaan GF, Beyer WEP, Schutten M, Olsen B, Osterhaus ADME, Fouchier RAM. 2007. Spatial, Temporal, and Species Variation in Prevalence of Influenza A Viruses in Wild Migratory Birds. *PLoS Pathogens* 3, e61.
- Munster VJ, Baas C, Lexmond P, Bestebroer TM, Guldemeester J, Beyer WEP, de Wit E, Schutten M, Rimmelzwaan GF, Osterhaus ADME and Fouchier ROM. 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.
- Newman SH, Siembieda J, Kock R, McCracken T, Khomenko S, and Mundkur T. 2010. FAO EMPRES Wildlife Unit Fact Sheet: Wildlife and H5N1 HPAI Virus - Current Knowledge. Animal Production and Health Division, Food and Agriculture Organization of the United Nations (available at <http://www.fao.org/avianflu/en/wildlife/index.html>).
- OCVO. 2010. National Avian Influenza Surveillance Dossier. Office of the Chief Veterinary Officer, Australian Government Department of Agriculture, Fisheries and Forestry, Canberra.
- Olsen B, Munster VJ, Wallensten A, Waldenström J, Osterhaus ADME and Fouchier RAM. 2006. Global Patterns of Influenza A Virus in Wild Birds. *Science* 312, 384-388.
- Selleck PW, Gleeson LJ, Hooper PT, Westbury HA and Hanson E. 1997. Identification and Characterisation of an H7N3 influenza A virus from an outbreak of virulent avian influenza in Victoria. *Australian Veterinary Journal* 75, 289-292.
- Selleck PW, Arzey G, Kirkland PD, Reece RL, Gould AR and Daniels PW. 2003. An outbreak of highly pathogenic avian influenza in Australia in 1997 caused by H7N4 virus. *Avian Diseases* 47, 806-811.
- Stallknecht DE, Nagy E, Bruce Hunter D and Slemmons RD. 2007. Avian Influenza. In: Thomas NJ, Hunter DB and Atkinson CT editors. *Infectious Diseases of Wild Birds*, Blackwell Publishing. pp. 108–130.
- Spackman E, Senne DA, Myers TJ, Bulaga LL, Garber LP, Perdue ML, Lohman K, Daum LT and Suarez DL. 2002. Development of a real-time reverse transcriptase PCR assay for Type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *Journal of Clinical Microbiology* 40, 3256–3260.
- Tracey JPR, Woods R, et al. 2004. The role of wild birds in the transmission of avian influenza for Australia: an ecological perspective. *Emu* 104, 109-124.
- Tracey J. 2010. Risk-based surveillance of avian influenza in Australia's wild birds. *Wildlife Research*. 37, 134–144.
- Turner AJ. 1976. The isolation of fowl plague virus in Victoria. *Australian Veterinary Journal* 52, 384.
- Wahlgren J. 2011. Influenza A viruses: an ecology review. *Infection Ecology and Epidemiology* 1, 6004.
- Westbury HA. 1997. History of highly pathogenic avian influenza in Australia. In: Swayne DE and Slemmons RD editors. *Proceedings of the 4th International Symposium on Avian Influenza*, May 29–31, Athens, Georgia. Symposium on Avian Influenza, US Animal Health Association: Richmond, VA. pp. 22–30.
- Zhou EM, Chan M, Heckert RA, Riva J and Cantin MF. 1998. Evaluation of a competitive ELISA for detection of antibodies against avian influenza virus nucleoprotein. *Avian Diseases* 42, 517–522.