The surveillance and risk assessment of wild birds in northern Australia for highly pathogenic avian influenza H5N1 virus

This thesis is presented for the degree of Doctor of Philosophy at Murdoch University

by

John M Curran

B.V.Sc.

2012

School of Veterinary and Biomedical Sciences

Faculty of Health Sciences

Murdoch University

Western Australia
Declaration

I declare that this thesis is my own account of my research and contains as its main content, work which has not previously been submitted for a degree at any tertiary education institution.

John Milford Curran
Abstract

Highly pathogenic avian influenza (HPAI), caused by infection with H5N1 virus, is a transboundary disease which has had a significant socio-economic impact on the poultry production systems of Eurasia, and spillover events with mortality in humans and wild birds. In northern Australia, prior to the current study there was poor understanding of the ecology of avian influenza viruses (AIV) and the risks of H5N1 transmission by wild birds. In this study, the biological pathways of risk for HPAI H5N1 by migratory birds were estimated as a negligible to very low risk to the wild birds of northern Australia. Following stochastic modelling the highest mean frequency of outbreaks was 1 year in 36 years (range 1 in 25-53 years; annual incidence of 0.028) for the Little Curlew (Numenius minutus), followed by the Sharp-tailed Sandpiper (Calidris acuminata) (1 in 56 years, range 36 to 91 years).

Three species of wild birds were challenged with a H6N2 low pathogenicity AIV (LPAIV). There was poor viral replication in the Ruddy Turnstones (Arenaria interpres) and Silver Gulls (Chroicocephalus novaehollandiae) with mostly low titre oropharyngeal (OP) excretion [median titre at 4 days post inoculation (DPI) of $10^{1.43}$ and $10^{2.09} 50\%$ embryo infectious dose (EID$_{50}$/0.1 mL respectively], with the exception of an OP sample from one Silver Gull ($10^{4.26}$ EID$_{50}$/0.1 mL at 2 DPI), and one cloacal sample from a Ruddy Turnstone ($10^{3.14}$ EID$_{50}$/0.1 mL at 10 DPI). In the Wandering Whistling Ducks (Dendrocygna arcuata), there was gastro-intestinal tropism with moderately high titre viral excretion to 6 DPI (highest median titre of $10^{4.58}$ EID$_{50}$/0.1 mL in cloacal swabs at 4 DPI). The anti-haemagglutinin (HA) antibody response was poor in the ducks and
declined from 19-56 DPI [highest haemagglutination inhibition (HI) test reciprocal geometric mean titre (GMT) of 16.1 at 19 DPI to a GMT of 3.7 at 56 DPI]. In the ducks after 42 DPI, nucleoprotein (NP) c-ELISA antibodies waned slowly from a median of 81% inhibition, and were long-lived to at least 8 months with a 57% median inhibition value.

The evaluation of a commercial NP c-ELISA, HI test, Taqman Type A RRT-PCR and embryonating chicken egg (ECE) virus isolation methods suggests high validity of these tests in wild birds, comparable to that reported in poultry. The NP c-ELISA in high AIV prevalence situations had a 100% diagnostic sensitivity (95% CI 81.5, 100) and in controls had 91% diagnostic specificity (95% CI 70.8, 98.9). In low AIV prevalence situations using a ≥60% inhibition threshold for positivity relative to the HI test, c-ELISA performed with 90.5% diagnostic sensitivity (95% CI 86.2, 93.8) and 41.2% diagnostic specificity (95% CI 38.1, 44.5). Assessment of the HI test suggests that a titre of ≥8 is a significant result in wild birds, and using this titre the HI test had 83.3% diagnostic sensitivity (95% CI 58.6, 96.4) in the challenged birds. The Type A RRT-PCR test performance for cloacal swabs had high diagnostic sensitivity that varied between 83.3-100% and diagnostic specificity that varied between 94.1-100% over 2-6 DPI when evaluated against ECE virus isolation, with substantial to outstanding agreement (Kappa statistic=0.8) and significant positive correlation (r_s=0.82). The recommended thresholds for the Type A RRT-PCR at the Australian Animal Health Laboratory (AAHL) in poultry of CT<37 for positivity with an intermediate threshold (CT 37-40) were found to be valid in wild birds. The ECE virus isolation method performed well with 89% of virus positive birds positive on the first passage.
The virological surveillance of 7,830 wild birds supports Australia’s current claim of freedom from HPAI H5N1 virus. The AIV prevalence was negligible in Charadriiformes (apparent or test prevalence, AP=0%; 95% CI 0, 0.09), and very low in Anseriformes (AP=0.03%; 95% CI 0, 0.16), with only one virus (H6N1) isolated from a Plumed Whistling Duck (*Dendrocygna eytoni*). Overall the NP c-ELISA seroprevalence was 3.5 times higher (Odds Ratio=4.7; 95% CI 4.1, 5.3) in Anseriformes (AP=31%; 95% CI 29.5, 32.6) compared to Charadriiformes (AP=8.8%; 95% CI 8, 9.7) indicating marked differences in the ecology of AIV. Moreover, analysis of NP seroprevalence data showed a higher AIV risk exposure profile in the Plumed Whistling Duck and eight species of migratory shorebirds, and spatiotemporal variations, with a two year cyclical periodicity in the waterfowl at Kununurra. The role of shorebirds in AIV ecology is more likely to be as spillover hosts in shared ecosystems with potential for sporadic global transmission of AIV, rather than being conventional reservoir hosts.
Communications

Presented at conferences:


Acknowledgments

This thesis had its synthesis in 1992, when my veterinary and ornithology skills connected with the testing of shorebirds, encouraged by Dr Trevor Ellis (co-supervisor) and Dr Clive Minton (eminent ornithologist), who have both continued to provide fantastic support over the last 20 years to this work. My principal supervisor at Murdoch University, Prof Ian Robertson has also given positive encouragement, outstanding technical and hands-on support especially to my virus challenge trial, good humour and statistical nous. Thanks to both Ian and Trevor for their diligent editing skills. At AAHL many staff have assisted, in particular Paul Selleck who has provided valuable editing and expert knowledge and facilitated my serological work without hesitation.

The surveillance of wild birds was funded by the AQIS/NAQS program, with Beth Cookson, Joe Schmidt, Tim Kerlin and many others contributing. The energy and enthusiasm of Broome ornithologist, Chris Hassell, was critical to the waterfowl surveys in all manner of places under extreme conditions and for access to his precious shorebirds many times. Thanks to the now defunct AB-CRC, Peta Edwards, Lisa Adams and Debby Cousins for encouragement and financial support. In Perth, the help of Mark O’Dea, John Parkinson and Jenny Hills with my trial and during my month long stint in their laboratory was a very rewarding experience. Thanks also to Adrian Boyle and many other AWSG volunteers. I also acknowledge last, but not least, my loving wife Kandy, for always encouraging me to take this on so late in my career and for being the primary breadwinner whilst I exited from paid employment.
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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AAHL</td>
<td>Australian Animal Health Laboratory</td>
</tr>
<tr>
<td>AD</td>
<td>average deviation</td>
</tr>
<tr>
<td>AGID</td>
<td>agar gel immunodiffusion test</td>
</tr>
<tr>
<td>AI/AIV(s)</td>
<td>avian influenza/avian influenza virus(es)</td>
</tr>
<tr>
<td>AP/App Prev</td>
<td>apparent (test) prevalence</td>
</tr>
<tr>
<td>AQIS</td>
<td>Australian Quarantine and Inspection Service</td>
</tr>
<tr>
<td>AWSG</td>
<td>Australasian Wader Studies Group</td>
</tr>
<tr>
<td>DAFWA</td>
<td>Department of Agriculture and Food Western Australia</td>
</tr>
<tr>
<td>DPI</td>
<td>days post inoculation</td>
</tr>
<tr>
<td>EAAF</td>
<td>East Asian-Australasian Flyway</td>
</tr>
<tr>
<td>ECE</td>
<td>embryonating chicken eggs</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EMPRES</td>
<td>FAO’s Emergency Prevention Programme for Transboundary Animal Diseases</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
</tr>
<tr>
<td>GMT</td>
<td>geometric mean titre</td>
</tr>
<tr>
<td>H/HA</td>
<td>haemagglutinin/haemagglutination</td>
</tr>
<tr>
<td>HI</td>
<td>haemagglutination inhibition test</td>
</tr>
<tr>
<td>HPAI</td>
<td>highly pathogenic avian influenza</td>
</tr>
<tr>
<td>HPNAIV</td>
<td>highly pathogenic notifiable avian influenza virus</td>
</tr>
<tr>
<td>LPAl</td>
<td>low pathogenicity AI</td>
</tr>
<tr>
<td>LPNAIV</td>
<td>low pathogenicity notifiable avian influenza virus</td>
</tr>
<tr>
<td>mAb</td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>NAQS</td>
<td>Northern Australia Quarantine Strategy</td>
</tr>
<tr>
<td>NI</td>
<td>neuraminidase inhibition</td>
</tr>
<tr>
<td>NDV</td>
<td>Newcastle Disease virus</td>
</tr>
<tr>
<td>NP</td>
<td>nucleoprotein</td>
</tr>
<tr>
<td>NT</td>
<td>Northern Territory (of Australia)</td>
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The World Organisation for Animal Health

OIE

OP oropharyngeal

PCR polymerase chain reaction

RRT-PCR real-time reverse transcription-PCR

PBST phosphate buffered saline Tween-20

QLD Queensland

RDE receptor destroying enzyme (II – Seiken)

RNA ribonucleic acid

SE standard error

SD standard deviation

TMB tetramethylbenzidine (Sigma T-2885)

USGS United States Geological Survey

VTM viral transport media

WA Western Australia

List of Unit Abbreviations

% percent

°C degree Celsius

CT cycle threshold

EID50 50% embryo infectious dose

mL millilitre

g grams

HAU haemagglutinating units

hr(s) hour(s)

km kilometre(s)

M molar

m metre(s)

min minutes

nm nanometres

nM nanomolar

OD optical density
ppm      parts per million
IU       international unit
secs     seconds
TCID\textsubscript{50}  50\% median tissue culture infective dose
µg       micrograms
µL       microlitre
µm       micrometre
x g      times gravity (centrifugal force)
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