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

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by

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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.

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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 C_T <37 for positivity with an intermediate threshold (C_T 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:

Curran JM (2008). Comparative risk profiles for HPAI in migratory shorebirds and waterfowl in northern Australia. Australian Biosecurity CRC for Emerging Infectious Diseases National Workshop, 24-26 June 2008, Bangkok, Thailand.

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Abbreviations

AAHL	Australian Animal Health Laboratory
AD	average deviation
AGID	agar gel immunodiffusion test
AI/AIV(s)	avian influenza/avian influenza virus(es)
AP/App Prev	apparent (test) prevalence
AQIS	Australian Quarantine and Inspection Service
AWSG	Australasian Wader Studies Group
DAFWA	Department of Agriculture and Food Western Australia
DPI	days post inoculation
EAAF	East Asian-Australasian Flyway
ECE	embryonating chicken eggs
ELISA	enzyme-linked immunosorbent assay
EMPRES	FAO's Emergency Prevention Programme for Transboundary Animal Diseases
FAO	Food and Agriculture Organization of the United Nations
GMT	geometric mean titre
H/HA	haemagglutinin/haemagglutination
HI	haemagglutination inhibition test
HPAI	highly pathogenic avian influenza
HPNAIV	highly pathogenic notifiable avian influenza virus
LPAI	low pathogenicity AI
LPNAIV	low pathogenicity notifiable avian influenza virus
mAb	monoclonal antibody
mRNA	messenger RNA
N/NA	neuraminidase
NAQS	Northern Australia Quarantine Strategy
NI	neuraminidase inhibition
NDV	Newcastle Disease virus
NP	nucleoprotein
NT	Northern Territory (of Australia)

OIE	The World Organisation for Animal Health
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
^{0}C	degree Celsius
C_T	cycle threshold
EID_{50}	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
$TCID_{50}$	50% median tissue culture infective dose
µg	micrograms
µL	microlitre
µm	micrometre
x g	times gravity (centrifugal force)

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