

**Studies on the diagnosis, epidemiology and control of
highly pathogenic H5N1 avian influenza**

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This thesis is presented for the degree of Doctor of Philosophy of

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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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Tze Hoong CHUA

Abstract

In late 2003 and early 2004, outbreaks of highly pathogenic avian influenza (HPAI) H5N1 occurred in domestic poultry across East Asia and Southeast Asia. Since May 2005, infection spread to wild birds and domestic poultry in Central and South Asia, Europe, Middle East and Africa. The rapid geographical spread of H5N1, the direct transmission of an avian virus to humans, the death and destruction of hundreds of millions of poultry with disease endemicity in many areas, the perpetuation of virus in apparently healthy ducks and paradoxically, its unusual virulence for waterfowl and mammals, and the constant viral evolution, highlighted the challenges the global community faced. This thesis addresses issues related to the diagnosis, epidemiology and control of H5N1 through research evaluating tools for rapid virus detection, field epidemiology studies and improved methods of vaccination in ducks as a disease control option. In particular, Chapter Two evaluates rapid antigen tests for H5N1 HPAI diagnosis in the field; Chapter Three identifies H5N1 infection in healthy village poultry in Bali and the possible role played by village poultry traders on H5N1 virus transmission; Chapter Four identifies risks for spread of viruses from live poultry markets in Bali; Chapter Five analyses the phylogenetic relationship of H5N1 viruses circulating in the village study sites during the 2006-2007 study period in Bali; and Chapter Six evaluates a recombinant baculovirus-expressed H5 vaccine against virulent H5N1 HPAI virus challenge in ducks for purpose of disease control.

Chapter Two describes a laboratory evaluation of five influenza antigen detection tests to estimate their diagnostic sensitivity. The evaluation was performed using close to 300 H5N1 positive swab samples that had been collected from field cases in Hong Kong. The results showed that the overall sensitivities of these tests ranged from 36.3% to 51.4% (95% confidence interval ranging from 31.0% to 57.0%). Analysis of test sensitivity indicated that these antigen detection tests could be used for rapid and preliminary flock

investigations of H5N1 outbreaks in sick and dead birds but should not be used for surveillance testing of clinically healthy birds. These tests offer a valuable role in disease investigation for example in rural village communities without immediate access to reference facilities. For the surveillance studies in villages (Chapter 3) and markets (Chapter 4) in Bali the evaluation studies of the rapid tests showed they were not suitable for detection of H5N1 infections in non-outbreak situations and consequently were not used for this purpose.

Chapters Three and Four describe field studies that were carried out to investigate the epidemiology of H5N1 in poultry in Bali, Indonesia. The surveillance recovered H5N1 HPAI virus at a low isolation rate (0.09% in chickens and 0.13% in ducks) in apparently healthy village poultry. A case-control study of village household flocks was performed. Using logistic regression analysis, the study identified risk factors that could influence the occurrence of H5N1 HPAI: the sale of poultry to collectors ($p < 0.01$), a poultry production system with access to backyard roaming birds ($p < 0.05$) and purchase of live poultry (chickens) ($p < 0.1$). To further investigate the H5N1 epidemiology, characteristics of live bird markets were studied through a questionnaire survey of market sellers. The survey found that live bird markets aggregate birds of different species, from different sources and locations, to be kept in close proximity, and this lack of biosecurity can contribute to H5N1 persistence and dissemination. Separately, molecular analysis of isolates from the surveillance showed the continuing evolution of H5N1 virus from 2004 till 2007. Isolates from the surveillance of apparently healthy birds shared close phylogenetic relationship with poultry viruses from outbreak cases (under subclade 2.1) and also contained the characteristic HPAI molecular pathotypes. Based on this finding, further research is needed to ascertain if asymptomatic chickens and ducks are H5N1 HPAI carriers and can become a transmission risk for poultry and humans.

Finally, as ducks were a source of H5N1 infection for other poultry in rural, endemic areas, Chapter Six describes a laboratory challenge study that was performed to investigate the efficacy of a recombinant baculovirus-expressed H5 vaccine. The study showed that the vaccine conferred protection from disease and mortality in ducks following challenge from an H5N1 HPAI virus. Vaccination in ducks resulted in elimination of respiratory virus shedding compared to unvaccinated control birds. The use of vaccines as a control strategy to break flock transmission and reduce the threat of ducks acting as a virus reservoir for other poultry is discussed. In addition, recombinant vaccine technologies offer a feasible method of production for affected developing countries without needing high biocontainment facilities or the expensive infrastructure required for producing vaccines via chicken embryos.

Publication

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Table of contents

Declaration	i
Abstract	ii
Publication	v
Acknowledgements	vi
Table of contents	x
List of tables and figures	xvi
Abbreviations	xix
Chapter 1 Literature review	1
1.1 General introduction	1
1.1.1 Avian influenza viruses	1
1.1.2 Host range and transmission of avian influenza	4
1.1.3 Clinical signs, pathology and pathogenesis	6
1.1.4 Diagnosis of AI, especially H5N1 HPAI	9
1.1.5 Host-pathogen relationship	12
1.1.6 Evolution of avian influenza virus	15
1.1.7 Mutation from LPAI to HPAI virus	18
1.1.8 Avian influenza as zoonoses	20
1.1.9 H5N1 human infection: risk factors and treatment	23
1.2 Spread of H5N1 in Asia and beyond	27
1.2.1 Influenza epicentre	27
1.2.2 H5N1 HPAI in Hong Kong, 1997	28
1.2.3 Events in Hong Kong, post-1997	32
1.2.4 Events in Asia, pre-2003	35
1.2.5 H5N1 HPAI in Asia, 2003-2004	37
1.2.6 Spread and transmission of H5N1	40
1.2.7 H5N1 HPAI in Asia, post-2004	45

1.2.8 H5N1 HPAI: spread beyond Asia, 2005	47
1.2.9 H5N1 HPAI: Europe, Africa, Middle East, 2006	50
1.2.10 H5N1 HPAI: 2007 till current	56
1.3 Role of different species in ecology of avian influenza	60
1.3.1 Wild birds as reservoir hosts of avian influenza	60
1.3.2 Virus persistence in nature	63
1.3.3 Transfer of virus between wild birds and domestic poultry	65
1.3.4 H5N1 HPAI in wild birds	66
1.3.5 Role of domestic ducks	68
1.3.6 H5N1 HPAI in domestic ducks	70
1.3.7 Role of chickens, quails and other gallinaceous poultry	73
1.3.8 Pigs as “mixing vessel” for influenza viruses	74
1.3.9 H5N1 HPAI in mammals	76
1.4 Molecular determinants of host range and pathogenicity	79
1.4.1 Host receptor specificity	79
1.4.2 Cleavage site of the haemagglutinin	82
1.4.3 Neuraminidase	84
1.4.4 Polymerase complex	85
1.4.5 Non-structural protein	87
1.4.6 Drug resistance mutations	89
1.5 Vaccination as a component of disease control	91
1.5.1 Use of vaccination in control of LPAI	91
1.5.2 Vaccination to control HPAI including H5N1	92
1.5.3 Current and new approaches on HPAI (H5N1) vaccines	94
1.6 Aims of thesis	98
Chapter 2 Performance evaluation of five detection tests for avian influenza antigen with various avian samples	100
2.0 Abstract	100
2.1 Introduction	101
2.2 Materials and methods	104

2.2.1 Samples for the test evaluations	104
2.2.2 Comparative assessment of the analytical sensitivity for the influenza A antigen tests	111
2.2.3 Statistical analysis	111
2.3 Results	113
2.3.1 Comparative sensitivity of the antigen detection tests	113
2.3.2 Effect of specimen type on test sensitivity	116
2.3.3 Effect of type of bird on test sensitivity	120
2.3.4 Effect of virus genotype on test sensitivities	123
2.3.5 Comparative analytical sensitivity of the influenza A-specific antigen detection tests	127
2.4 Discussion	128
Chapter 3 Investigation of the epidemiology of highly pathogenic avian influenza (H5N1) virus in village poultry in Bali	140
3.1 Introduction	140
3.2 Materials & Methods	142
3.2.1 Animal and human ethics approvals	142
3.2.2 Virological and serological surveillance	142
3.2.3 Questionnaire survey	146
3.2.4 Epidemiological analysis of questionnaires	147
3.3 Results	151
3.3.1 Virological surveillance	151
3.3.2 Serological surveillance	153
3.3.3 Respondents' background	154
3.3.4 Univariable analyses of questionnaire response	155
3.3.5 Logistic regression analysis of questionnaire response	161
3.3.6 Summary of survey response to descriptive questions	164
3.4 Discussion	166
3.4.1 Case definition and inapparent infections	166
3.4.2 Transmission risks from poultry traders	171

3.4.3 Risk from live poultry movement	173
3.4.4 Risk associated with disposal of dead birds and trading sick birds	173
3.4.5 Role of ducks in transmission pathways	174
3.4.6 Other risk factors	175
3.4.7 Villager attitudes to hygiene measures with respect to H5N1	176
3.4.8 Effectiveness of vaccination coverage and the H5 vaccination programme	176
3.4.9 Conclusion	178
Chapter 4 Live bird markets, can they spread avian influenza viruses?	179
4.1 Introduction	179
4.2 Materials and methods	181
4.2.1 Animal and human ethics approvals	181
4.2.2 Virus surveillance	181
4.2.3 Questionnaire survey in markets	183
4.2.4 Seeking expert opinion from veterinary staff	185
4.3 Results	186
4.3.1 Virological surveillance	186
4.3.2 Questionnaire interview responses on market characteristics	189
4.3.3 Expert opinions on market disease risk	194
4.4 Discussion	195
Chapter 5 Circulation of different H5N1 virus groups in poultry in Bali from 2004 to 2007	201
5.1 Introduction	201
5.2 Materials & Methods	202
5.2.1 Viruses isolated from village and market surveillance in Bali	202
5.2.2 Genomic characterisation of the village H5 isolates	203
5.2.3 Molecular analysis of H5N1 virus sequence data	204
5.3 Results	206

5.4 Discussion	218
----------------	-----

Chapter 6 Efficacy of a recombinant baculovirus-expressed vaccine in protecting ducks against a highly pathogenic H5N1 virus **225**

6.1 Introduction	225
------------------	-----

6.2 Materials and Methods	226
---------------------------	-----

6.2.1 Animal ethics and biosafety approvals	226
---	-----

6.2.2 Vaccines and virus	226
--------------------------	-----

6.2.3 Source of ducks	228
-----------------------	-----

6.2.4 Duck accommodation for vaccination and challenge studies	228
--	-----

6.2.5 Determination of duck challenge dose	229
--	-----

6.2.6 Vaccination and H5N1 challenge procedures	230
---	-----

6.2.7 Serology	232
----------------	-----

6.2.8 Virus isolation	232
-----------------------	-----

6.2.9 Histopathology of dead birds	233
------------------------------------	-----

6.2.10 Statistical analysis	234
-----------------------------	-----

6.3 Results	235
-------------	-----

6.3.1 Disease and mortality, first experiment	235
---	-----

6.3.2 Virus isolation, first experiment	236
---	-----

6.3.3 Serology, first experiment	236
----------------------------------	-----

6.3.4 Disease and mortality, second experiment	237
--	-----

6.3.5 Virus isolation, second experiment	238
--	-----

6.3.6 Serology, second experiment	238
-----------------------------------	-----

6.3.7 Gross and histopathology of dead birds	245
--	-----

6.4 Discussion	247
----------------	-----

Chapter 7 General discussion **257**

Appendices	264
Appendix 1 Village communities in Bali where sampling surveillance were conducted, 2006-2007	264
Appendix 2 Map of Bali districts	269
Appendix 3 Sample of questionnaire used in Bali village household study	270
Appendix 4 Sample of questionnaire used in Bali market study	275
Bibliography	278

List of tables and figures

Tables

1.1	Major HPAI outbreaks, 1959-2008.	13
1.2	Countries reporting H5N1 HPAI in poultry and in humans since late 2003 to the World Animal Health and World Health Organisations.	38
2.1	Avian influenza H5N1 culture-positive samples examined and summary results of rapid antigen detection tests.	114
2.2	Sensitivities, measured as percent positive compared with virus culture for rapid immunoassays and antigen capture ELISAs with undiluted H5N1 samples, compared with total samples.	116
2.3	Comparison of antigen detection test sensitivity with different H5N1 specimens.	118
2.4	Effect of bird type (dead chickens compared with dead waterbirds) on the sensitivity of rapid immunoassays and antigen capture ELISA tests.	122
2.5	Comparison of the effect of H5N1 genotype on the sensitivities of the antigen detection tests.	124
2.6	Differences in sensitivities of the antigen detection tests for viruses of different H5N1 Z genotypes between early and late 2002.	126
2.7	Comparison of analytical sensitivity for tests 1, 2, and 4 for influenza A antigen detection with a low-pathogenicity avian influenza virus (A/Eurasian Coot/Perth/2727/83).	127
3.1	Results of virological surveillance in village households, November 2006 to July 2007 in the districts of Tabanan, Bangli and Gianyar of Bali.	152
3.2	H5 antibody profiles by HI tests of 34 unvaccinated chickens that showed presence of H5 antibody during the village surveillance.	154
3.3	Cross-tabulation of categorical household variables by poultry flock cases and results of the univariable analysis, using an original case classification for Model A.	157
3.4	Comparison of flock sizes of main poultry types between cases and control for Model A.	160

3.5	Summary of univariable analyses of household variables where $p \leq 0.25$, using a modified case classification for Model B.	160
3.6	Factors associated with poultry flock cases identified through logistic regression analyses, based on two different case classifications (Model A and Model B).	163
4.1	Results of virological surveillance (Avian Influenza and Newcastle Disease virus) on pooled cloacal and throat swabs in live bird markets in Bali, September 2007 and February 2008.	188
4.2	Sources of market birds in the Bali market study.	191
5.1	H5N1 viruses from Bali examined in the molecular study.	207
5.2	Comparison of HA cleavage sequence examples of major H5 clades	221
6.1	Summary of disease and mortality in ducks on days post-VN/1203/04 challenge.	240
6.2	Efficacy of recombinant H5 vaccine in ducks challenged with $10^{4.3}$ EID ₅₀ S VN/1203/04 (first experiment).	241
6.3	Efficacy of recombinant H5 vaccine in ducks challenged with $10^{5.3}$ EID ₅₀ S VN/1203/04, and compared with an inactivated H5N2 vaccine (second experiment).	243

Figures

2.1	Photographs of antigen detection test kits	110
3.1	Poultry population by districts.	143
3.2	H5 antibody responses in 33 communities that had practised vaccination.	153
3.3	Household flock sizes and distribution of poultry types in village households of 518 respondents.	155
3.4	Epidemic curve of HPAI (H5N1) outbreaks in Bali, 2003-7.	169
5.1	Amino acid sequence alignment of the HA genes from H5N1 isolates from Bali, Indonesia.	213
5.2	Amino acid sequence alignment of the NA genes from H5N1 isolates from Bali and from Gs/Gd/96.	215

5.3a	Phylogenetic tree of HA genes of representative Indonesia viruses.	216
5.3b	Phylogenetic tree of HA genes of representative H5N1 viruses.	217
6.1	Oropharyngeal virus re-isolation (first challenge experiment).	242
6.2	Oropharyngeal virus re-isolation (second challenge experiment).	244

Abbreviations

AAHL	Australian Animal Health Laboratory
EID ₅₀	50% embryo infectious dose
ELISA	enzyme-linked immunosorbent assay
GMT	geometric mean titre
Gs/Gd/96	Goose/Guangdong/1/96 (H5N1)
HA	haemagglutinin
HI	haemagglutination inhibition
HPAI	highly pathogenic avian influenza virus
HRP	horseradish peroxidase
IVPI	intravenous pathogenicity index
LPAI	low pathogenic avian influenza virus
NA	neuraminidase
NASBA	nucleic acid sequence-based amplification
NIDVD	National Institute of Diagnostics and Vaccine Development in Infectious Disease
ND	Newcastle Disease
NP	nucleoprotein
OD	optical density
OIE	Office Internationale des Epizooties
pers. comm.	personal communications
RT-PCR	reverse transcription polymerase chain reaction
TCID ₅₀	50% tissue culture infective dose
T+C	tracheal and cloacal
VTM	viral transport media