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

Tze Hoong CHUA, BVSc (Hons)

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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 endemnicity 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

Chua, T. H., T. M. Ellis, C. W. Wong, Y. Guan, S. X. Ge, G. Peng, C. Lamichhane, C. Maliadis, S. W. Tan, P. Selleck, and J. Parkinson (2007) Performance evaluation of five detection tests for avian influenza antigen with various avian samples. *Avian Dis* **51** (1):96-105.

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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