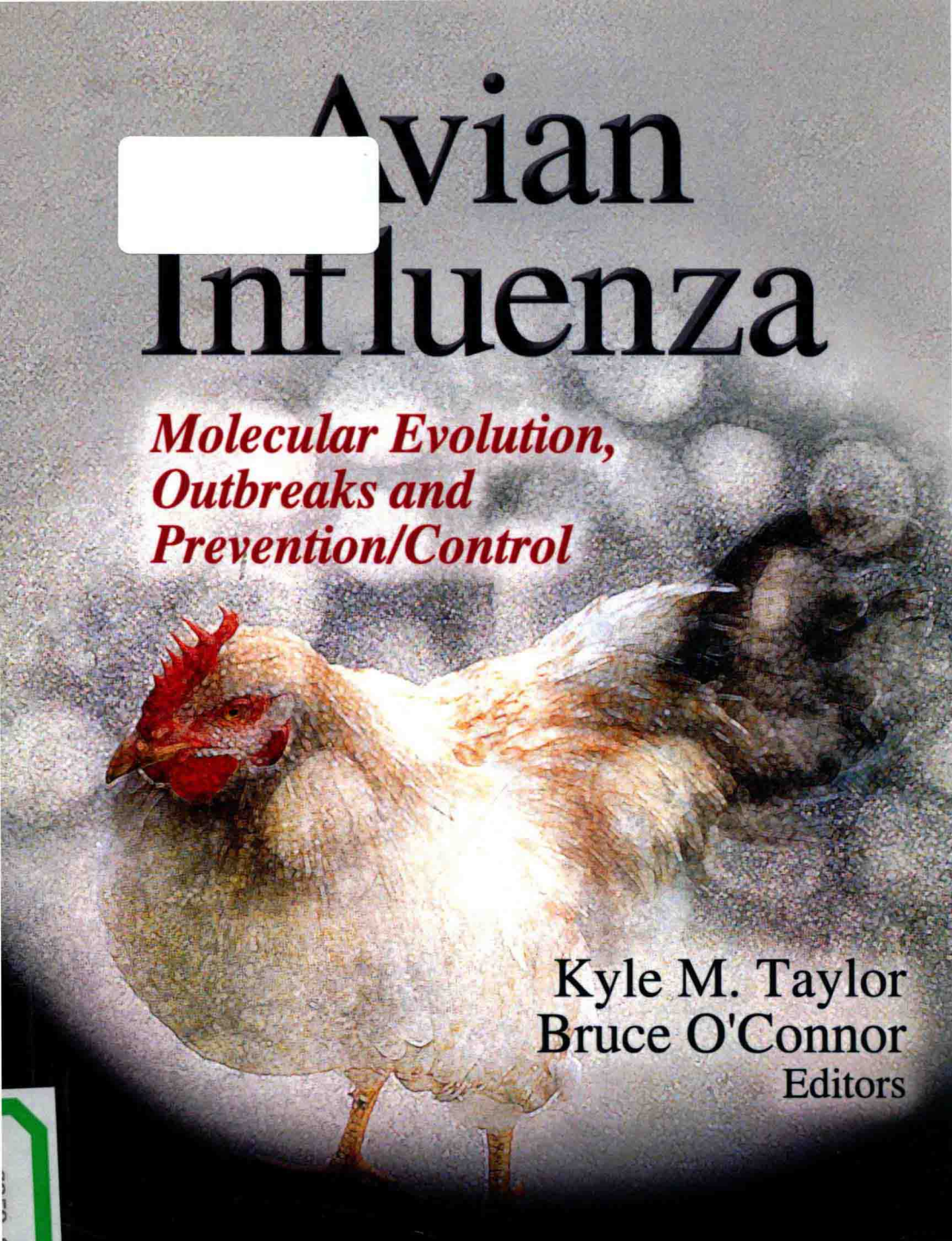
Avian Influenza

Molecular Evolution, Outbreaks and Prevention/Control



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AVIAN INFLUENZA

MOLECULAR EVOLUTION, OUTBREAKS AND PREVENTION/CONTROL

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PREFACE

Avian influenza or bird flu refers to "influenza caused by viruses adapted to birds." Of the greatest concern is the highly pathogenic avian influenza (HPAI). Most human contractions of the avian flu are a result of either handling dead infected birds or from contact with infected fluids. In this book, the authors discuss the molecular evolution, outbreaks and prevention/control of avian influenza. Topics include the risk assessment of highly pathogenic avian influenza virus infections through water; biosecurity measures against highly pathogenic avian influenza (HPAI) in free-range flocks and commercial poultry in developing countries; outbreak control and viral evolution of the highly pathogenic H5N1 avian influenza in Thailand; and the changes in perceptions and attitudes that were identified in a follow-up survey conducted when bird flu was not the focus on widespread media coverage in Australia.

Chapter 1 – Wild waterfowl are considered as the natural reservoir of all influenza A virus subtypes, including H5N1. Influenza A viruses replicate preferentially in the gastrointestinal tract of waterfowl, high concentrations are excreted in feces, and the viruses can be transmitted via the fecal-oral route among the waterfowl. Infected waterfowl can contaminate open water bodies, including drinking water sources and recreational areas, and the oral ingestion or aspiration of water containing influenza A virus could be a possible mode of transmission to humans. Quantitative microbial risk assessment (QMRA) framework is a powerful tool to understand how to control pandemics mediated by environmental reservoirs or human-to-human transmission (e.g. calculating risk of infection due to a low dose). Essential steps in the QMRA process are, exposure assessment and dose-response analysis. Recently, H5N1 influenza risk assessment models (1) to estimate the probability of human

infection from H5N1 through water and (2) to describe mortality of experimental animals exposed to H5N1 (time-dependent dose-response model) were developed. These models will be useful to evaluate the risks of infection under various transmission scenarios and contribute to prevention of a future human influenza pandemic caused by this lethal virus.

Chapter 2 – The emerging and re-emerging of infectious diseases over the last few decades demonstrate the potential for introduction of epidemic illnesses such as avian influenza through global migration of animals and humans. Recent outbreaks of a highly pathogenic avian influenza (HPAI) strain H5N1 in Asia and Africa have caused severe impacts on the poultry industries worldwide, both through bird mortality and morbidity and the resultant trade restrictions and negative demand shocks. There is also a considerable global concern that the virus could mutate into a form that can be passed between humans, which poses a significant risk of leading to a global pandemic. The risk 'mitigation' measures and risk 'propagation' practices in free-range flocks could influence introduction and maintenance of low pathogenic avian influenza (LPAI) with consequent depression in immunity of the free-range flocks, mutation and development of HPAI. These enormous potential liabilities have led to significant global investments in the disease prevention and control. However, poultry producers have roles to play in the prevention and control of avian influenza.

In this study, a review biosecurity measures practice in free-range flocks and commercial poultry operations in developing countries. The scientific literature on the prevention/control of highly pathogenic avian influenza of the past years is reviewed. The review findings are plausible as birds from free-range flocks have more opportunities of contact with wild birds that serve as reservoirs of low-pathogenic avian influenza strains than the commercial poultry, thus providing them with constant challenge of flock immunity. The development of efficient and effective biosecurity measures against avian influenza on commercial farms requires adequate placements of barriers to provide segregation, cleaning and disinfection, while concerted community established sanitary measures are needed for free-range poultry flocks in the developing economies. Good biosecurity levels on the farms and in the flocks ultimately lower costs in the production cycle, and flock welfare is always enhanced.

Chapter 3 – Thailand is known for its success in controlling the highly pathogenic H5N1 avian influenza epidemic. Despite the explosive outbreak in 2003-2004 similar to other countries in the East and Southeast Asian region, the epidemic was bought down under control in 2006 with the elimination of

infectious sources by "stamping out", strengthened biosecurity measures, and periodic active surveillance as the main control strategies. The initial epidemic in 2003-2005 involved mainly medium to large scale farming, resulting in massive economic loss. After 2006, sporadic cases occurred seasonally in backyard poultry in certain repeated outbreak areas involving limited number of poultry. The last animal outbreak was reported in 2008, and the last indigenous human case was detected in 2006. The reduction of viral population size was also evidenced by the reduced viral sequence diversity after 2006. The viral sequences showed little changes without evidences of positive selection during this low level endemic period and no known human-adapted mutations were observed. Similarity of viral sequences among outbreak seasons indicated that the virus was maintained in a local reservoir between outbreak seasons. Virus of similar lineage was occasionally isolated from local wild birds and migratory birds. Although some of these birds migrate in a route covering Southeast Asia to the epidemic hot spots in Central Asia, the similarity of viral sequences in these birds to the local virus suggested that the birds acquired the virus locally rather than carrying new viruses into the country. The small reservoir size in a limited area suggested that the virus can be eradicated from the country. While the current situation in the country is well under control, new kindle from undetected local reservoirs and import of new viral strains through human or wildlife activities are still a threat.

Chapter 4 – A survey of 200 Australian adults in May 2006 found reasonable levels of awareness, but low levels of concern, regarding bird flu. This paper reports on the changes in perceptions and attitudes that were identified in a follow-up survey conducted when bird flu was not the focus of widespread media coverage.

A computer assisted telephone survey was conducted in August and September 2006. A total of 5,565 eligible households were contacted and 805 interviews completed (response rate of 14.5%).

Bird flu fell from fourth to seventh most-frequently mentioned infectious disease. The majority of respondents were in favour of the government implementing quarantine procedures in the event of an outbreak, but less in favour of the government closing schools and offering people experimental vaccines or drugs. Respondents had low levels of awareness of preventive actions, but were generally willing to engage in these when they were identified.

We found that within four months of the initial high levels of concern bird flu was "off the radar" for the majority of the Australian population. One of the most important findings was that the general public appeared willing to engage in the appropriate preventive and protective behaviours, in the 'unlikely' event of a bird flu outbreak in Australia, but was lacking awareness of what these behaviours are.

Our results suggest that the Australian government will face a number of significant communication challenges in the event of an influenza pandemic. Not the least of these will be the need to communicate risk at the same time as educating people about appropriate preventive behaviours.

CONTENTS

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Chapter 1

RISK ASSESSMENT OF HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS INFECTIONS THROUGH WATER

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ABSTRACT

Wild waterfowl are considered as the natural reservoir of all influenza A virus subtypes, including H5N1. Influenza A viruses replicate preferentially in the gastrointestinal tract of waterfowl, high concentrations are excreted in feces, and the viruses can be transmitted via the fecal-oral route among the waterfowl. Infected waterfowl can contaminate open water bodies, including drinking water sources and recreational areas, and the oral ingestion or aspiration of water containing influenza A virus could be a possible mode of transmission to humans. Quantitative microbial risk assessment (QMRA) framework is a powerful tool to understand how to control pandemics mediated by environmental reservoirs or human-to-human transmission (e.g. calculating risk of infection due to a low dose). Essential steps in the QMRA process are, exposure assessment and dose-response analysis. Recently, H5N1 influenza risk assessment models (1) to estimate the probability of human

infection from H5N1 through water and (2) to describe mortality of experimental animals exposed to H5N1 (time-dependent dose-response model) were developed. These models will be useful to evaluate the risks of infection under various transmission scenarios and contribute to prevention of a future human influenza pandemic caused by this lethal virus.

1. INTRODUCTION

Influenza A viruses are members of the family *Orthomyxoviridae*, which comprises enveloped viruses with segmented, negative-sense RNA genomes (Wright et al. 2007). Based on the antigenicity of the two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), influenza A viruses are currently divided into 16 HA and 9 NA subtypes, designated as H1-H16 and N1-N9. All subtypes of influenza A viruses have been isolated from waterfowl, and they are the natural reservoir of influenza A viruses and asymptomatic virus carriers (Webser et al. 1992). Avian influenza A viruses replicate not only in the respiratory tract but also in the gastrointestinal tract in waterfowl and are thus shed in high concentrations in the feces (Webster et al. 1978). Avian influenza viruses have been isolated from water bodies where waterfowl gather and can persist for a long period of time in water. Infected waterfowl can contaminate open water bodies, including drinking water sources and recreational areas, and the oral ingestion or aspiration of water containing influenza A virus could be a possible mode of transmission to humans, although most human infection cases of H5N1 highly pathogenic avian influenza virus had a history of very close contact with infected birds, and inhalation of infectious droplets or aerosols was probably the most common route of infection (Brankston et al. 2007).

This chapter discusses the potential risk of influenza virus infection through water and the prevention/control of waterborne influenza outbreaks.

2. INFLUENZA VIRUS IN WATER

Detection Methods

Virus concentration is an essential step to detect viruses at low levels in water. Roepke et al. (1989) modified the **vir**us **ad**sorption-**el**ution (VIRADEL)

utilizing 1MDS filter (Cuno, Meriden, CT, USA), which was used for the detection of human enteric viruses in large volumes of water (Farrah et al. 1976), and evaluated for the concentration of influenza virus from water. The VIRADEL procedure (primary concentration) was combined with the chicken erythrocyte adsorption technique (secondary concentration), which was able to concentrate influenza virus for up to 3200-fold from 100-L tap water (Roepke et al., 1989).

However, influenza virus is not recovered as efficiently as enteric viruses, probably because the structure of influenza virus is different from that of enteric virus. The methods based on virus adsorption onto formalin-fixed chicken blood cells have been proposed for concentration of influenza viruses in environmental water (Khalenkov et al. 2008, Dovas et al. 2010), sometimes in combination with the filter adsorption (Sivanandan et al. 1991).

Several methods have been employed to detect influenza viruses in water. Isolation in embryotic specific-pathogen-free (SPF) eggs followed by reverse transcription-polymerase chain reaction (RT-PCR) assay is highly sensitive for detection of infectious virus particles (Khalenkov et al. 2008). Early techniques of isolating the influenza virus from lake water used unconcentrated water samples along with isolation in allantoic or amniotic cavities of embryonated chicken eggs or in tissue culture, such as Madin–Darby canine kidney (MDCK) cells, followed by hemagglutination inhibition or virus neutralization assays to confirm the presence of virus. Later, immunofluorescence methods and PCR-based assays were applied. At present, virus detection methods that use different PCR-based techniques, such as real-time PCR detection of different segments of influenza virus genes by using specific primers and probes with simultaneous subtyping (Stone et al. 2004), are most popular.

In our recent evaluation, H1N1 and H5N3 influenza viruses were not recovered as efficiently as enteric viruses by Mg-method (Katayama et al. 2002), Al-method (Haramoto et al. 2004), and 1-MDS method, because the structure of influenza virus is different from that of enteric virus. Modified Mg-method, which utilizes elution buffer (pH 7.9) containing 0.5% Tween 80, provided reasonably high recovery efficiency of up to 17% (Kitajima 2011).

Occurrence

Previous studies describing the occurrence of influenza viruses in water are summarized in Table 1.

Table 1. Occurrence of influenza viruses in water

Detection methods	Water type	Detection of indigenous influenza viruses				Rerefence
		Subtype	Country/Region	Titer[a]	Detection	
None	Lake water	H7N2, H4N1	Canada	NA	Egg isolation	Hinshaw et al. 1979
1MDS	Surface water	H13N2	Minnesota	NA	Egg isolation	Sivanandan et al. 1991
Chicken erythrocytes	Pond/ lake water	H4N6, H3N8, H7N3	Alaska	$10^{1.8}$ to $10^{2.8}$ EID_{50}/ml	Egg isolation	Ito et al. 1995
None	Lake ice	H1	Russia	NA	RT-nested PCR	Zhang et al. 2006
NanoCeram	Pond water	Non-H5 AIV	France	3×10^{1} to 9×10^{3} $TCID_{50}$-equivalent/L	RT-qPCR	Deboosere et al. 2011
PEG precipitation	Lake water	H10N8	China	NA	Egg isolation	Zhang et al. 2011
Ultrafiltration	Sewage	Influenza A	Netherlands	2.6×10^{5} copies/L	RT-qPCR	Heijnen and Medema 2011
None	Surface water	Influenza A	California	C_T value: 38.9±0.2	RT-qPCR	Hénaux et al. 2012

[a]EID_{50}, 50% egg infectious dose; $TCID_{50}$, 50% tissue culture infectious dose; C_T, cylcle threshold.

The concentrations of influenza viruses excreted by infected birds in environmental water are considered to be quite low because of dilution with water bodies. However, it has been reported that influenza viruses can be detected from lake water even without concentration when a number of wild waterfowls are present (Hinshaw et al. 1979, Webster et al. 1992). Influenza A viruses have been isolated from unconcentrated lake water on the shores of Canadian lakes where wild ducks had congregated before winter migration (Hinshaw et al. 1979). A recent study reported that 12 out of 597 (2.0%) unconcentrated water samples collected from 10 wetlands in two regions of the California Central Valley were positive for influenza A virus RNA by RT-qPCR targeting matrix gene (Hénaux et al. 2012).

Table 2. Detection of influenza viruses in human feces

Virus type	Remarks	References
Seasonal A	• Viral RNA was detected in feces by RT-PCR.	Wootton et al. 2006
Influenza B	• Viral RNA was detected in feces by RT-PCR.	Wootton et al. 2006
H3N2	• Viral RNA was detected in feces by real-time RT-PCR; 1.7×10^{4}~8.0×10^{7} copies/g-stool (n=6).	Chan et al. 2009
H5N1	• Infectious virus was detected in a rectal swab. • Viral RNA was also detected by real-time RT-PCR; 9.8×10^{4} copies/ml-rectal swab (n=1).	de Jong et al. 2005
H5N1	• Viral RNA in fecal swab was detected by real-time RT-PCR; 8.6×10^{2}~1.7×10^{6} copies/ml-VTM[a] (n=4). Infectious virus was also detected in fecal swabs.	Buchy et al. 2007
H1N1 2009 pdm	• Viral RNA was detected by real-time RT-PCR; 1.44×10^{4} copies/ml-stool (mean, n=4). Infectious virus was also detected in a stool. • Viral RNA was detected in urine by real-time RT-PCR but infectious virus was not detected.	To et al. 2010
H1N1 2009 pdm	• Viral RNA was detected from fecal samples by real-time RT-PCR in 16/65 (24.6%) of hospitalized individuals.	Yoo et al. 2010

[a]VTM, virus transfer medium.

However, viable influenza virus was not isolated with embryonating eggs, which suggests that the influenza virus concentration in water was low and/or the virus was inactivated by environmental factors, such as temperature and UV (Hénaux et al. 2012). The VIRADEL procedure was used to concentrate influenza virus, and low pathogenic avian influenza virus (H13N2) was isolated from 500 L of lake water in Minnesota (Sivanandan et al. 1991). Zhang et al. (2011) isolated an H10N8 influenza virus from lake water in China using polyethylene glycol (PEG) precipitation. It is possible that influenza viruses are frozen and preserved in ice or in lake water. Zhang et al. (2006) detected H1 influenza virus gene from 20 out of 373 ice meltwater samples collected from three northeastern Siberian lakes that are visited by large numbers of migratory birds. Results obtained in this study indicate that influenza A virus RNA is preserved in high concentrations in lake ice, which might facilitate genetic reassortment and/or recombination between the viruses

shed during the previous year and the viruses newly acquired by birds that spent winter months in the south (Zhang et al. 2006). Since several studies reported viral shedding in stool of patients infected with influenza viruses (Table 2), influenza viruses could potentially be present in municipal sewage water via feces excreted by infected individuals. In April 2009, a novel influenza virus A (H1N1) emerged in Mexico and California. Typical flu-like symptoms as well as gastrointestinal symptoms (vomiting, diarrhea, and abdominal pain) were also frequently observed in patients infected with pandemic influenza A (H1N1) 2009 virus, and this virus was detected in feces of infected patients (Yoo et al. 2010, To et al. 2010). Heijnen and Medema (2011) reported the results of monitoring of influenza viruses in sewage and surface water; although influenza A viruses were detected, the pandemic influenza A (H1N1) virus was not detected. Similarly, raw sewage samples collected over a year in Tokyo, Japan and Arizona, US were tested for the presence of influenza virus matrix (M) gene by real-time RT-PCR, but no amplification was observed from any samples (Kitajima M, unpublished data). These results imply that the water cycle does not play a relevant in spreading influenza A virus, including the pandemic influenza A (H1N1) virus.

Persistence

Avian influenza viruses can persist for a long period of time in water, although little information is available for the subtype H5N1. In a study by Ito et al. (1995) that obtained 7 positives for influenza A virus out of 13 water samples (54%) collected at a lake where ducks were nesting in summer. The positive rate remained high (14%) in the following autumn after ducks had migrated, which suggests that the viral particles were able to persist in water.

Table 3. Persistence of influenza viruses in water

Water type	Subtype	Temperature (°C)	pH	Salinity (ppt)	Findings	Reference
River water	H3N6	22	6.8	-	Infective up to 4 days	Webster et al. 1978
River water	H3N6	0	6.8	-	Infective over 30 days	Webster et al. 1978
Distilled water	H3N8, H4N6, H6N2, H12N5, H10N7	17		0	Infective for 207 days	Stallknecht et al. 1990a