THE ECOLOGY OF INFECTIOUS PATHOGENS IN A LONG DISTANCE MIGRATORY BIRD, THE BLUE-WINGED TEAL (*Anas discors*): FROM INDIVIDUALS TO POPULATIONS

A Thesis Submitted to the College of

Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Veterinary Pathology

University of Saskatchewan

Saskatoon

By

RODOLFO A. NALLAR-GUTIERREZ

Permission to Use

In presenting this thesis in partial fulfilment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professors who supervised my thesis work, Dr. Catherine Soos and Dr. Frederick A. Leighton, or, in their absence, by the Head of the Department of Veterinary Pathology or the Dean of the Western College of Veterinary Medicine. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Rodolfo A. Nallar-Gutierrez

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Veterinary Pathology
Western College of Veterinary Medicine
University of Saskatchewan
52 Campus Drive
Saskatoon, Saskatchewan S7H 5B4

ABSTRACT

The aim of this study is to improve our understanding of the ecology, spatiotemporal patterns, and risk of infectious pathogens of migratory waterfowl, using the Blue-winged teal (*Anas discors*, BWTE), as a model. From 2007-2010, 1,869 BWTE were sampled in the prairie provinces (Alberta, Saskatchewan and Manitoba, Canada) to examine infection status and/or evidence of previous exposure to avian influenza virus (AIV), West Nile virus (WNV), and avian paramyxovirus-1 (APMV-1), in relation to host demographic variables (age, sex, body condition, exposure to other pathogens), other ecological variables such as local waterfowl breeding population density and local pond density, and year. The probability of AIV infection depended on an interaction between age and AIV antibody status. Hatch year birds with antibodies to AIV were more likely to be infected, suggesting an antibody response to an active infection. After hatch year birds with antibodies to AIV were less likely to be infected, suggesting immunity resulting from previous exposure. AIV infection was positively associated with local BWTE density, supporting the hypothesis of density dependent transmission. Exposure to WNV and APMV-1 were also associated with age and year. Furthermore, the probability of WNV exposure was positively associated with local pond density rather than host population density, likely because ponds provide suitable breeding habitat for mosquitoes, the primary vectors for transmission.

We also investigated large-scale spatiotemporal trends in apparent prevalence of AIV across Canada and the United States throughout the year, using data from national avian influenza surveillance programs in Canada and the US in 2007-2010. Our analyses revealed that age, sex, year of sampling, flyway, latitude, and season (categorized by stages of the BWTE annual life cycle) were all important variables in predicting probability of AIV infection. There was an interaction between age and season. During late summer staging (August) and fall migration

(September-October), hatch year birds were more likely to be infected than after hatch year birds, however there was no difference between age categories for the remainder of the year (winter, spring migration, and breeding season). Probability of infection increased non-linearly with latitude, and was highest in summer, corresponding with the beginning of fall migration when densities of birds and the proportion of susceptible hatch year birds in the population are highest. Birds in the Pacific, Central and Mississippi flyways were significantly more likely to be infected compared to those in the Atlantic flyway. Observed trends in seasonal, annual, and geographic patterns of AIV infection in BWTE across Canada and the US were primarily driven by the dynamics of AIV infection in hatch year birds. Our results demonstrate demographic as well as seasonal, latitudinal and flyway trends across Canada and the US.

This research provided further evidence for the role of wild dabbling ducks, particularly BWTE, in the maintenance and ecology of AIV. This improved understanding of the role of BWTE as natural hosts, and the geographic, demographic and temporal variables that affect infection and transmission parameters, moves us closer to understanding the overall ecology of the virus at the individual, population and continental levels. This knowledge, in turn, will permit development of better tools to predict and perhaps to prevent possible outbreaks in domestic animals as well as in humans.

ACKNOWLEDGMENTS

I would like to thank Drs. Catherine Soos and Ted Leighton for their invaluable guidance, support, and friendship during my years in Canada involved in this research. I could not have had better supervisors and teachers. All the challenges they proposed to me, made me a better professional. I thank my advisory committee, Drs. Tasha Epp, Trent Bollinger, and Keith Hobson for their priceless support and advice. I also thank Drs. Beverly Kidney and Susantha Gomis for their work as Graduate Chair during my study.

I thank Dr. Zsuzsanna Papp for her invaluable help doing and re-doing the models and for introducing me to the world of statistics. Thank you so much Zsuzsa! I had the privilege to be involved in this project and had the opportunity to work with wonderful people in field and lab. I especially thank research technicians Hollie Remenda, Molly Lux, and Wendy van Dijk from Environment Canada; Robbin Lindsay and Antonia Dibernardo from the National Microbiology Laboratory-Public Health Agency of Canada; and John Pasick and Yohannes Berhane from the National Centre for Foreign Animal Disease - Canadian Food Inspection Agency, for all their teaching, guidance, and experiences shared. I also thank the various field biologists from the Canadian Wildlife Service (Jim Leafloor, Darin Walker, Marc Schuster, Keith McAloney and many others), Ontario Ministry of Natural Resources (Tore Buchanan, Chris Davies), and USFWS (Phil Thorpe, Jim Wortham, John Solberg, Walt Rhodes, Jenny Hoskins and others) for assistance with waterfowl capture, banding, and sampling Blue-winged teal across Canada. I thank all summer students and volunteers for their assistance in the field and lab, including Allison Murray, Josanne Verhagen, Kenna Halford, Ashlee Albright, Melissa Domoslai, Tatiana Santander, and Esteban Guevara.

I am very grateful to Canada's Interagency Wild Bird Influenza Survey, the US Early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds

(USDA-APHIS), and the Avian Influenza Surveillance Program of the US Fish and Wildlife Service and USGS for permitting the use of their valuable surveillance data. In particular I would like to thank Thomas DeLiberto, Mark Lutman and Seth Swafford from the USDA-APHIS; Jonathan Sleeman, Hon Ip, and Robert Dusek, and Jeff Hall from the USGS National Wildlife Health Center; and Samantha Gibbs from the USFWS.

I also thank Dr. Gary Wobeser, Chen-Chi Chen, Erin Silbernagel, Ian Shirley, Tyler Moss, Sandy Mayes, and Nadine Kozakevich who were always a great fount of invaluable resources and troubleshooting for me. Thank you so much for all your help and patience. I would like to thank Sergio Sanchez and Predrag Novakovic for those long hours of deep philosophy; Orleigh Bogle, Jimena Yapura and Maria F. Mejia who helped me with proof-reading to improve this thesis; the nice group of fellow Latin graduate students for your wonderful camaraderie; and Iain Rose, Hussein Juma and Takako Travis for their friendship and support during those years away from home.

I have to mention the people in Bolivia who were supporting me at different stages of my thesis. Thank you so much to Lilian Painter and Rob Wallace for their sponsorship to get the GFP-WCS scholarship to start my graduate studies. Nuria Bernal, Lisa Paye, Enrique Domic, Ariel Reinaga and Teddy Siles helped me with language and GIS, thank you all.

Finally and most importantly, I am so grateful to my family. My wife, Rocio, who interrupted her career to be part of this project, my son Brahim and my baby Rodito are the incandescent engine of my life. I thank my mom, dad, and my brother Bra for supporting me in my decisions and encouraging me during both the best and the hardest moments. Gracias por su apoyo, amor y entusiasmo, ustedes son fuente de inspiracion.

This research was supported by (in alphabetic order) Alberta Conservation Association, Canada's Inter-agency Wild Bird Influenza Survey, Canadian Cooperative Wildlife Health Centre, Canadian Food Inspection Agency, Ducks Unlimited Canada's Institute for Wetland and Waterfowl Research, Environment Canada; Graduate Fellowship Program of the Wildlife Conservation Society; the University of Saskatchewan (Graduate Studies Enhancement Funds, Wildlife Health Research Funds) and the USDA – APHIS.

DEDICATION

To Rocio, Brahim & Rodito…
Gracias por su apoyo incondicional.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AHY	after hatch year
AIV	avian influenza virus
APMV	avian paramyxovirus
BWTE	Blue-winged teal
cELISA	competitive enzyme-linked immunosorbent assay
ELISA	enzyme-linked immunosorbent assay
HPAIV	highly pathogenic avian influenza virus
HY	hatch year
LPAIV	low pathogenic avian influenza virus
PCR	polymerase chain reaction
RRT-PCR	Real time polymerase chain reaction
US	United States
WNV	West Nile virus

CHAPTER 1
GENERAL INTRODUCTION AND LITERATURE REVIEW

Ecology of diseases and the importance of epidemiological surveillance in migratory waterfowl

Infectious diseases in migratory waterfowl, such as avian influenza (AIV), West Nile virus (WNV) and avian paramyxovirus (APMV), have the potential to cause negative environmental, social, and economic impacts (Guan *et al*., 2004; Takekawa *et al*., 2010). Some of these diseases can cause high mortality in migratory birds, or affect reproduction (Wobeser, 1997; Takekawa *et al*., 2010), both of which can have serious consequences for biodiversity, conservation and ecosystem health.

Long-term studies in migratory birds have uncovered a wide range of information about the ecology of waterfowl. However, the host-pathogen ecological dynamics among migratory birds have so far received comparatively little scientific attention. Study of these dynamics is complicated due to the difficulty in studying migratory species that travel long distances, often across multiple countries or continents, associated differences in spatiotemporal variables and niches occupied at breeding, overwintering, and stopover sites (Krauss *et al*., 2004), dissimilar intrinsic host traits between and within species (Hill *et al*., 2010), and multiple stressors or pressures impacting populations, including habitat loss and alteration due to industrial or agricultural practices (Altizer *et al*., 2011; Wobeser, 1997), hunting pressure (Raftovich *et al*., 2010), exposure to contaminants, and other anthropogenic stressors.

Long distance migratory birds travel thousands of kilometers from breeding to wintering areas and vice-versa every year (Reed *et al*., 2003). A key characteristic of migration, particularly in waterfowl species, is that millions of birds from diverse areas often converge on specific staging or stop-over sites during both spring and fall migration, after which they disperse

to numerous locations for breeding or over-winter habitat. Due to high densities of birds at these locations and the multiple sources of birds, often of different species, these sites are potential locations for the transmission and mixing of pathogens (Webster *et al*., 2007; Reed *et al*., 2003), with subsequent wide geographic dispersal. Migratory birds have the potential to spread pathogens among different ecosystems along their migratory routes, including pathogens such as H5N1 highly pathogenic avian influenza (HPAI) (Guan *et al*., 2004), and WNV (Owen *et al*., 2006) which also have implications for human and domestic animal health.

Effective early warning systems that might reduce the negative impacts of pathogens shared among wild animals, domestic animals and humans rely on an understanding of the ecology of such pathogens in wild populations (Rhyan & Spraker, 2010). Since the spread of HPAI H5N1 in the eastern hemisphere, and of WNV in North America, many studies have been carried out to determine the role of wild birds in transmission of pathogens (Olsen *et al*., 2006; Komar *et al*., 2003). These studies have provided valuable information about the pathogenesis, molecular evolution, and potential risk to public, domestic animal, and wildlife health. Other studies have examined bird migration in the perpetuation and spread of pathogens (Beldomenico & Uhart, 2008; Rhyan & Spraker, 2010). Such studies contribute to understanding the ecology of pathogens in these populations, and improve our capacity to predict, prevent, or mitigate the socio-economic and environmental consequences of some emerging pathogens. In the present study, we examined spatiotemporal distribution and environmental and demographic variables (risk factors) at the individual, population, and continental levels associated with exposure to infectious pathogens (AIV, WNV and APMV) in Blue-winged Teal.

The Blue-winged teal

The Blue-winged teal (*Anas discors*, BWTE) is a highly sociable dabbling duck of the subfamily Anatinae (family Anatidae), and has the largest distribution and range among North

American migratory ducks, and goes through Central to South America, with some vagrants as far South as Argentina (Botero & Rusch, 1988; Botero, 1992; Szymanski & Dubovsky, 2010). Its breeding range extends from Alaska east to Nova Scotia and south to Texas (Figure 1). The northern prairies and prairies of central North America are the principal nesting and breeding areas for BWTE (Rohwer *et al*., 2002).

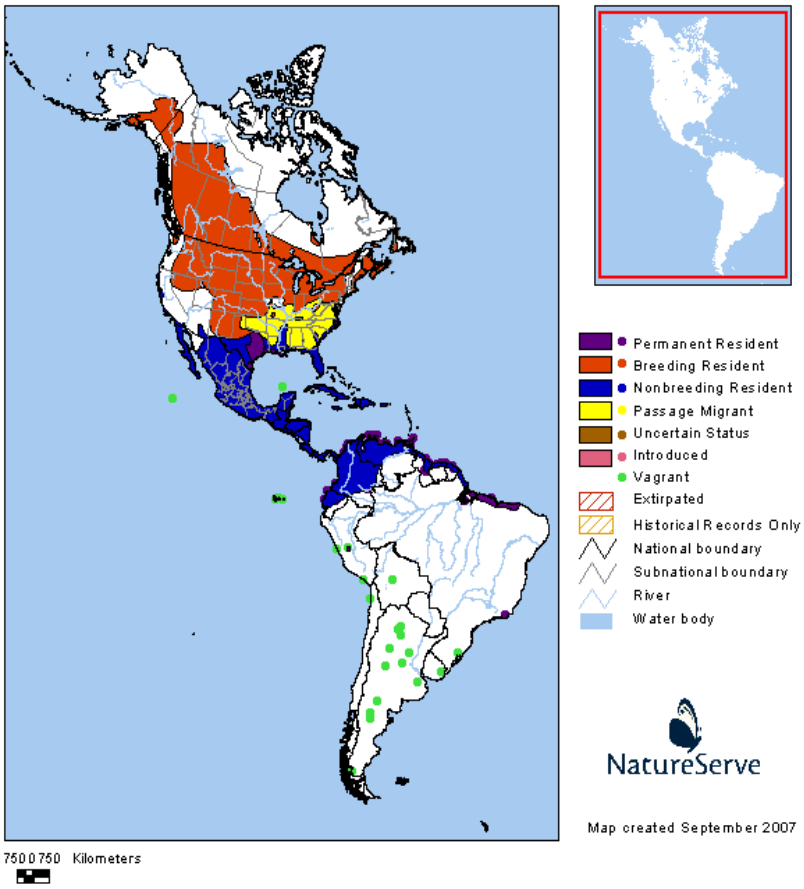

Figure 1-1. Blue-winged Teal distribution map showing breeding and migratory patterns. (Map created by Natureserve, 2007.)

The BWTE is one of the earliest dabbling duck species to fly south in the fall. As soon as hatch year birds are able to fly for long distances, they gather in flocks at the end of summer in late August and early September in preparation for fall migration, which occurs in September to November. BWTE overwinter in warm areas in southern latitudes of the United States, Mexico, Central America, northern South America and the Caribbean islands, from November to

February (Rohwer *et al* 2002). Most pairs are formed during northward migration in spring (from March to May), before arrival on breeding areas (Rohwer *et al.*, 2002). The BWTE is the latest duck species to arrive in the prairies in late April and May, (Botero & Rusch, 1988; Szymanski & Dubovsky, 2010), and are also latest to begin nesting (Rohwer *et al.*, 2002). Males are territorial and intolerant to other pairs during nesting, egg-laying and incubation, which occur from May to July (Rohwer *et al*., 2002). Females lay clutches of six to fourteen eggs per season; eggs are incubated for 19 to 29 days, and all chicks hatch synchronously. Juveniles fly at 40 days and reach sexual maturity in the following breeding season (Palmer, 1976).

The BWTE has the second most abundant breeding population of ducks in North America with a long-term average of 4.7 million individuals annually, compared with the more abundant Mallard (*Anas platyrhynchos*) which has a long-term population average of 7.5 million (Zimper *et al*., 2010). The BWTE is the fifth most hunted species in the United States and the eighth most hunted species in Canada, with an estimated annual harvest average of 1 million and 30,000 individuals, respectively (Raftovich *et al*., 2010).

Blue-winged teal feed in shallow water and rarely dive. Their diet is mainly (~70%) composed of plant material, including seeds of grasses, sedges, and both seeds and leaves of pondweeds. The remainder of their diet (~30%) consists of animal matter, including mollusks, insects, and crustaceans (Palmer, 1976).

Avian influenza virus

In recent years, avian influenza has received considerable attention worldwide because it represents one of the greatest concerns for large-scale outbreaks in poultry and people. Influenza viruses belong to the Orthomyxoviridae family, and are subclassified as influenza virus A, B, and C. The causal agent of avian influenza is an influenza A virus (Webster *et al*., 1992; Webster *et al*., 2007). Influenza A viruses are further subclassified according to two glycoproteins on the

surface of the virus: H or hemagglutinin (rod-shaped trimers) and N or neuraminidase (mushroom-shaped tetrameters). Avian influenza viruses (AIV) are classified according to their combination of H and N proteins, for example: H7N3, H1N1, H5N2, etc. (Horimoto & Kawaoka, 2001; Garcia-Garcia & Ramos, 2006; Olsen *et al*., 2006). The international nomenclature system applied to influenza viruses includes the host of origin (excluding humans), geographical location of origin, strain number, year of isolation and the H and N. [e.g., A/cinnamon teal/Bolivia/4537/01 (H7N3)] where "A" is the influenza virus type, Cinnamon teal is the host, Bolivia is the geographical location, 4537 is the laboratory number given by the laboratory which isolated the virus, and 01 represents the year of the isolation (2001) (Chanock *et al*., 1972).

At present, 17 HA and 9 NA have been described in different combinations and species (Webster *et al*., 1992; Tong *et al*., 2012). Most of the 153 possible combinations of HA and NA have been isolated from wild birds. Currently, subtypes H13, H14, H15 and H17 have never been isolated from wild ducks (Olsen *et al*., 2006; Krauss *et al*., 2004). Although little work has been done in some places around the world, it is believed that AIV are ubiquitous. In addition to the classification of AIV by their H and N protein composition, they also are classified as low (LP) and high (HP) in pathogenicity or virulence to domestic chickens (Alexander, 2000). Genetic variants of AIV are continuously produced from existing strains by genetic mutation (genetic drift), classical recombination, insertion, deletion, and reassortment (genetic shift) (Webster 2007).

In wild birds, the orders Anseriformes (waterfowl) and Charadriiformes (shorebirds and gulls) are the most significant known natural reservoirs for low pathogenicity strains of AI virus (LPAIV) and may be the original source of influenza A viruses that infect a variety of animals,

including poultry, horses, pigs, sea mammals and humans (Krauss *et al*., 2004; Webster *et al*., 2007). High prevalences of LPAIV are commonly reported in dabbling ducks (Parmley *et al*., 2008; Pasick *et al*., 2010; Olsen *et al*., 2006). The virus is excreted in high concentrations in feces, so the principal route of transmission is thought to be the fecal-oral route, with water playing an important role as a medium of virus transmission (Webster *et al*., 1992). In addition, flocking (Garamszegi & Moller, 2007), sexual behaviour (Garamszegi & Moller, 2007; Hegyi *et al*., 2009) and feeding behaviour, e.g. filter-feeding in shallow water (Hill *et al*., 2010) may also favor or facilitate the transmission of AIV. Furthermore, long distance migration and the potential for AIV to remain infectious for long periods of time in fresh water are considered the most important characteristics in nature for the perpetuation and transmission of the virus (Garamszegi & Moller, 2007; Stallknecht & Brown, 2009).

Apparent prevalence of influenza viruses in surveys of wild ducks in North America has been high in northern post-breeding areas during the staging period prior to southward migration at the end of summer and beginning of fall, and low during northward migration and during the breeding season (Parmley *et al*., 2008; Pasick *et al*., 2010; Sharp *et al*., 1993; Hinshaw *et al*., 1985; Hinshaw *et al*., 1980; Krauss *et al*., 2004). Long term studies in Canada have shown cyclical patterns of AIV, with prevalence peaking every 2 to 3 years (Sharp *et al*., 1993; Krauss *et al*., 2004; Hinshaw *et al*., 1985; Hinshaw *et al*., 1980; Wilcox *et al*., 2011). These seasonal and annual patterns of apparent prevalence found in dabbling ducks suggest that the virus persists in duck populations, either entirely through transmission among ducks or in association with persistence in the environment (Olsen *et al*., 2006; Rohani *et al*. 2009; Henaux and Samuel 2011).

West Nile Virus

Since its emergence in North America in 1999, West Nile virus (WNV) has caused declines in local populations of many species of wild birds, (LaDeau *et al*., 2007, Foppa *et al*., 2011), and clinical sickness and fatality in humans (Hayes & Gubler, 2006; Artsob *et al*., 2009) and horses (Epp *et al*., 2007). The introduction of this virus into the western hemisphere served as a warning sign to public health officials worldwide by showing that such pathogens can emerge and re-emerge anywhere (Komar, 2003; Epp *et al*., 2007).

WNV is a mosquito-borne virus of the family Flaviviridae, a family that also includes Japanese encephalitis, dengue, and yellow fever viruses (Georgiev, 2009; Nash *et al*., 2001). It is transmitted between birds, which amplify the virus, and mosquitoes, especially *Culex* species, with occasional spill-over to horses and humans which are dead end hosts (Hayes & Gubler, 2006.)

WNV was first identified and described in the 1930s and was known to be widespread in Africa, Europe, and the Middle East before 1999. It was first isolated and identified in the western hemisphere in 1999, in the New York City metropolitan area (Nash *et al*., 2001). By the end of 2002, the virus had spread throughout most of Canada and the United States, affecting both animals and humans. By 2006, it was isolated in Argentina, first in horses and then in wild birds (Morales *et al*., 2006).

Over 200 wild bird species in the Americas have been recorded infected with the virus (National Wildlife Health Center/USGS, 2011). Passeriformes, especially birds of the family Corvidae, may have been one of the most significantly impacted. An analysis of American crow populations showed a decline of up to 45% in 6 regions across the United States (LaDeau *et al*., 2007). In the case of waterfowl, the National Wildlife Health Center reported that 31 species of the family Anatidae were found infected with WNV (National Wildlife Health Center/USGS,

2011), however, little is known about the impact of this virus on their populations. Studies have found hatch year ducks susceptible to both fatal and non-fatal infections with WNV (Komar *et al*., 2003; Shirafuji *et al*., 2009).

Waterfowl species were not included in the WNV surveillance program in Canada. However, the rapid expansion of WNV in America created significant concern in Japan and Korea where surveillance studies detected seroprevalences in adult wild ducks ranging in Japan (Saito *et al*., 2009) and South Korea (Yeh *et al*., 2011) providing evidence that migratory ducks were exposed at some point along their migratory routes.

Avian paramyxovirus

Avian paramyxovirus-1 (APMV-1) is globally widespread and has been isolated from wild birds, especially aquatic birds (Wobeser *et al*., 1993; Kuiken *et al*., 1998; Alexander, 2009), poultry and occasionally humans (Alexander, 2009). This virus belongs to the genus *Avulavirus* of the family Paramyxoviridae. The genome of APMV is composed of a single filament of RNA with negative polarity which codes for six proteins (Alexander, 2000).

Highly pathogenic APMV-1 causes vast economic loss to the poultry industry and commerce of poultry products due to morbidity and mortality of affected birds and to trading restrictions which are applied to areas and countries where outbreaks have occurred (Alexander, 2009). Highly pathogenic APMV-1 viruses are also called “Newcastle Disease Virus.” Due to the potential risk of massive and rapid spread, highly pathogenic APMV-1 (Newcastle Disease virus) is classified by the World Organization for Animal Health (OIE) as a notifiable disease (OIE, 2005). Previously considered as being exotic to North America, highly pathogenic APMV-1 was discovered in breeding colonies of double-crested cormorants in North America between 1990 and 2000 (Wobeser *et al*., 1993; Kuiken *et al*., 1998). Outbreaks of APMV-1 in double-crested cormorant colonies represent one of the most important incidents in wild bird species in

which this pathogen has caused large die-offs. Also, APMV-1 has been detected regularly in European cormorant species and Rock Pigeon (Leighton & Heckert, 2007). The virus is transmitted to susceptible birds by feces, body fluids and vertically through eggs. Contaminated inanimate objects can serve as sources of infection. The majority of affected birds in reported mortalities were hatch-year birds (Leighton & Heckert, 2007).

Surveillance of AIV in wild ducks has also resulted in the isolation of APMV including serotypes 1, 2, 3, 4, 6, 7, 8, and 9 (Stallknecht *et al*., 1991; Hanson *et al*., 2005; Goekjian *et al*., 2011). Furthermore, a study in Japan showed that after several cycles of infection in chickens with a low pathogenicity strain of APMV-1 isolated from a wild goose, the virus became highly pathogenic for chickens, demonstrating the possible role of wild waterfowl in the transmission of the low pathogenic virus to poultry which then might evolve into highly pathogenic strains within poultry populations (Shengqing *et al*., 2002). However, the role of waterfowl and low pathogenic APMV-1 strains in the epidemiology of highly pathogenic APMV-1 remains unclear.

Thesis Summary

Ethics Statement

Birds were captured, banded and sampled under the authority of Environment Canada – Canadian Wildlife Service Scientific Research Permit (CWS07-005), Saskatchewan Ministry of Environment Special Permit (09FW131, renewed annually), University Committee on Animal Care and Supply (UCACS), Animal Use Protocol Number (20070039), Migratory Bird Sanctuary permit (for working at Last Mountain Lake; renewed annually), National Wildlife Area permit (for working at Last Mountain Lake; renewed annually) and Environment Canada Migratory Bird Banding permit (Soos, 10458R). Bird capture, handling and sampling procedures were approved by the University of Saskatchewan's Animal Research Ethics Board, and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

Objectives

The general objective of my research was to investigate the spatiotemporal distribution and ecological variables associated with the likelihood of infection or exposure to AIV, WNV and APMV-1 viruses in Blue-winged teal, using individual to population-based approaches. I investigated the role of host demographic factors (age, sex, body condition, exposure to other pathogens), ecological factors such as population density and pond density, and annual variation in exposure to AIV, WNV and APMV-1 viruses in BWTE in the prairie provinces. I also investigated annual, seasonal, spatial, and demographic factors associated with AIV infection in BWTE sampled in North America, by analyzing surveillance data from national avian influenza surveillance programs in Canada and the United States.

Thesis Topics

1. Ecological determinants of avian influenza virus, West Nile virus and avian paramyxovirus infection and exposure in Blue-winged teal (*Anas discors*) in the Canadian Prairies.

(i) determine the proportion of Blue-winged teal infected and/or previously exposed to AIV, WNV, and APMV-1 viruses, in the three Canadian Prairie Provinces, between 2007 and 2010;

(ii) examine infection and/or exposure status for each of the above pathogens in relation to age, sex, body condition, population density, pond density, year, and infection/exposure to other pathogens;

2. Demographic and spatiotemporal patterns associated with avian influenza infection in Blue-winged teal (*Anas discors*) at the continental scale

(iii) investigate demographic (age, sex), spatial (latitudinal, flyway), and temporal (annual, seasonal) factors affecting low pathogenicity AIV infection in Blue-winged teal sampled throughout their annual life cycle in Canada and the United States;

(iv) graphically illustrate geographical and temporal trends in prevalence of low pathogenic AIV infection in Blue-winged teal in Canada and the United States.

Rationale or importance

A better understanding of the ecology of pathogens in wild migratory birds will improve our capacity to predict, prevent, or mitigate their potential negative socio-economic and environmental consequences.

CHAPTER 2
ECOLOGICAL DETERMINANTS OF AVIAN INFLUENZA VIRUS, WEST NILE VIRUS AND AVIAN PARAMYXOVIRUS INFECTION AND EXPOSURE IN BLUE-WINGED TEAL (*ANAS DISCORS*) IN THE CANADIAN PRAIRIES

Abstract

Although numerous surveillance programs and epidemiological studies have been conducted on avian influenza virus (AIV), West Nile virus (WNV), and avian paramyxovirus (APMV) in wild birds, our understanding of the ecology of these pathogens in migratory waterfowl remains limited. We investigated ecological drivers of infection with these pathogens in Blue-winged teal (*Anas discors*, BWTE) in the Canadian prairies, one of the most important breeding and staging areas for migratory waterfowl in North America. We used logistic regression models to examine infection and/or exposure to each of the above pathogens in relation to host demographic variables (age, sex, body condition, exposure to other pathogens), and other ecological variables such as local waterfowl breeding population density, local pond density, and year. AIV infection in BWTE was associated with individual host factors such as age and serological status, regional or population-level factors such as local BWTE population density, and year. The probability of AIV infection depended on an interaction between age and AIV antibody status. Hatch year birds with antibodies to AIV were more likely to be infected, suggesting an antibody response to an active infection. After hatch year birds with antibodies to AIV were less likely to be infected, suggesting immunity resulting from previous exposure. AIV infection was positively associated with local BWTE density, supporting the hypothesis of density dependent transmission. Exposure to WNV and APMV-1 were also associated with age and year. Furthermore, the probability of WNV exposure was positively associated with local pond density rather than host

population density, likely because ponds provide suitable breeding habitat for mosquitoes, the primary vectors for transmission. Our findings highlight the importance of spatiotemporal, environmental, and host factors at the individual and population levels, all of which may have impacts on infection dynamics of pathogens in wild populations.

Keywords: Avian influenza, avian paramyxovirus, Blue-winged teal, disease ecology, migratory waterfowl, molecular diagnostics, serology, West Nile virus.

Introduction

Since the spread of H5N1 highly pathogenic avian influenza (HPAI) in the eastern hemisphere, and the introduction and spread of West Nile virus (WNV) in North America, numerous surveillance programs and studies have been undertaken to detect the occurrence, distribution, or spread of these pathogens in wild bird populations (Parmley *et al*., 2008; Saito *et al.*, 2009; Gaidet *et al*., 2010). Birds that migrate long distances may be exposed more frequently and to a broader range of pathogens compared to non-migratory birds, and thus may have the potential to be long-distance carriers of infectious pathogens (Reed *et al*., 2003; Altizer *et al*., 2011). Migratory waterfowl are ideal hosts in which to study the ecology of infectious diseases because they aggregate in high densities during different stages of their life cycle, and travel long distances. Furthermore, free-ranging waterfowl, particularly those of the family Anatidae, are natural reservoirs for a wide range of avian influenza viruses (AIV) and avian paramyxoviruses (APMV), both of which are pathogens of broad significance to public and/or domestic animal and wildlife health (Hinshaw *et al*., 1980; Webster *et al*., 1992). Although numerous studies of AIV, WNV and APMV-1 in free-ranging or captive birds have been conducted, our understanding of the ecology of these pathogens in migratory ducks remains limited.

Understanding the ecology of these pathogens in migratory waterfowl is required to assess risks to wildlife, domestic animal, and human health, and to devise management responses to these or other pathogens that can infect or be carried by migratory birds.

The Canadian prairies, located in the provinces of Alberta, Saskatchewan, and Manitoba, form one of the most important hubs in North America of flyway intersection and shared habitat for migratory waterfowl (North American Waterfowl Management Plan, 2004). Hundreds of thousands of waterfowl of numerous species from many different flyways converge in this region annually for breeding or staging. Subsequently, birds disperse southward on winter migration via several different flyways and to numerous winter destinations. Hence, the Canadian prairies are a key area for potential cross-infection among birds that have come from a variety of geographic locations in North, Central and South America, and they are also a key area from which pathogens can disperse to numerous locations in the western hemisphere.

The Blue-winged teal (*Anas discors*, BWTE) is an ideal host species for studying the ecology of the above pathogens because it makes extensive use of the Canadian prairies for breeding and staging, is gregarious, often forming large flocks which come in contact with shorebirds and other waterfowl, and has the largest migratory range among dabbling ducks (subfamily Anatinae) in the western hemisphere (Botero & Rusch, 1988; Szymanski & Dubovsky, 2013). This species represents the second most abundant breeding population of ducks in North America, with an estimated population of 6.3 million individuals in 2010 (U.S. Fish and Wildlife Service, 2010). All these factors may increase opportunities for exposure, transmission, and movement of a range of pathogens.

Our objective was to identify demographic and ecological risk factors associated with infection and/or exposure to AIV, WNV, and APMV-1 in Blue-winged teal in the three Prairie

Provinces of Canada, from 2007-2010. For each pathogen, host age, sex, body condition, exposure to other pathogens, year, breeding population density and local pond density were examined as potential factors affecting the probability of infection or exposure to the target pathogens.

Material and methods

Field methods

From 2007 to 2010, Blue-winged teal were sampled in August, prior to fall migration, at several sites within the Canadian Prairies, including Frank Lake and the regions around Brooks, Alberta; Last Mountain Lake, Saskatchewan; and Delta Marsh, Manitoba (Figure 2-1). Birds were captured in collaboration with the Canadian Wildlife Service (CWS) and US Fish and Wildlife Service (USFWS) during annual banding programs using standard bait trap methods. For each bird, information on location, date, band number, age, sex, mass and head-bill length were recorded. Oral and cloacal swabs were placed together in a single vial and stored as described by Parmley *et al.* (2011). Blood samples were collected by jugular venipuncture and stored on frozen gel packs. At the end of each day, all blood samples were centrifuged, and serum was harvested and frozen at -20C until further testing.

Laboratory analysis

Swabs were analyzed by standardized methods at regional veterinary diagnostic laboratories within Canada's Influenza Virus Laboratory Network (Parmley *et al*., 2008; Pasick *et al*., 2010) for the presence of influenza A virus nucleic acid using a reverse transcriptase real-time PCR (RRT-PCR) which targets the matrix 1 gene (Spackman *et al*., 2002). We tested sera for the presence of antibodies to AIV nucleoprotein using a competitive enzyme-linked immunosorbent assay (cELISA) at the Canadian Food Inspection Agency, National Centre for Foreign Animal

Disease, in Winnipeg, MB, using methods described by Yang *et al.*(2008). Values exceeding 30% inhibition in relation to the controls were considered positive (Yang *et al*., 2008).

Serum antibodies to WNV were measured at the National Microbiology Laboratory, Public Health Agency of Canada, in Winnipeg, MB. Samples were first screened with a cELISA for flaviviruses using a mouse anti-West Nile/Kunjin virus monoclonal antibody MAB8152 (Millipore TM, Single Oak Drive, Temecula, CA). Samples with inhibition values above 30% were considered positive (Blitvich *et al*., 2003). All cELISA-positive samples were confirmed using Plaque-reduction neutralization (PRNT) specific for WNV; samples that neutralized ≥90% of virus were considered positive (Blitvich *et al*., 2003; Drebot *et al*., 2003).

Serum antibodies against avian paramyxovirus-1 (APMV-1) were measured using hemagglutination inhibition assays (HI) at the Poultry Diagnostic and Research Center, University of Georgia in Athens, GA (Alexander, 2000).

Bird population and pond densities

We obtained data from the Waterfowl Breeding Population and Habitat Survey for 2007-2010 to estimate spring breeding population and spring pond densities (USFWS, 2010) for each of the regions in which ducks were sampled (Table 2-1). Total duck population density and BWTE density were defined as the number of dabbling ducks or BWTE estimated per km^2 for each stratum surveyed during the breeding season (USFWS, 2010). Similarly, pond density was defined as the number of standing water bodies estimated per km^2 for each stratum surveyed during the breeding season (USFWS, 2010). For our analyses, we assumed that breeding population and pond density estimates in spring were correlated with densities at the time of sampling in August.

Statistical analysis

Individuals with missing data on sex, age, or diagnostic test results were excluded from analysis. Descriptive statistics were calculated to estimate overall apparent prevalence (proportion of infected birds) and/or apparent seroprevalence (proportion of birds with antibodies) of the selected pathogens. Each of the four outcome variables (AIV infection, AIV antibody status, WNV antibody status, and APMV-1 antibody status) was examined in relation to demographic and ecological factors listed in Table 2-1. We constructed generalized linear models with a binomial distribution, using a logit link function based on maximum likelihood estimation, using the program R (version 2.14.1; R Core Team, 2012). Model selection was carried out using the Akaike information criterion corrected for small sample size (AICc; Anderson, 2008).

For each outcome, a set of global models was built, including as many biologically relevant explanatory variables as possible, based on two criteria: 1. the variables had an initial association with the outcome variable in univariate models; and 2. the variables were not collinear with each other (Murray and Conner, 2009). Because of high correlation between pond density, duck-density, BWTE density and province (Pearson or phi correlation coefficient > 0.4), these variables were not entered together into the same models. A correlation coefficient higher than 0.4 can be problematic in ecological data, potentially causing inaccurate estimates (Murray & Conner, 2009). The residuals of BWTE density from its linear regression on pond density (Figure S2-1) were used to create an alternate measure of BWTE population density (Table 2-1) which could be combined with pond density in models. Nested models derived from global models were included in each model set to show relative importance of variables. Informative variables were defined as those that improved the model's AICc (Anderson, 2007). If models were within 2 AIC units of each other, then the simplest one was chosen. We calculated p values

based on 95% confidence intervals (C.I.) while AIC-based model selection was based on 85% C.I. (Arnold, 2009).

Table 2-1. List of outcome and explanatory variables used in models exploring determinants of infection or exposure to avian influenza virus (AIV), West Nile virus (WNV), and avian paramyxovirus-1 (APMV-1), in Blue-winged teal in the Canadian prairies (2007-10).

Variable	Type	Description	Categories/Units
Year	Categorical, explanatory	Year of sampling	2007- 2010
Pond density	Continuous, explanatory	Suitable breeding pond density estimated by aerial surveys conducted in spring	# ponds/km^2
Duck density	Continuous, explanatory	Total duck breeding population density estimated by aerial surveys in spring	Total # ducks/km^2
BWTE density	Continuous, explanatory	Blue-winged teal breeding population density estimated by aerial surveys in spring	Total # BWTE/km^2
Residuals of BWTE density	Continuous, explanatory	Expressed as the residuals of BWTE density from its linear regression on pond density	
Sex	Categorical, explanatory	Gender of bird	Female / Male
Age	Categorical, explanatory	Age of bird at capture	Hatch year / after hatch year
Body condition index (BCI)	Continuous, explanatory	Measure incorporating mass and body size to define the energy capital accumulated in the body (Peig & Green, 2009)	
Head-bill length	Continuous, explanatory	Standard measure of head to bill length	mm
AIV infection	Categorical, outcome and explanatory	Current infection status, as determined using RRT-PCR of the matrix gene	Positive / Negative
AIV antibody status (AIV-ab)	Categorical, outcome and explanatory	Avian Influenza Virus nucleoprotein-specific antibodies; positive suggests previous exposure	Positive / Negative
WNV antibody status (WNV-ab)	Categorical, outcome and explanatory	West Nile Virus-specific antibodies; positive suggests previous exposure	Positive / Negative
APMV antibody status (APMV-ab)	Categorical, outcome and explanatory	Avian paramyxovirus-specific antibodies; positive suggests previous exposure	Positive / Negative

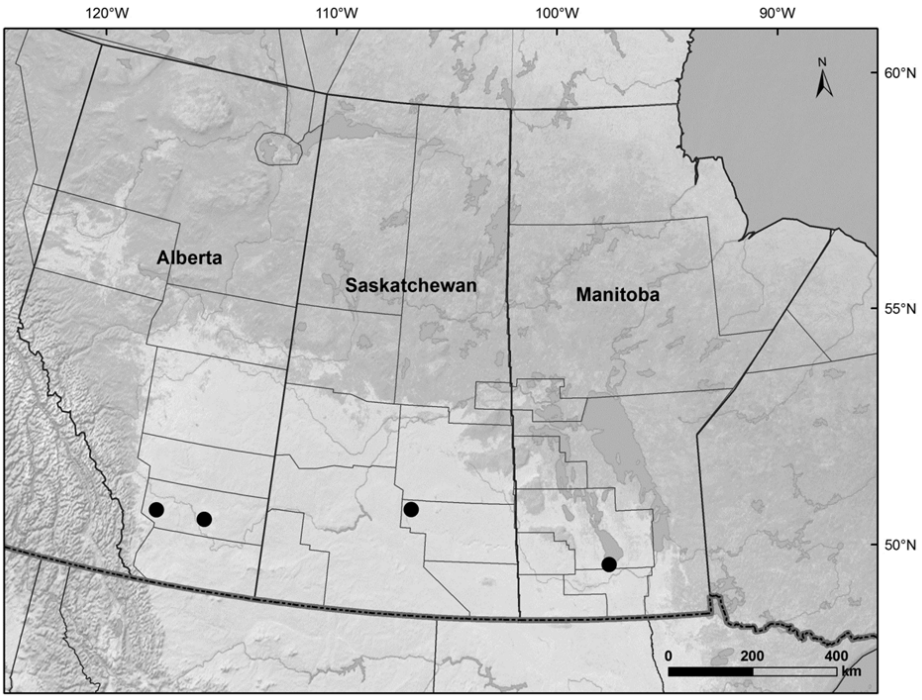

Figure 2-1. Black dots indicate locations of capture sites for Blue-winged teal sampled the Canadian Prairies in 2007-2010. Thick black lines delineate provincial/territorial boundaries. Thin lines indicate strata used for estimating pond density and waterfowl breeding population densities by the Waterfowl Breeding Population and Habitat Survey. Thick line indicates Canada-US border.

Results

From 2007-2010, we captured, banded and sampled 1,971 Blue-winged teal in Alberta, Manitoba, and Saskatchewan. Individuals excluded from statistical analyses due to missing data or insufficient serum for sample analysis included 126 individuals for AIV analyses, 102 for WNV analyses, and 154 for APMV analyses.

Risk factors associated with avian influenza virus infection and exposure

Overall, 18.8% of BWTE sampled in the Canadian prairies between 2007 and 2010 were infected with AIV, and 47.2% had antibodies to AIV (Table 2-2). Of birds seropositive for AIV (n = 871), 111 (12.7%) were infected with AIV, 53 (6%) were seropositive for WNV, 71 (8%) were seropositive for APMV, and 6 (0.7%) birds were seropositive for all three pathogens. Results of our model selection exercise are shown in Tables S1 and S2. Based on the best supported model for AIV infection (Table 2-3), there was an interaction between age and AIV serological status (AIV-Ab). Hatch year (HY) birds with antibodies to AIV were more likely to be infected with AIV compared to seronegative HY birds, and after hatch year (AHY) birds with antibodies to AIV were slightly less likely to be infected compared to seronegative AHY birds (Figure 2-2). Interestingly, seronegative AHY birds were still less likely to be infected with AIV compared to seronegative HY birds. Sex was not informative in our final model although it initially appeared to play a role when examined on its own, with females apparently more likely to be infected (Table 2-2, S2-1). This association was spurious, resulting from the large proportion of HY birds among the females sampled, and thus was lost when combined in models with age (Table S2-1).

Our model showed annual variability in estimated prevalence of AIV infection, being lowest in 2008, then increasing over the following two years, with the highest prevalence estimated in 2010 (Figure 2-3). Probability of AIV infection was positively associated with the residuals of

BWTE density regressed on pond density, which explained more of the variation in AIV status than did BWTE density (Table S2-1). Total duck density, pond density, head-bill length and body condition index (BCI, Table 2-1) did not have initial associations with AIV, and therefore were excluded from model sets. There was also no association between AIV infection and previous exposure to WNV or APMV-1.

The probability of a bird having antibodies to AIV was higher in AHY birds compared to HY birds (Table 2-4, Figure 2-4). Head-bill length was also informative, in that larger birds were more likely to have antibodies to AIV. Similar to models examining AIV infection status, there was annual variation in estimated seroprevalence of AIV. In this case, lowest seroprevalences in both age classes were observed in 2009, following a year (2008) of almost zero prevalence of infection (Figure 2-3). Density variables (pond density, BWTE density, total duck density or residuals of BWTE density on pond density), BCI, and previous exposure to WNV or APMV-1 were not informative in predicting AIV antibody status (Table S2-2).

Risk factors affecting WNV antibody status

Overall seroprevalence of WNV in BWTE sampled in the Canadian Prairies in 2007-10 was 4.2% (Table 2-2). Of birds seropositive for WNV (n = 76), 12 (15.7%) were infected with AIV, 53 (69.7%) were seropositive for AIV, and 9 (11.8%) were seropositive for APMV. Candidate models examined to explain variation in probability of WNV exposure are presented in Table S2-3. AHY birds were more likely to be seropositive for WNV compared to HY birds (Table 2-5). Only slight annual variation was detected in WNV seroprevalence, being lowest in 2010. BWTE were more likely to be seropositive for WNV with increasing spring pond density (Table 2-5), which explained more of the variation in WNV antibody status compared to all measures of population density (Table S2-3). Sex, head-bill length, and previous exposure to AIV or APMV-1 had no initial association with WNV antibody status (results not shown).

Risk factors affecting APMV-1 antibody status

Overall seroprevalence of APMV-1 was 6.3% (Table 2-2). Of birds seropositive for APMV-1 (n = 115), 16 (13.9%) were infected with AIV, 71 (61.7%) were seropositive for AIV, and 9 (7.8%) were seropositive for WNV. Candidate models explored to evaluate factors affecting probability of APMV-1 exposure are presented in Table S4-2. In our best-supported model (Table 2-6), the chance that a bird had antibodies to APMV-1 was higher in AHY birds compared to HY birds, and influenced by year of collection, with 2009 having the highest apparent seroprevalence (Table 2-6). Density variables (pond, BWTE, and total duck density) as well as sex, head-bill length, BCI, and exposure to other pathogens were not associated with APMV-1 antibody status (results not shown).

Table 2-2. Raw data showing overall apparent prevalence of avian influenza virus (AIV) and apparent seroprevalence of AIV, West Nile virus (WNV) and avian paramyxovirus-1 (APMV-1) in Blue-winged teal in the Canadian prairies, 2007-10.

	AIV	AIV-Ab	WNV-Ab	APMV-Ab
N	1869	1846	1869	1817
Overall positive (%)	351 (18.8)	817 (47.2)	78 (4.2)	115 (6.3)
Number positive by sex				
M (%)	207/1209(17.1)	644/1194 (53.9)	53/1209 (4.4)	74/1169 (6.3)
F (%)	144/660 (21.8)	227/652 (34.8)	24/660 (3.6)	41/648 (6.3)
Numer positive by age				
HY (%)	282/939 (30.3)	162/929 (17.4)	15/939 (1.6)	32/918 (3.5)
AHY (%)	69/930 (7.4)	709/917 (77.3)	62/930 (6.7)	83/899(9.2)
Number positive by year				
2007 (%)	141/534 (26.4)	190/523 (36.3)	25/534 (4.7)	20/534 (3.7)
2008 (%)	2/436 (0.5)	292/429 (68.1)	27/436 (6.2)	30/436 (6.9)
2009 (%)	34/304 (11.2)	144/299 (48.1)	17/304 (5.6)	33/304 (10.9)
2010 (%)	174/595 (29.2)	245/595 (41.2)	8/595 (1.3)	32/543 (5.9)

Abbreviations: M = male, F = female, HY = hatch year, AHY = after hatch year, AIV = Avian influenza virus infection, AIV-ab = avian influenza virus antibodies (to nucleoprotein), APMV-ab = avian paramyxovirus antibodies, WNV-ab = West Nile virus antibodies

Table 2-3. Summary of best-supported model to estimate AIV infection probability in Blue-winged teal sampled in the Canadian prairies from 2007 to 2010. AIV infection was determined using RRT-PCR on oral and cloacal swab samples (n = 1846).

Variables	β	SE	P
AIV-ab (ref=Neg)			
Pos	0.500	0.211	0.0186
Age (ref=HY)			
AHY	-0.936	0.256	0.0003
AIV-Ab (Pos)*Age(AHY)	-0.989	0.354	0.0053
Year (ref=2007)			
2008	-5.0240	0.735	<0.0001
2009	-0.8224	0.229	0.0003
2010	0.7045	0.167	<0.0001
BWTE Density residuals on Pond density	0.5715	0.044	<0.0001
Intercept	-0.742	0.135	<0.0001

Abbreviations: β = coefficient estimate, SE = standard error, P = p-value based on Wald chi-square, ref= reference category, AIV = avian influenza virus, AIV-Ab = antibodies against avian influenza virus nucleoprotein, pos = positive, HY = hatch year, AHY = after hatch year

Table 2-4. Summary of best-supported model to estimate AIV exposure probability of Blue-winged teal sampled in the Canadian prairies from 2007 to 2010, based on AIV antibody status as measured by cELISA (n =1846).

Variables	*B*	SE	P
Age (ref = HY)			
AHY	2.845	0.136	<0.0001
Year (ref = 2007)			
2008	-0.212	0.183	0.243
2009	-0.767	0.194	<0.0001
2010	-0.373	0.158	0.018
Head to bill	0.039	0.019	0.043
Intercept	-4.52	1.572	0.004

Abbreviations: β = coefficient estimate, SE = standard error, P = p-value based on Wald chi-square, ref= reference category, AIV = avian influenza virus, HY = hatch year, AHY = after hatch year

Table 2-5. Summary of best-supported model to estimate WNV exposure probability of Blue-winged teal sampled in the Canadian prairies from 2007 to 2010, based on antibody status as measured by cELISA and confirmed by Plaque-reduction neutralization specific for WNV (n = 1869).

Variables	*B*	SE	P
Age (ref = HY)			
AHY	1.882	0.337	<0.001
Pond density	0.138	0.038	<0.001
Year (ref = 2007)			
2008	-0.278	0.320	0.382
2009	-0.258	0.346	0.457
2010	-1.363	0.425	0.001
Intercept	-5.392	0.538	<0.001

Abbreviations: β = coefficient estimate, SE = standard error, P = p-value based on Wald chi-square, ref= reference, WNV = West Nile virus, HY = hatch year, AHY = after hatch year

Table 2-6. Summary of best-supported model to estimate APMV-1 exposure probability of Blue-winged teal sampled in the Canadian prairies from 2007 to 2010, based on APMV-1 antibody status as measured using hemagglutination inhibition assays (n = 1817).

Variables	*B*	SE	P
Age (ref = HY)			
AHY	0.956	0.230	<0.0001
Year (ref = 2007)			
2008	0.144	0.315	0.646
2009	0.786	0.305	0.010
2010	0.333	0.295	0.259
Intercept	-3.579	0.251	<0.0001

Abbreviations: β = coefficient estimate, SE = standard error, P = p-value based on Wald chi-square, ref= reference, HY = hatch year, AHY = after hatch year

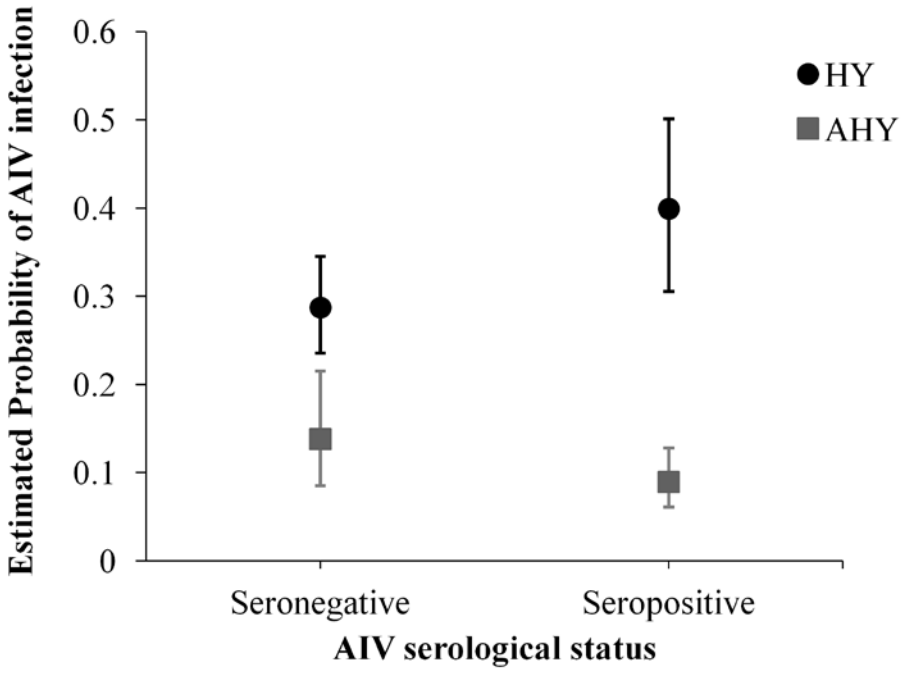

Figure 2-2. Model-estimated probability of avian influenza virus (AIV) infection (95% CI, error bar) in Blue-winged teal, illustrating the interaction between age and antibody status. Estimates of infection probability are based on our best supported model (Table 2-3) with year set to 2007 and residual BWTE density set to its mean. HY = hatch year birds, AHY = after hatch year birds.

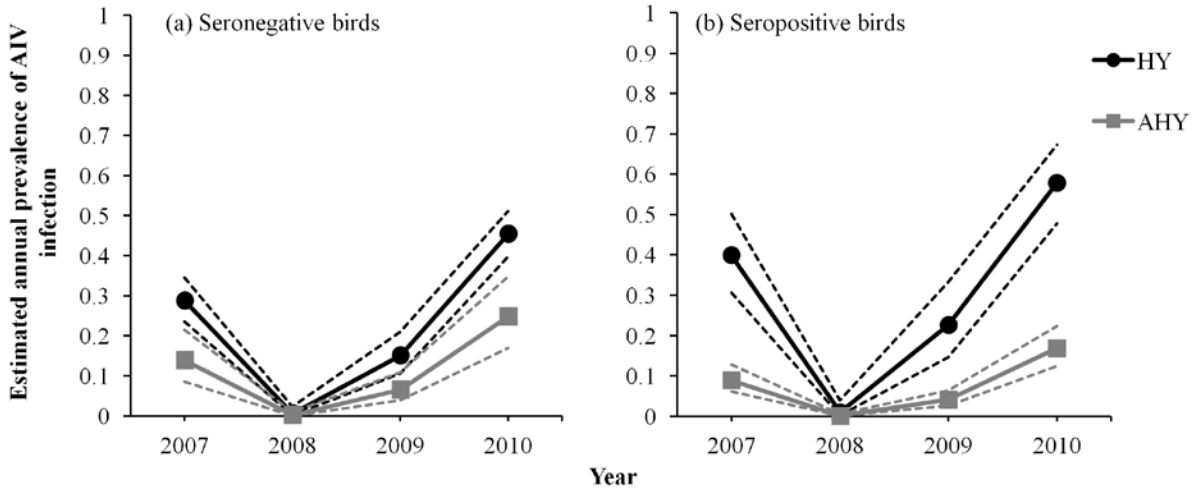

Figure 2-3. Estimated annual seroprevalence of avian influenza virus (AIV) infection in Blue-winged teal in the Canadian prairies from 2007 to 2010, based on our best supported model (Table 2-3). Dashed lines represent 95% CI. For these estimates, residual density was set to its mean. AIV-Ab (-) = negative on cELISA for antibodies to AIV, AIV-Ab (+) = antibody-positive for AIV.

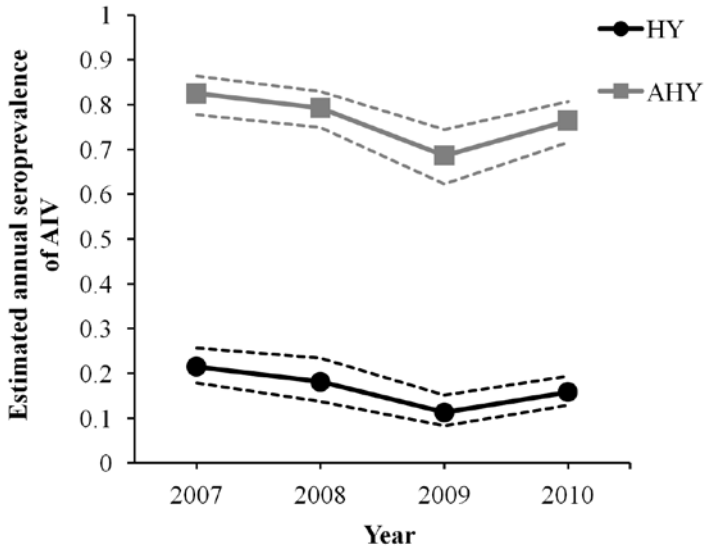

Figure 2-4. Estimated annual seroprevalence of avian influenza virus (AIV) in Blue-winged teal in the Canadian prairies from 2007 to 2010, based on our best supported model shown in Table 2-4. Dashed lines represent 95% CI. For these estimates, head to bill was set to its mean value. HY = hatch year birds, AHY = after hatch year birds.

Discussion

This study identified ecological determinants of infection and/or exposure to AIV, WNV, and APMV-1 in BWTE in the three Prairie Provinces of Canada. AIV infection in BWTE was associated with individual factors such as age and antibody status, regional or population-level factors such as local BWTE population density, and year. Similar to AIV, exposure to WNV and APMV-1 were both associated with age and year, and WNV was positively associated with pond density rather than host population density.

Avian Influenza Virus

Although numerous studies have been undertaken to investigate AIV infection in wild migratory birds, very few studies have examined infection in relation to serological data in wild birds (e.g., Hoye *et al*, 2011). Similar to studies in other species (e.g., Hinshaw *et al*., 1985; Sharp *et al*., 1993; Parmley *et al*., 2008), HY BWTE were more likely to be infected with AIV compared to AHY birds, likely due to naïve immune systems and lack of prior exposure to the virus. The latter is supported by our findings which show that adults were much more likely to have antibodies to AIV nucleoprotein compared to HY birds. A unique finding in our study was the interaction between age and antibody status. Infection was detected more often in HY BWTE with antibodies to AIV than in HY birds without antibodies, suggesting that at the time of sampling, these birds were actively producing antibodies in response to their current infections. This is plausible because three-month-old mallard ducks experimentally challenged with LPAIV shed virus continuously for 12 days post inoculation (+/- 1 day) and intermittently for an additional 3.7 days (+/- 3.1 days), while producing detectable antibodies one week post inoculation (Jourdain *et al.*,2010). In contrast, adult BWTE with antibodies to AIV were less likely to have detectable infection, suggesting that antibodies produced in response to previous infection conferred immunity against subsequent infection. This is supported by previous

experimental studies which have found that prior exposure to a LPAIV provides immunity to homosubtypic as well as heterosubtypic re-infection with LPAI or HPAI viruses in mallards (Fereidouni *et al.,* 2010; Jourdain *et al., 2*010). Similar cross protection has been described for other waterfowl species (e.g., Berhane *et al.*, 2010; Hoye *et al.*, 2011). AHY birds with both AIV infection and serum antibodies may, like the HY birds, have developed those antibodies in response to an ongoing infection, or may have had antibodies from previous exposure to a strain of AIV that was not protective for the strain detected at sampling. Interestingly, among the seronegative birds, HY birds were still more likely to be infected compared to adult birds. It is possible that this apparently higher resistance to infection in seronegative adults was due to more developed cell-mediated or innate immunity in adults compared to HY birds, or due to antibody responses not detected by our methods.

Our study did not find an association between sex and AIV infection. The role attributed to sex in the risk of infection varies among studies, with some showing either males (e.g., Parmley *et al*., 2005; Farnsworth *et al*., 2012) or females (e.g., Runstadler *et al.*, 2007) to have a higher probability of infection, and others, like ours, showing no effect of sex (e.g., Ferro *et al*., 2010; Soos *et al.*, 2012). The positive association between AIV antibody status and head-bill length suggests that larger birds were more likely to have been previously exposed. In HY birds, this may have been related to age at sampling, in that older (i.e., larger) HY birds were more likely to have been exposed and developed antibodies compared to younger HY birds. In AHY birds, the association may have been either a reflection of individual quality, in that larger birds are more likely to have developed an immune response, or a reflection of an association between size and frequency of social interactions (e.g., aggressive encounters) that may increase probability of exposure.

Higher apparent prevalences were observed in 2007 and 2010 than in 2008 and 2009, which may be suggestive of a cyclical pattern of AIV infection in the population. Long term studies in North America and Europe have detected similar temporal patterns of AIV infection in wild ducks, with peaks occurring every 2 to 3 years (Hinshaw *et al*., 1985; Sharp *et al*., 1993; Krauss *et al*., 2004; Munster *et al*., 2007). Hinshaw *et al.* (1985) hypothesized that cyclical periodicity of AIV prevalence in a population may have an immunologic basis. Our results show some support of this hypothesis because the year with the lowest probability of infection with AIV (2008) was followed by the year with the lowest seroprevalence (2009), possibly due to the low exposure in the previous year (Figures 2-3 and 2-4). This was then followed by the year with the highest apparent prevalence of infection detected by our study (2010), possibly resulting from the comparatively low proportion of individuals with evidence of AIV-specific immunity in the previous year (Figure 2-4). Longer term studies would be required to determine whether this pattern is similar in subsequent cycles.

AIV infection was positively associated with the residuals of BWTE population density regressed on pond density, providing support for the hypothesis of density dependent transmission in temperate regions. While Gaidet *et al*. (2011) found a similar association in Africa, this is the first report of a link between AIV infection and regional waterfowl breeding population density in North America. This positive association may have been caused by direct transmission of virus among individuals, or through indirect transmission of virus via the fecal-oral route. Higher waterfowl densities in a given wetland will result in higher concentrations of fecal material, thereby increasing the frequency of contacts between susceptible birds and virus. Interestingly, total duck density was not associated with AIV infection, suggesting that BWTE are more likely to become exposed from conspecifics rather than from other species. This is

plausible because BWTE are the latest ducks to arrive in the prairies during spring migration, and they nest later than most other dabbling duck species counted in breeding population surveys (e.g., *A. platyrhynchos*, *A. acuta*, and northern*A. clypeata*; Rohwer *et al.*, 2002). During the breeding season, most interactions are intraspecific, and interactions with other much larger duck species in the community tend to be casual or non-aggressive (Connelly & Ball, 1984). The seemingly lower spatial and temporal overlap with other species during timing of migration and nesting, may account for why AIV is associated with BWTE density rather than total duck density.

West Nile Virus

The ecology of WNV in migratory waterfowl is poorly understood. To our knowledge, this is the first study examining WNV exposure in free-ranging waterfowl in Canada. Our results show that the probability of exposure to WNV was influenced by age, pond density and year (Table 2-5). AHY birds were 6 times more likely to be seropositive for WNV compared to HY birds, which is likely a reflection of the increased probability of exposure to WNV with age, and provides evidence that at least some BWTE can survive infection with WNV. Seroprevalences were low despite sampling birds in August, which corresponds with timing of peak mosquito abundance and infection rates (Chen *et al*., 2012), as well as with annual peaks in human cases (Public Health Agency of Canada, 2011). It is possible that low seroprevalences resulted from high mortality rates associated with exposure to the virus. For instance, of the 88 birds that died during a WNV outbreak in a commercial waterfowl operation in the United States, 16 were BWTE (Meece *et al*., 2006). Thus, BWTE appear to be susceptible to fatal WNV infection, however it is unknown what the mortality rate or case fatality rate is for this species. Surveillance studies in the eastern hemisphere have also reported low (Saito *et al*., 2009; Yeh *et al*., 2011) to zero (Lindh *et al*., 2008) seroprevalence of WNV in wild waterfowl, thus perhaps

some species of wild waterfowl may be less likely to become exposed compared to other species due to differences in behavior, habitat preferences or the ability to replicate the virus.

Komar *et al*. (2003) found that mallard ducks (*Anas platyrhynchos*) and Canada geese (*Branta canadensis*) were not competent reservoir hosts for WNV, as they had low levels of viremia, and exhibited no clinical signs or mortality in experimental trials. However, other studies have since reported that numerous species of waterfowl, including dabbling ducks (e.g., *Anas* spp.), diving ducks (e.g., Barrow's goldeneye (*Bucephala islandica*), lesser scaup (*Aythya affinis*), geese (e.g., *Branta* spp and *Chen* spp.), and captive bred ducks (e.g., Rouen ducks) are susceptible to WNV, exhibiting clinical signs of disease and mortality in captivity (Meece *et al*., 2006; Wojnarowicz *et al*., 2007; Himsworth *et al*., 2009). It is also possible that the low seroprevalence in BWTE is a function of their relative attractiveness as hosts for vector mosquitoes. The primary enzootic vector of WNV in the Canadian prairies, *Culex tarsalis*, may select other avian or mammalian hosts as blood meal sources over BWTE, despite their widespread availability. Recent studies in California (Thiemann *et al*., 2012) and Colorado (Kent *et al*., 2009) demonstrate that *Cx. tarsalis* is a generalist feeder, but blood meals from waterfowl appear to be rare.

The lowest seroprevalence of WNV in BWTE was observed in 2010, which also was the year with the lowest number of reported cases in humans in the prairies, and a year with low infection rates in mosquitoes and birds (Public Health Agency of Canada, 2010). Spring pond density was an important risk factor for exposure to WNV in BWTE. Larger numbers of ponds in a given area may increase the amount of suitable developmental habitat for mosquitoes, the primary vectors for transmission. Not all wetlands are suitable for large scale production of vector mosquitoes. For example, in South Dakota, Chuang *et al.* (2011) found a positive association between wetlands and *Aedes vexans*, however they found no relationship with *Cx. tarsalis*.

Temporary or semi-permanent wetlands are likely more important as developmental sites for vector mosquitoes than permanent wetlands, and account for most of the variation in our annual spring pond density estimates. Further studies would be required to clarify the observed relationship between spring pond densities and WNV exposure in the Canadian prairies, and a better understanding of drivers of WNV amplification need to incorporate other parameters such as accumulated heat units, timing and patterns of rainfall and seasonal patterns of mosquito population dynamics, which are beyond the scope of the current study.

APMV-1

There is limited information on the ecology of APMV-1 in Blue-winged teal and waterfowl in general. Overall seroprevalence appeared low each year, similar to other studies which found low infection rates for APMV-1 in BWTE, as well as low infection rates or seroprevalence in other dabbling duck species in North America and Europe (e.g., Vickers and Hanson, 1982; Stallknecht *et al*., 1991; Maldonado *et al*., 1995; Goekjian *et al*., 2011), while high prevalences for other avian paramyxoviruses (APMV- 8, 6 and 9) were found in Spain (Maldonado *et al*., 1995).

Similar to our AIV and WNV serology results, APMV-1 seroprevalence was significantly higher in AHY birds compared to HY birds, probably due to adults being more likely to have had opportunities for previous exposure to APMV-1. As for AIV, these antibodies may also be protective given that lower prevalences of APMV-1 infection have been observed in adult birds compared to young (Stallknecht *et al*., 1991; Sharp *et al*., 1993).

Year was also an important variable, with the highest seroprevalence observed in 2009, and little difference between 2007, 2008, and 2010. Though this temporal pattern appeared opposite to that observed with AIV seroprevalence, which was lowest in 2009, there was no relationship between AIV antibody status and APMV-1 antibody status in our best-supported models.

Our results provide new insight into the ecology and epidemiology of infectious diseases in migratory waterfowl in the Canadian prairies, one of the most important breeding and staging areas in North America. Our findings highlight the importance of spatiotemporal, environmental, and host-specific factors at the individual, regional, and population levels, all of which have potential impacts on the dynamics of infectious diseases.

Supplementary information

Table S2-1. Models explored to explain variation in avian influenza virus (AIV) infection in Blue-winged teal in the Canadian prairies, from 2007 to 2010 (n=1846). Non-informative variables are in italics.

Global and nested models	# of parameters	AICc
Age + AIV-Ab + AIV-Ab*Age + Year + BWTE Density Residuals	8	1290.6
Age + AIV-Ab + AIV-Ab*Age + Year + BWTE Density Residuals + *Sex*	9	1292.3
Age + AIV-Ab + AIV-Ab*Age + Year + BWTE Density	8	1430.6
Age + AIV-Ab + AIV-Ab*Age + Year + BWTE Density + *Sex*	9	1432.2
Age + AIV-Ab + AIV-Ab*Age + Year	7	1489.9
Age + AIV-Ab + AIV-Ab*Age + BWTE Density Residuals	5	1504.3
Year	4	1570.1
Age + AIV-Ab + AIV-Ab*Age + BWTE Density	5	1600.5
Age + AIV-Ab + AIV-Ab*Age	4	1614.5
Age + AIV-Ab	3	1627.2
Age	2	1627.5
BWTE Density Residuals	2	1783.1
AIV-Ab	2	1752.3
BWTE Density	2	1789.3
Sex	2	1787.5
Null	1	1792.1

AIV-Ab: avian influenza virus antibody status

Table S2-2. Models explored to explain variation in avian influenza virus antibody status in Blue-winged teal in the Canadian prairies, from 2007 to 2010 (n=1846). Non-informative variables are in italics.

Global and nested models	# of parameters	AICc
Age + Year + Head to bill	**6**	**1834.2**
Age + Year + Head to bill + *Pond Density*	7	1834.2
Age + Year + Head to bill + *Duck density*	7	1834.3
Age + Year + Head to bill + *BWTE Density Residuals*	7	1835.8
Age + Year + Head to bill + *Duck Density* + *Sex*	8	1836.1
Age + Year	5	1836.3
Age + Year + Head to bill + *Pond Density* + *Sex*	8	1836.0
Age + Year + *Pond Density*	6	1836.5
Age + Year + *Duck Density*	6	1836.9
Age + Year + *BWTE Density Residuals*	6	1838.1
Age + Year + Head to bill + *BWTE Density Residuals* + *Sex*	7	1837.7
Age	2	1845.8
Year	4	2451.2
BWTE Density Residuals	2	2472.3
Sex	2	2494.6
Head to bill	2	2495.6
Duck Density	2	2537.8
Pond Density	2	2551.7
Null (intercept)	1	2555.2

Table S2-3. Models explored to explain variation in West Nile virus antibody status in Blue-winged teal in the Canadian prairies, from 2007 to 2010 (n=1869). Non-informative variables are in italics.

Global and nested models	# of parameters	AICc
Age + Year + Pond Density	6	**579.1**
Age + Year +Pond Density + *BCI*	7	581.0
Age + Year + Pond Density + *BWTE Density Residuals*	7	581.0
Age + Year + Pond Density + *BWTE Density Residuals* + *BCI*	8	582.9
Age + Year + BWTE Density	6	583.9
Age + Year + Duck Density + BCI	7	585.3
Age + Year + BWTE Density + *BCI*	7	585.7
Age + Pond Density	3	586.4
Age + BWTE Density	3	587.3
Age + Duck Density	3	589.9
Age + Year	5	591.4
Age + Year + *Duck Density*	6	592.6
Age + Year + *BWTE Density Residuals*	6	592.9
Age + Year + *BWTE Density Residuals* + *BCI*	7	594.1
Age	2	601.9
Age + *BWTE Density Residuals*	3	602.0
Duck Density	2	618.2
BWTE Density	2	619.0
Year	4	621.5
BWTE Density Residuals	2	628.4
Pond Density	2	630.4
BCI	2	631.4
Null	1	637.6

BCI: body condition index

Table S2-4. Models explored to explain variation in avian paramyxovirus-1 antibody status in Blue-winged teal in the Canadian prairies from 2007-2010 (n=1817). Non-informative variables are in italics.

Global and nested models	# of parameters	AICc
Age + Year	5	832.8
Age + Year + *BWTE Density Residuals*	6	834.5
Age	2	835.3
Age + *BWTE Density Residuals*	3	837.2
Year	4	849.2
BWTE Density Residuals	2	857.6
Null	1	859.4

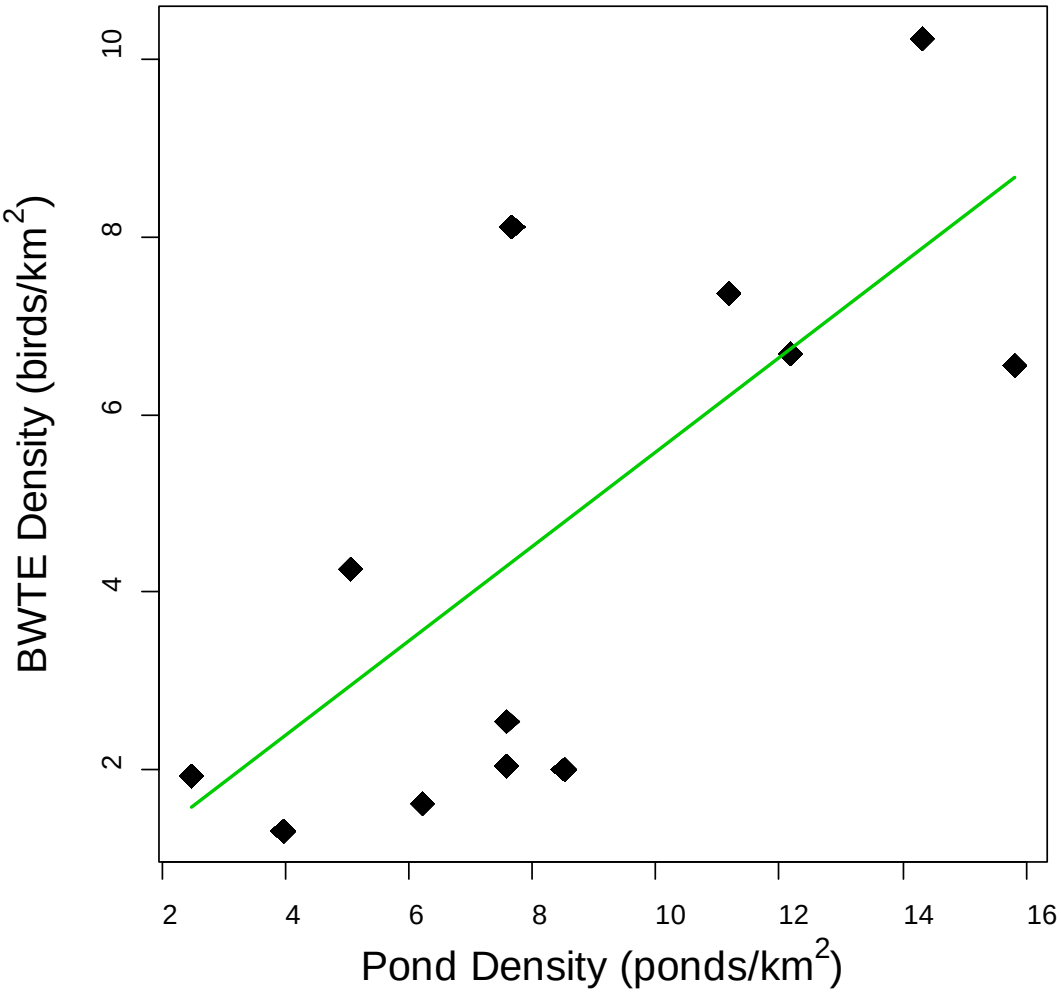

Figure S2-1. Relationship between Blue-winged teal density and pond density within strata surveyed for the Waterfowl Breeding Population and Habitat Survey in Alberta, Saskatchewan, and Manitoba in 2007-10.

CHAPTER 3
DEMOGRAPHIC AND SPATIOTEMPORAL PATTERNS OF AVIAN INFLUENZA INFECTION AT THE CONTINENTAL SCALE, AND IN RELATION TO ANNUAL LIFE CYCLE OF A MIGRATORY HOST

ABSTRACT

Since the spread of highly pathogenic avian influenza (HPAI) H5N1 in the eastern hemisphere, numerous surveillance programs and studies have been undertaken to detect the occurrence, distribution, or spread of low pathogenic avian influenza viruses (AIV) in wild bird populations worldwide. To identify ecological determinants and spatiotemporal patterns of AIV infection in long distance migratory waterfowl in North America, we fitted generalized linear models with binominal distribution to analyze results from 13,574 Blue-winged teal (*Anas discors*, BWTE) sampled in 2007 to 2010 as part of Canada's Inter-agency Wild Bird Influenza Survey, the US Early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds (US Department of Agriculture), and the Avian Influenza Surveillance Program of the US Fish and Wildlife Service and US Geological Survey. Our analyses revealed that age, sex, year, flyway, latitude, and season (categorized based on stage of BWTE annual life cycle) were all important variables in predicting the probability of AIV infection. There was an interaction between age and season. During late summer staging (August) and fall migration (September-October), hatch year birds were more likely to be infected than after hatch year birds, however there was no difference between age categories for the remainder of the year (winter, spring migration, and incubation), likely due to maturing immune systems and newly acquired immunity of HY birds. Probability of infection increased non-linearly with latitude, and was highest in summer just prior to fall migration when densities of birds and the proportion of susceptible hatch year birds in the population are highest. Birds in the Pacific, Central and

Mississippi flyways were significantly more likely to be infected compared to those in the Atlantic flyway. Seasonal cycles and spatial variation of AIV infection were largely driven by the dynamics of AIV infection in HY birds, which had significantly more prominent cycles and spatial variation in infection compared to AHY birds. Our results demonstrate clear demographic as well as seasonal, latitudinal and flyway trends across Canada and the US, while illustrating the importance of migratory host life cycle and age in driving cyclical patterns of prevalence. This study provides new insight into the ecology of low pathogenic AIV in migratory waterfowl at the continental scale.

Introduction

Wild birds, particularly waterfowl of the order Anseriformes, are considered the natural reservoir for most subtypes of low pathogenic avian influenza viruses (AIV; Webster *et al*., 1992; Takekawa *et al*., 2010). Low pathogenic AIVs (LPAIVs) do not cause significant disease in wild ducks; however, H5 and H7 subtypes have the potential to evolve into highly pathogenic AIV (HPAIV) when introduced into domestic bird populations (Webster *et al* 1992). HPAIVs cause large scale mortality in domestic bird populations, and some may cause serious illness in humans (Horimoto & Kawaoka, 2001). In response to the emergence and spread of H5N1 HPAIV in Asia, Europe, and Africa, numerous large scale surveillance programs have been initiated worldwide. Although the primary objective of most of these surveillance programs was the early detection of H5N1 HPAIV, these programs have resulted in a large amount of valuable data on LPAIVs. To our knowledge, only a few studies have examined large scale spatiotemporal patterns and ecological determinants of AIV infection in waterfowl at the continental scale, particularly along migratory flyways or across seasons (e.g., Gaidet *et al* 2011). These types of analyses are essential not only for increasing our understanding of AIV

ecology in wild birds at continental levels, but also for enhancing future surveillance and response efforts, potentially identifying key locations and time periods for AIV infection risk.

In this study, we make use of data generated by surveillance programs conducted in Canada and the United States, to examine temporal, spatial, and demographic trends of AIV infection in migratory waterfowl. Previous studies have detected high prevalences of AIV in waterfowl prior to fall migration when large numbers of birds aggregate, favoring transmission of virus through the oral-fecal route (Hinshaw *et al*., 1980; Hinshaw *et al*., 1985; Webster *et al*., 1992; Krauss *et al*., 2004), while detecting decreasing prevalences as birds migrate further south (Stallknecht *et al*., 1990; Munster *et al*., 2007). Here we analyze four years of surveillance data to determine the effects of year, flyway, latitude, season (as defined by stage of annual cycle of BWTE), and demographic factors (age and sex) on the probability of AIV infection in Blue-winged teal (*Anas discors;* BWTE). This species provides an ideal model for studying determinants of AIV infection in waterfowl, because it has the largest migratory range among dabbling ducks (Gammonley & Fredrickson, 1995), individuals are highly gregarious, and are commonly infected with LPAI viruses (Parmley *et al*., 2008; Pasick *et al*., 2010; Wilcox *et al*., 2011).

Methods

Data was obtained courtesy of Canada's Inter-agency Wild Bird Influenza Survey (2007-10), the US Early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds (US Department of Agriculture, 2007-10), and the Avian Influenza Surveillance Program of the US Fish and Wildlife Service (2007-10) (USFWS, 2007; Parmley *et al*., 2008; Deliberto *et al*., 2009). Sampling of BWTE was distributed across 42 states in the US and 9 provinces in Canada (Figure 3-1). Samples from all three surveys were analyzed for the presence of the matrix protein gene segment common to all influenza A viruses using the real-time reverse transcriptase polymerase chain reaction (RRT-PCR) assay described by Spackman *et al*. (2002).

Statistical analyses

Descriptive analyses were conducted in Excel (Microsoft office Excel, 2007), and inferential statistical analyses were performed in R (version 2.15.2; R Core Team, 2012). Records missing any field or laboratory data were excluded from analysis. To investigate determinants of AIV infection, we built generalized linear models with binominal distribution based on maximum-likelihood approximation, using explanatory variables described in Table 3-1. To better understand temporal trends of AIV infection in BWTE, season categories were created based on the annual life cycle of BWTE: August (staging prior to fall migration), September-October (fall migration), and November-July (a pooled category created due to small sample sizes, including wintering stage and spring migration (Nov-April), and breeding period (May-July) (Rohwer *et al*., 2002). Migratory flyways were classified using the four admistrative categories based on waterfowl migration corridors: Pacific, Central, Mississippi, and Atlantic Flyway (www.flyways.us, 2008). The assumption of linearity for the continuous variable "latitude" was examined using two methods: categorization and inclusion of a quadratic term. Both methods revealed a similar non-linear association, thus we included the quadratic term in our final model set. Latitude was standardized to have a mean of 0 and a SD of 1 before including in models and before creating the quadratic term. Akaike information criterion corrected for small sample size (AICc) was used for model selection. If models were within 2 AIC units of each other, then the simplest one was chosen. We calculated *p* values based on 95% confidence intervals (C.I.) while AIC-based model selection is based on 85% C.I. (Arnold, 2010).

To create maps illustrating probability of AIV infection, predicted probability of AIV infection for each individual was calculated based on the best supported model. These values were group-averaged across sexes and years for each sampling location, and then were interpolated with the spatial analysis tool in ArcGIS, using Natural Neighbor interpolation

procedures to obtain values for unsampled locations based on known surface values of adjacent sites (ArcGIS® 9: ArcEditor ® 9.3.1 Redlands, CA) (Ledoux and Gold 2005).

Table 3-1. List of outcome and explanatory variables used in models examining demographic and spatiotemporal determinants of low pathogenic avian influenza virus infection in Blue-winged teal in Canada and the United States, 2007-10.

Variable	Type	Definition	Description
AIV infection	Categorical, outcome variable	Positive suggests current infection based on RRT-PCR	Binomial outcome – positive ornegative
Sex	Categorical, explanatory	Gender of bird	Female or Male
Age	Categorical, explanatory	Age of bird at capture	Hatch year or after hatch year
Year	Categorical, explanatory	Year of sampling	2007- 2010
Flyway	Categorical, explanatory	Migratory flyway where sampling occurred (www.flyways.us 2008)	Atlantic, Mississippi, Central, or Pacific
Latitude	Continuous, explanatory	Latitude of sampling location	Decimal degrees, standardized
Blue-winged teal stage of annual life cycle – "Season"	Categorical, explanatory	Stage of life cycle of BWTE (Rohwer *et al.* 2002)	- August: Staging, End of Moult, Preparation for fall migration; - Sept-Oct: Fall migration - Nov-July: Wintering, Spring migration, breeding period

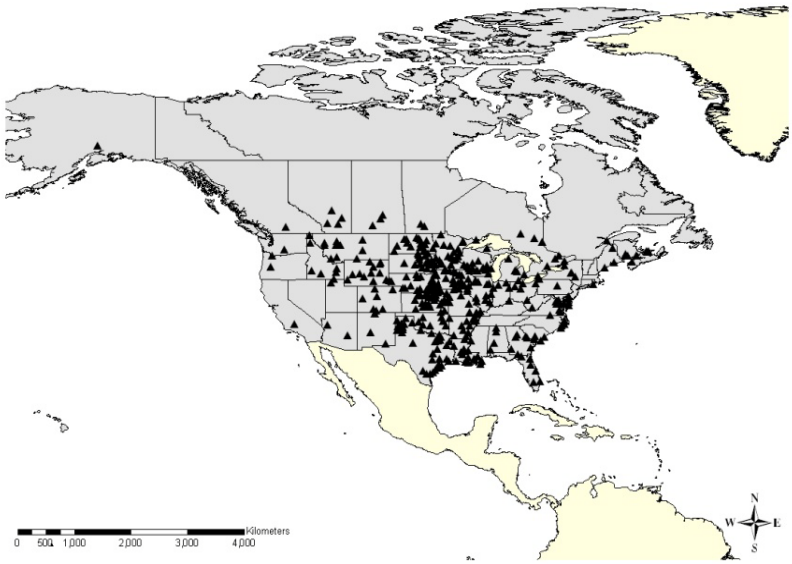

Figure 3-1. Locations of capture sites of Blue-winged teal sampled for low pathogenic avian influenza virus across Canada and the United States, from 2007 to 2010.

Results

Descriptive statistics. Records of 13,574 BWTE tested for AIV infection in Canada and the US from 2007 to 2010 were obtained. Apparent prevalence of AIV infection was 17.8% overall, 18.2% in Canada (n = 2,989), and 17.8% in the US (n = 10,585). Proportions of AIV infection by sex and age are shown in Table 3-2. Seasonal and annual variation in apparent prevalence of AIV was observed, appearing lowest from November to July (9.5%, n = 1,528), and highest in August (22.9%, n = 3,330) and during fall migration, September-October (19.3%, n = 8,716). Higher apparent prevalence was observed in birds captured in Central (19.6%, n = 8,230) and Mississippi flyways (15.6%, n = 4,923), compared to birds sampled in the Atlantic (7%, n = 381) and Pacific (12%, n = 40) flyways.

Modelling the ecology of low pathogenic AIV infection in Blue-winged teal. Results of our model selection exercise are presented in Table S1-3. In our best-supported model (Table 3-3),

there was an interaction between age and stage of annual cycle. HY birds were significantly more likely to be positive for AIV compared to AHY birds in August just prior to fall migration (Figures 3-2 and 3-3). This effect declined but was still significant in September-October (fall migration), and not significant in November-July (winter, spring migration, breeding; Figure 3-3). In HY birds, the probability of being infected with AIV was highest in August and declined significantly in subsequent stages of the annual cycle until the following August (Figures 3-2 and 3-3). A much less prominent seasonally cyclical pattern was observed in AHY BWTE which, unlike the HY birds, had the lowest probability of infection in August, with no difference between Sept-Oct and Nov-July (Table 3-3, Figure 3-3). Males were 27% more likely to be infected than females when controlling for age and season.

Year was an informative variable, with highest risk of infection in 2007 and 2010 (Table 3-3, Figure 3-2). Migratory flyway was a strong predictor of AIV infection. Blue-winged teal were much more likely to test positive for AIV in the Central and Mississippi Flyways compared to birds in the Atlantic Flyway (Table 3-3). The probability of AIV infection was positively associated with latitude up to about 43º north, after which there was no further increase in risk of infection with latitude, and a slight declining trend beyond ~48º north (for HY birds in August,Table 3-3, Figure 3-4). The seasonal and spatial trends discussed above are further illustrated in Figures 3-5 and 3-6, which show the spatial distribution of predicted probability of AIV infection in HY and AHY birds, respectively. Whereas latitudinal, seasonal and flyway trends appear to be very prominent in HY birds, these trends are much less apparent in AHY birds which not only have significantly lower apparent prevalences, but also have much less spatiotemporal variation in AIV infection probability.

Table 3-2. Apparent prevalence of low pathogenic avian influenza virus in BWTE (*Anas discors*) in Canada and the United States, 2007-2010.

		Proportion pos by sex		Proportion pos by age	
	Overall	**F**	**M**	**HY**	**AHY**
n	13,574	7,491	6,083	9,113	4,461
% of AIV	17.8	17.6	18.0	21.4	10.4

Abbreviations: pos = positive, M = male, F = female, HY = hatch year, AHY = after hatch year, AIV = Avian influenza matrix protein gene,

Table 3-3. Best-supported logistic regression model fitted to assess the ability of various demographic and spatiotemporal factors to explain variation in AIV infection probability in Blue-winged teal in Canada and the US, 2007-2010 (n=13,574).

Variables	β	SE	P
Intercept	-2.569	0.224	<0.0001
Age (ref = HY)	-1.423	0.109	<0.0001
Sex (ref = Female)	0.239	0.050	<0.0001
Season (ref =August)			
Sept-Oct	-0.370	0.079	<0.0001
Nov-July	-0.777	0.148	<0.0001
Year (ref = 2007)			
2008	-0.312	0.069	<0.0001
2009	0.035	0.068	0.609
2010	0.329	0.065	<0.0001
Latitude	0.185	0.037	<0.0001
(Latitude)^2	-0.129	0.030	<0.0001
Flyway (ref= Atlantic)			
Pacific	1.170	0.529	0.027
Central	1.625	0.206	<0.0001
Mississippi	1.399	0.207	<0.0001
Age *Season			
HY- Sept-Oct	0.907	0.132	<0.0001
HY- Nov-July	1.228	0.211	<0.0001

Abbreviations: β = coefficient estimate, SE = standard error, P = p-value based on Wald chi-square, intervals, ref= reference, HY = hatch year, AHY = after hatch year. Latitude is standardized to have a mean of 0 and standard deviation of 1. The quadratic term is the standardized latitude squared.

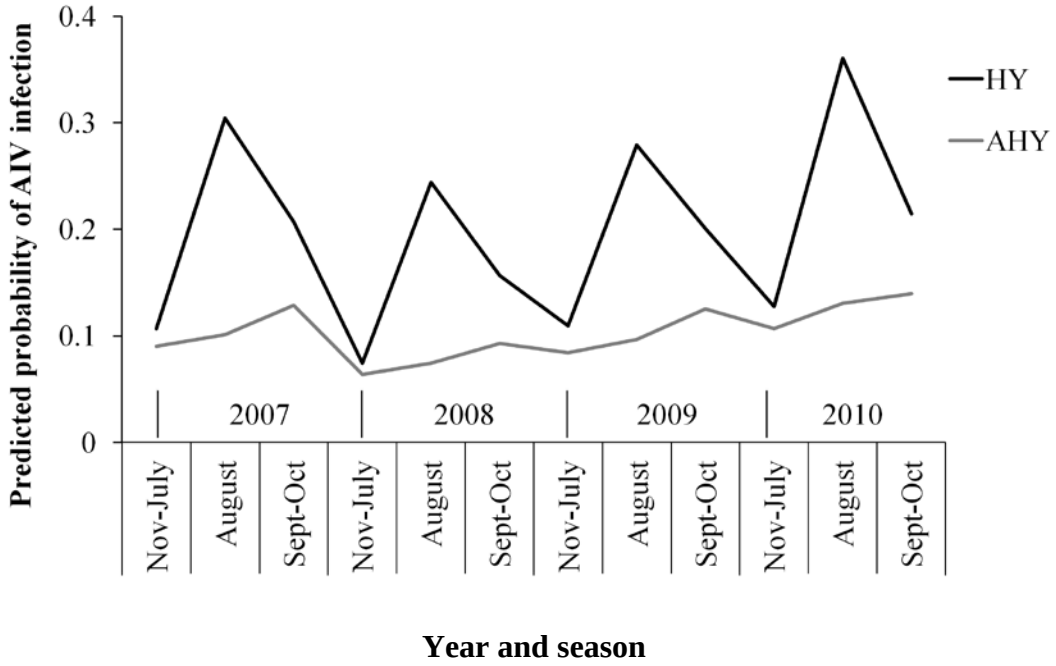

Figure 3-2 Annual and seasonal predicted probability of avian influenza virus infection in BWTE in Canada and the US from 2007-2010. Predictions are based on the best-supported model (Table 3-3) and are averaged across both sexes, all flyways and latitudes of data. Hatch year (HY) and after hatch year (AHY) age groups shown separately.

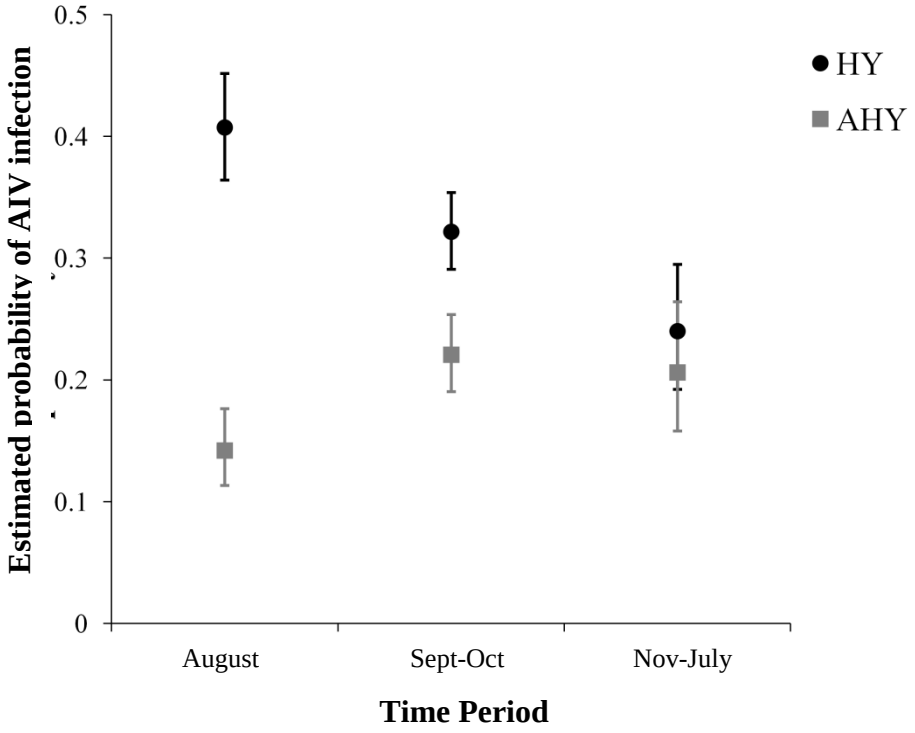

Figure 3-3. Estimated probability of avian influenza virus (AIV) infection (95% CI, error bar) in Blue-winged teal, illustrating interaction between age and season. Predictions are based on the best-supported model (Table 3-3) with other explanatory variables set at male (Sex), 2010 (Year), central flyway (Flyway) and mean latitude. Hatch year: HY, after hatch year: AHY.

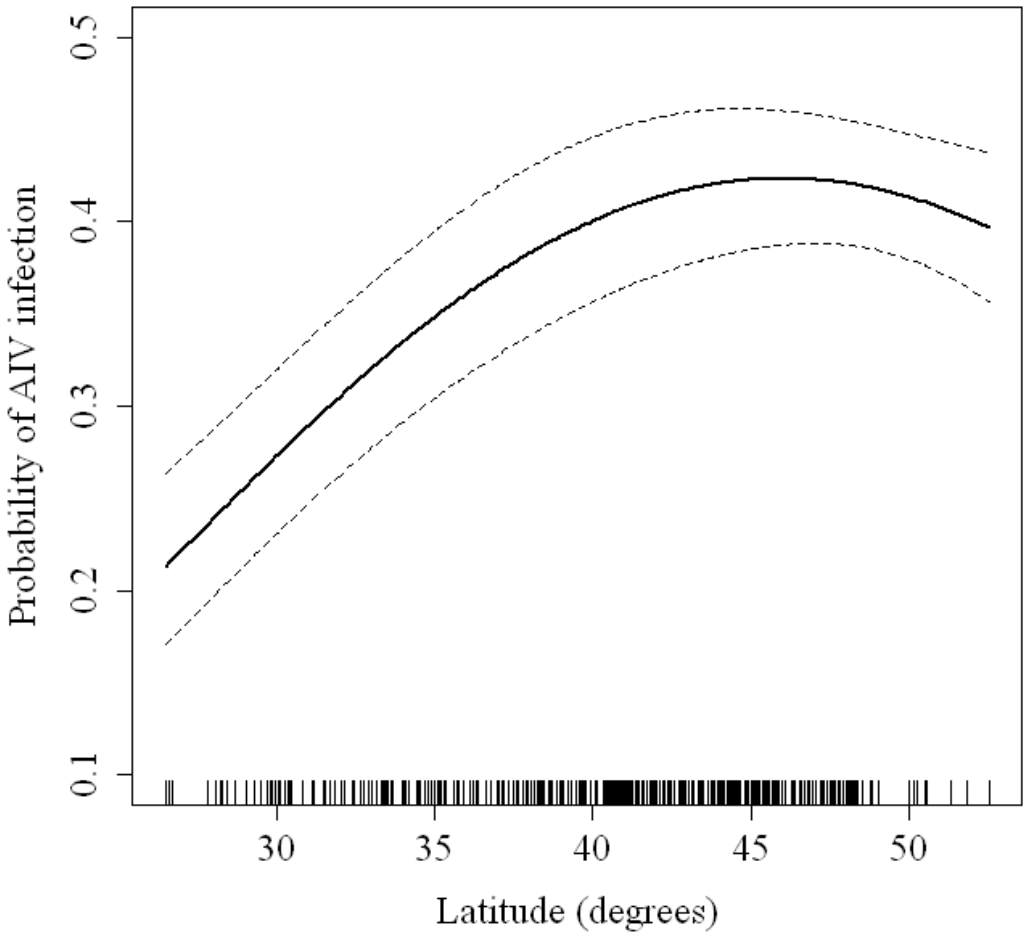

Figure 3-4. Probability of avian influenza virus (AIV) infection (95% CI, dashed lines) in Blue-winged teal as a function of latitude of sampling location. Probability was estimated based on the best-supported model (Table 3-3) with Age, Sex, Flyway, Season and Year set at hatch year, male, central, August and 2010 categories, respectively.

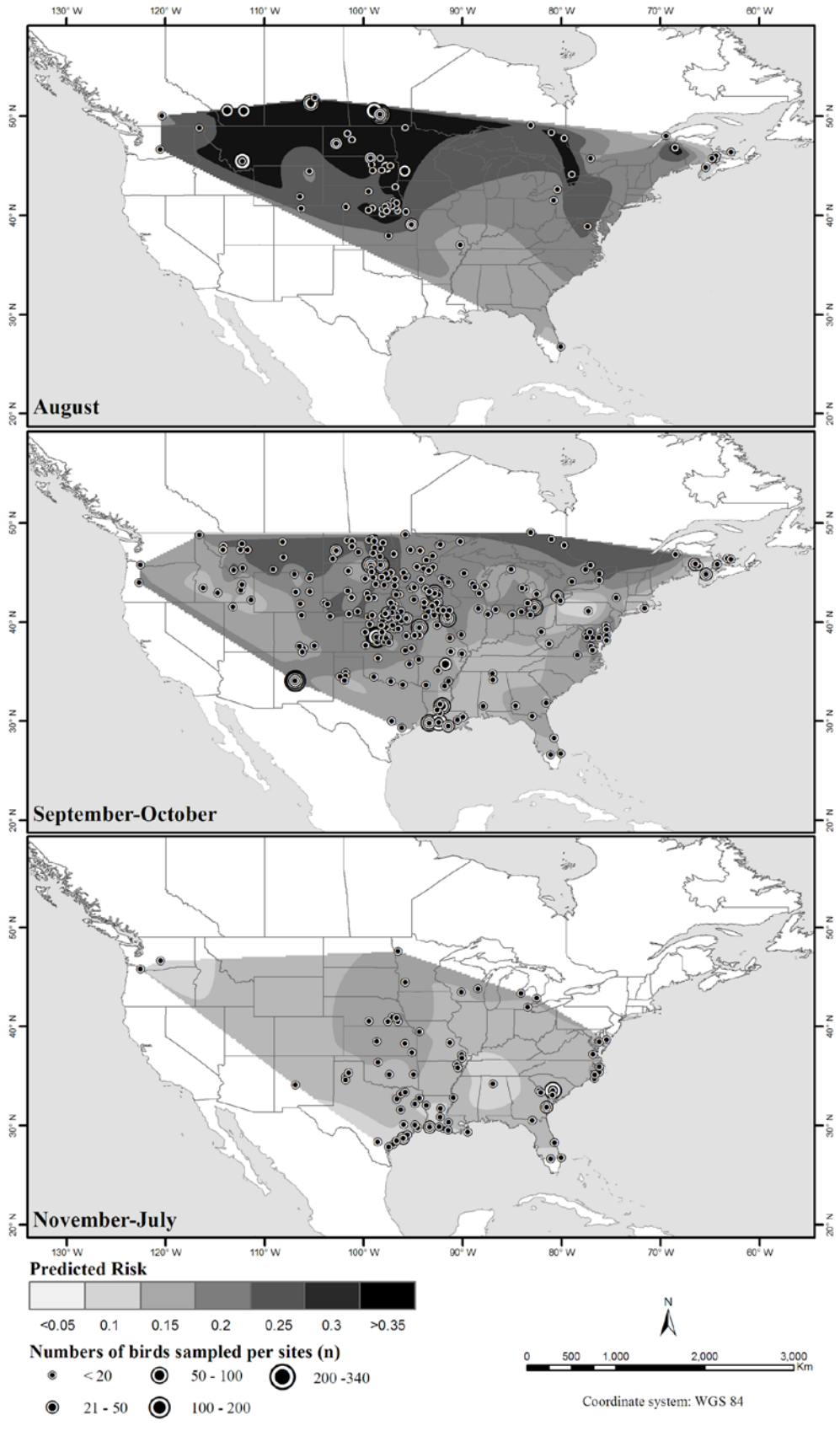
130° W
120° W
110° W
100° W
90° W
80° W
70° W
60° W
50° N
40° N
30° N
20° N
August
September-October
November-July
Predicted Risk
<0.05
0.1
0.15
0.2
0.25
0.3
>0.35
Numbers of birds sampled per sites (n)
< 20
21 - 50
50 - 100
100 - 200
200 -340
N
0
500
1,000
2,000
3,000
Km
Coordinate system: WGS 84

Figure 3-5. Predicted risk of avian influenza virus infection in hatch year (HY) Blue-winged teal in Canada and the US from 2007-2010 at different stages of the annual life cycle, using natural neighbor interpolation spatial analysis. AIV prediction values were calculated for all HY birds based on the best-supported model (Table 3-3), and averaged across all years and both sexes for each sampling site (circles).

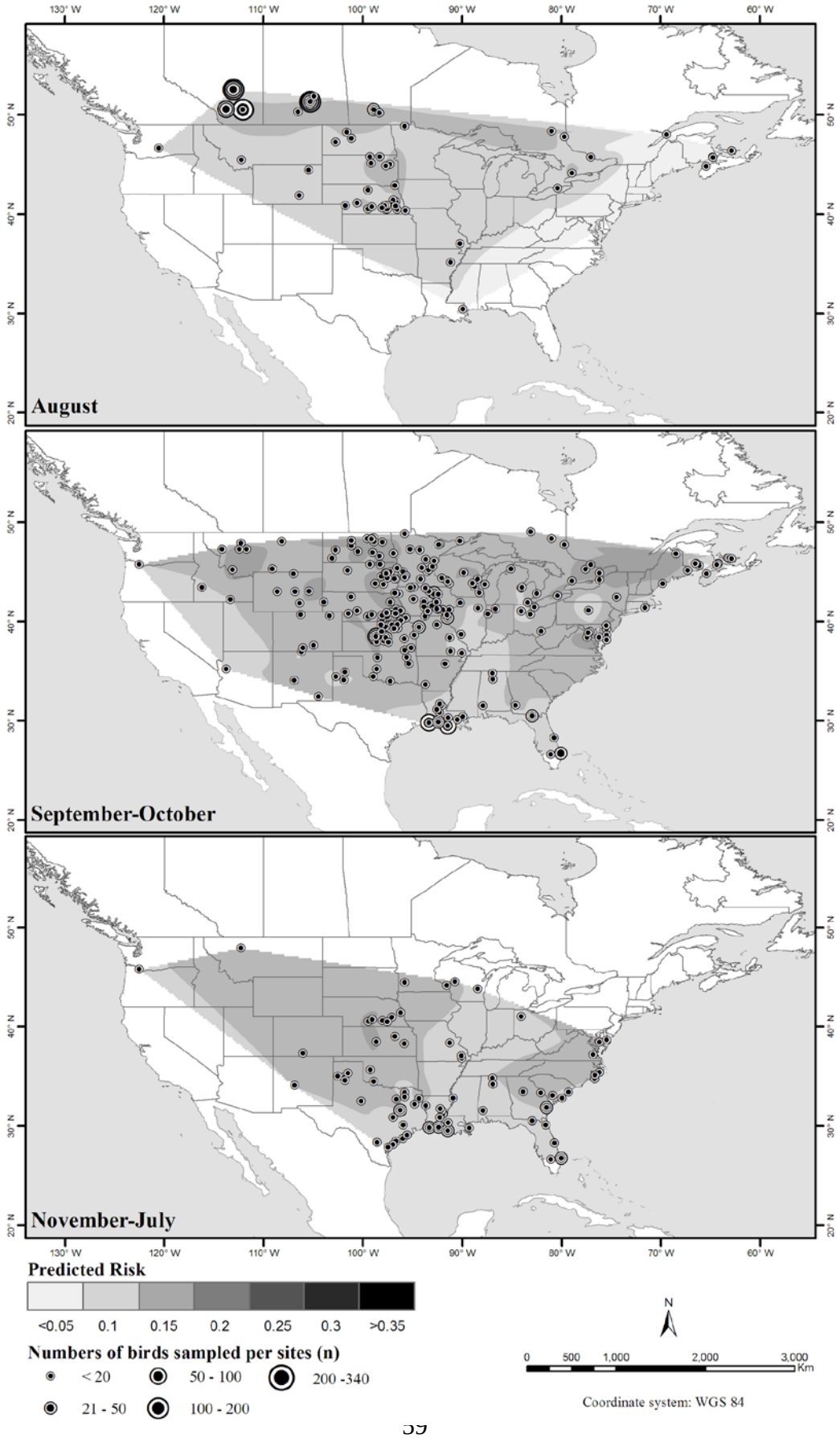
130° W
120° W
110° W
100° W
90° W
80° W
70° W
60° W
50° N
40° N
30° N
20° N
August
September-October
November-July
Predicted Risk
<0.05
0.1
0.15
0.2
0.25
0.3
>0.35
Numbers of birds sampled per sites (n)
< 20
21 - 50
50 - 100
100 - 200
200 -340
N
0
500
1,000
2,000
3,000
Km
Coordinate system: WGS 84

Figure 3-6. Predicted risk of avian influenza virus infection in after hatch year (AHY) Blue-winged teal in Canada and the US from 2007-2010 at different stages of the annual life cycle, using natural neighbor interpolation spatial analysis. AIV prediction values were calculated for all AHY birds based on the best-supported model (Table 3-3), and averaged across all years and both sexes for each sampling site (circles).

Discussion

Using data collected during surveillance programs in Canada and the United States from 2007 to 2010, we identified demographic as well as seasonal, latitudinal, flyway, and annual trends in AIV infection in BWTE in North America, while illustrating the importance of migratory host annual cycle and age in driving seasonal cycles in prevalence.

A unique finding of our study was an interaction between age and season (as defined by stage of annual cycle of BWTE), in which HY BWTE were more likely to be infected with AIV compared to adults as observed in previous studies (Ip *et al*., 2008; Parmley *et al*., 2008; Hoye *et al*., 2010), but only from August to October (staging and fall migration). By November (wintering stage) and the rest of the annual cycle (including spring migration and the following breeding season), HY birds were no longer more likely to be infected compared to AHY birds, likely due to maturing immune systems and newly acquired immunity to AIV, resulting in similar levels of resistance as in adults.

Seasonal cycles of AIV infection were largely driven by the dynamics of AIV infection in HY birds which had significantly more prominent cycles compared to AHY birds, which had comparatively little temporal variation in AIV infection (Figures 3-2, and 3-5 vs 3-6). HY birds had the highest estimated prevalences in August compared to other stages in the annual cycle as that is when they are most vulnerable to infection, having no previously acquired immunity. Not only is August the period with the highest HY:AHY ratio in the population, it is also the period with the highest population densities and mixing of waterfowl of different species from numerous locations, and thus, it is likely the period with the highest contact rates for transmission between infected and susceptible individuals. Interestingly, AHY BWTE were least likely to be infected in August and more likely to be infected in Sept-through July. It is possible that, at the time of sampling, AHY birds had already developed immunity to viruses circulating

in August, and were more vulnerable to novel strains encountered during the subsequent stages in the annual cycle. The decreasing trend as birds migrate south for wintering would likely be due to a combination of the increased proportion of HY birds becoming immune, and a reduction in contact rates among ducks due to decreasing population densities, resulting in reduced rates of transmission (Stallknecht *et al*., 1990b).

Males were slightly more likely to be infected with AIV compared to females, as observed by other studies (e.g., Farnsworth *et al* 2012, Parmley *et al* 2010), suggesting an innate difference in their vulnerability to infection possibly due to physiological (e.g., hormonal) and/or behavioural (e.g., aggression, gregariousness) differences (Garamszegi & Moller, 2007).

Latitude may be a complex variable in our models, important partially through its association with season, which drives the movement of birds northward and southward, but also because of changes in environmental or climatic factors with latitude. Environmental temperatures are cooler and more variable with increasing latitudes. AIVs have been shown to persist for long periods at cooler temperatures (Stallknecht *et al*., 1990a), and the northern hemisphere has been implicated as a potential environmental reservoir for AIV, with cold temperatures increasing the potential for viruses to survive overwinter (Henaux & Samuel, 2011). The positive association between AIV infection and latitude was not linear, with no additional increased risk in AIV infection beyond ~43 degrees north, beyond which there was a plateau and slightly declining trend. Although colder temperatures are generally better for virus survival, it has been demonstrated experimentally that extremely cold temperatures (below -30º C) may decrease virus survival in the environment (Shoham *et al*., 2012), which may explain this non-linear trend. In addition, latitudinal patterns were most prominent in August and during fall migration, particularly in HY birds, thus the latitudinal trend may have been driven in part by age and host

annual life cycle, and associated decreases in host density and increases in immunity as birds migrate southward. BWTE in the Central and Mississippi flyways were more likely to be infected with AIV compared to birds in the Atlantic flyway. This pattern may have been a reflection of BWTE population density. The highest density of breeding Blue-winged teal occurs in the central prairies of the US and Canada, spanning southeastern SK, southwestern MB, and the Dakotas which encompass the most important breeding areas for this species (Szymanski & Dubovsky, 2010).

Ours is one of few studies that have examined large scale spatiotemporal patterns and ecological determinants of AIV infection in waterfowl at the continental scale and across seasons. This study not only provides further evidence for the role of demographic and spatiotemporal factors such as latitude, flyway, and season, it illustrates the importance and interaction of host migratory ecology and age in driving seasonal and geographic patterns of prevalence. Similar approaches to analyzing available surveillance data for other migratory waterfowl species in North America may reveal similar spatiotemporal patterns in AIV infection. Studies such as this would be enhanced significantly by using an inter-continental approach, incorporating more data from wintering areas further south, particularly for species like BWTE which have wintering ranges extending into Central and South America.

This study enhances our knowledge of the ecology of low pathogenic AIVs in wild migratory waterfowl, and provides key information that can be used to enhance future surveillance and response efforts, potentially identifying key locations and time periods for AIV infection risk for this particular species.

Supplementary information

Table S3-1. Models fitted to explain variation in AIV infection probability in Blue-winged teal sampled in the US and Canada as part of national surveillance programs from 2007 to 2010 (n = 13,574).

Global and nested models	# parameters	AICc
Age + Sex + Age*Season + Season + Year + Flyway + Latitude + Latitude^2	15	12068.6
Age + Sex + Age*Sex + Age*Season + Sex*Season + Age*Sex*Season + Year + Flyway + Latitude + Latitude^2	19	12070.1
Age + Sex + Age*Sex + Season + Year + Flyway + Latitude + Latitude^2	14	12106.7
Age + Sex + Season + Year + Flyway + Latitude + Latitude^2	13	12124.6
Age + Sex + Sex*Season + Season + Year + Flyway + Latitude + Latitude^2	15	12125.3
Age + Sex + Season + Year + Flyway + Latitude	12	12153.6
Age + Sex + Season + Year + Flyway	11	12184.8
Age + Sex + Season + Year	8	12259.4
Age + Season + Age*Season	6	12268.5
Age + Season	4	12317.8
Age + Sex + Season	5	12318.2
Age + Sex + Age*Sex	4	12443.9
Age + Sex	3	12452.1
Age	2	12453.5
Latitude + Latitude^2	3	12536.3
Latitude	2	12577.6
Season	3	12580.5
Year	4	12598.3
Flyway	4	12648.5
Null	1	12716.2
Sex	2	12717.9

CHAPTER 4
GENERAL DISCUSION

This thesis covers a portion of an ongoing project investigating risk factors, spatiotemporal distribution, movement, and origins of infection and exposure to avian influenza virus (AIV), West Nile Virus (WNV) and avian paramyxovirus-1 (APMV-1) in the Blue-winged teal (*Anas discors,* BWTE), using individual to population and continental approaches. In this chapter, I summarize the ecological determinants of AIV infection and exposure to AIV, WNV and APMV-1 in the Canadian Prairie Provinces of Alberta, Saskatchewan and Manitoba, and the demographic and spatiotemporal patterns of AIV found in populations of BWTE in Canada and the US during a 4-year period between 2007 and 2010.

This research has:

- provided quantitative information on the proportion of the BWTE population infected and previously exposed to AIV, WNV and APMV-1; and identified demographic and ecological risk factors associated with infection and exposure of BWTE to these viruses in Alberta, Manitoba and Saskatchewan in the period between 2007 and 2010 (Chapter 2).
- examined temporal, spatial, and demographic trends of AIV infection throughout the annual life cycle of the BWTE (Chapter 3).

Avian influenza: from individuals to populations

Overall, this study demonstrated a relatively high apparent prevalence of AIV in BWTE populations in Canada and the United States, and revealed that AIV infection in BWTE is influenced importantly by demographic, environmental, geographic and temporal variables.

The importance of age and immunity: drivers of cycles in seasonal, annual, and spatial variation in prevalence

Age was one of the most important variables predicting AIV infection and exposure in BWTE. As per other studies (Parmley *et al*., 2008; Pasick *et al*., 2010; Hoye *et al*., 2011; Ip *et al*., 2008), hatch year (HY) birds were significantly more likely to be infected compared to after hatch year (AHY) birds (Figures 2-3 and 3-3), and also were much less likely to have antibodies to AIV compared to AHY birds (Figure 2-4). The naïve immune system of HY birds increases their risk of becoming infected with AIV, and also for becoming sources of infection, shedding AIV for longer periods compared to adult birds which have a higher chance of being immune due to previous exposure to these viruses, making them resistant to further infection (Jourdain *et al*., 2010). We also found that AHY BWTE with antibodies to AIV were slightly less likely to be infected with AIV compared to AHY without antibodies, suggesting the expected protective role of antibodies in preventing further infection. Interestingly, HY birds infected with AIV were more likely to have antibodies than were uninfected HY birds. These data provide evidence for an SIR (susceptible-infectious-recovered) dynamic for AIV infection in BWTE (Anderson & May, 1979). HY birds are initially in the sub-population of birds that are susceptible to AIV infection. Once infected, HY birds shed virus while mounting an immune response that moves them quickly into the subpopulation of birds that are resistant to subsequent infection.

The importance of age became more prominent when examining spatiotemporal patterns at the continental level across seasons, throughout the annual life cycle of the BWTE. Seasonal cycles of AIV prevalence in BWTE were largely driven by HY birds, with likelihood of infection being highest in HY birds in August, coinciding with pre-migratory aggregation, declining during fall migration in September and October, with lowest prevalences for the rest of the year

spanning winter, spring migration, and the following breeding season (Figures 3-2 and 3-5). In November through July, there was no difference in probability of infection between AHY and HY birds (Figure 3-3), likely because immune systems of HY birds have matured to the point of having similar resistance as in adults. Annual variation observed at both the population and continental levels may also have been largely driven by HY birds (Figures 2-3 and 3-2), as peaks in AIV prevalence were much higher and/or more prominent in HY birds compared to AHY birds. At the population level, the prevalence of AIV infection in BWTE appeared to have a biennial cycle of higher and lower infection rates. This biennial pattern may have been driven in part by biennial fluctuations in the prevalence of antibodies in the BWTE population. The proportion of HY birds in the population may be a key parameter driving the magnitude of seasonal and annual cycles observed in this and previous studies. A larger HY:AHY ratio would increase the proportion of naïve individuals in the population that are susceptible to infection, and become sources of infection and spread.

Age and host annual life cycle (season) were also likely important in partially driving the effects of latitude and flyway on AIV infection risk, because latitudinal and flyway trends were significantly more prominent in HY birds compared to AHY birds, throughout the annual cycle (Figure 3-5 vs 3-6). Thus, the role of age (i.e., for HY birds) is not only important for increasing risk of AIV infection within individuals, but it also plays an important role in driving observed seasonal and annual cycles, as well as spatial variation in AIV prevalence at the population and continental levels. Latitude remained significant in the model when combined with season and age, thus the latitudinal pattern is likely not just a reflection of season, annual life cycle BWTE, and annual production of HY birds, but also a reflection of environmental or climatic variables

that favour longer survival of AIV in the environment (e.g, cooler climates with increasing latitudes).

Population density – density dependence of infection

Another important factor driving annual as well as spatial variation (including variation in both latitude and flyway) is density of the waterfowl population, as observed in the prairie provinces, where we determined that AIV infection was highly correlated with BWTE population density (Table 2-3). Further evidence for the role of population density is that we observed higher risk of AIV infection in HY BWTE at northern latitudes in August, particularly in the Central and Mississippi flyways, covering southern areas of Alberta, Saskatchewan, Manitoba, Montana and the Dakotas (Figure 3-5), which encompasses the most important and highest density breeding areas for this species (Szymanski & Dubovsky, 2010).

The role of sex is variable

Although sex was not a significant variable for predicting AIV infection in BWTE in the Canadian Prairies, it was important when analyzing a larger scale dataset spanning Canada and the United States, with male BWTE significantly more likely to be positive compared to females. This has been observed in other studies focusing on waterfowl species (Parmley *et al*., 2008; Ip *et al*., 2008), and may be caused by behavioral differences which increase the likelihood of exposure to the virus among males. For example, males may have more frequent aggressive encounters. After breeding, males undergo a moult migration while females incubate eggs, and therefore males travel longer distances, are exposed to a wider range of habitats, and are more gregarious, thus increasing opportunities for transmission and exposure.

The findings of this thesis improve our understanding of the ecology of AIV in wild ducks, which are the most important reservoir of AIV worldwide. The pattern of decreasing apparent prevalence of infection with AIV during fall migration and over winter is likely due to a

combination of different factors such as increased resistance of ducks to infection over time, lower population densities of BWTE as they fly south reducing the rates of transmission and exposure during this time, and warmer conditions which reduce the viability of virus in the environment (Rohwer *et al*., 2002). Peaks in annual prevalence may be related to a combination of the proportion of naïve HY birds in the population and waterfowl population densities. Spatial patterns are a reflection of host annual life cycles (season), proportion of HY birds in the population, population densities, and climatic and other environmental factors.

The temporal and geographic variability of AIV in BWTE found in this thesis suggest that this species is a suitable host for LPAI viruses throughout the year. Other studies of AIV in various species of dabbling ducks in the northern hemisphere have shown similar temporal patterns of AIV infection, with high prevalences during the pre-migration period and lower prevalences on wintering grounds and during spring migration (Krauss *et al*., 2004, Hanson *et al*., 2005). Year-round presence of AIV infection in BWTE and other dabbling ducks may be a result of several factors such as dabbling duck feeding behavior, which favours the ingestion of AIV excreted in the surface water by other feeding ducks (Webster *et al*., 1992), the survival of AIV viruses for long periods of time in water (Stallknecht & Brown, 2009, Henaux & Samuel, 2011), and the gregarious behavior of these species such that they cluster together on the landscape (Hill *et al*., 2012; Gunnarsson *et al*., 2012).

West Nile virus and avian paramyxovirus in the Blue-winged Teal

There is limited information about the ecology of WNV and APMV-1 in waterfowl in general. Overall, we found a very low prevalence of antibodies against WNV and APMV-1, with 4.2% and 6.3% seropositive, respectively. Because of this low seroprevalence, BWTE are not a

good host species in which to study WNV and APMV-1. However, our data provided some insights into determinants associated with exposure to these pathogens.

We identified age of bird, year of sampling and pond density as important factors which influenced the presence of antibodies against WNV. In the case of APMV-1, age of the birds and year of sampling were the main factors associated with presence of antibodies. The chance that a bird had antibodies to WNV or APMV-1 was higher in AHY birds compared to HY birds, and also was influenced by year of collection. Further, in the case of WNV, BWTE were more likely to be seropositive at higher pond densities rather than at higher population densities, likely because more ponds in a given area may increase the amount of suitable developmental habitat for mosquitoes, the primary vectors for transmission.

Important questions remain about the ecology of WNV and APMV-1 in BWTE. For WNV, the role of BWTE as host and as a potential carrier is not known, nor is it known how important the virus is for the survival of BWTE during breeding season and before their migration. The same questions apply to the role of BWTE in the ecology of the APMV-1 virus strains. It would be interesting to replicate this analysis using additional variables such as antibodies and infection measured together in the same bird, and to differentiate among strains of APMV-1 (Maldonado *et al*., 1995).

Surveillance and management implications

Both influenza A viruses and WNV have the potential to cause both epidemic disease in people and animals and associated social, economic and environmental damage. Public and animal health services face the challenge and complexity of maintaining surveillance networks for important pathogens under conditions of fiscal restraint. Thus, in order to allocate budgets efficiently, sooner or later active surveillance programs must establish priorities for surveillance. This thesis provides tools and background information for choosing variables to measure within

surveillance programs involving wild birds. This thesis emphasizes the utility of collaborative, multidisciplinary work using surveillance data from different agencies that had been collected for other purposes. However, one of the weaknesses of the analyses we conducted was the low number of samples taken during winter and spring in southern locations. Large sample sizes are essential for analyses at the population and continental levels.

Conclusion and Future direction

This research provided further evidence for the role of wild dabbling ducks, particularly BWTE, in the maintenance and ecology of AIV. This improved understanding of the role of BWTE as natural hosts, and the geographic, demographic and temporal variables that affect infection and transmission moves us closer to deciphering the overall ecology of the virus and its transmission and spatiotemporal patterns at the individual, population and continental levels. This knowledge, in turn, will permit development of better tools to predict and perhaps to prevent possible outbreaks in domestic animals as well as in humans. For example, our results suggest that surveillance programs which focus on identifying AIV strains circulating in populations should focus on sampling HY BWTE, in the Prairie pothole region during the staging period (e.g., August), before fall migration.

Like any other research project, our findings resulted in new questions to be answered. Future research in this same general area should aim at constructing more accurate predictive maps to determine the relationships among several risk factors and the overall risk of higher prevalence of infection in a specific area. In order to achieve this objective, new statistical models are needed that include additional variables such as environmental parameters that may influence survival of AIV in environment or alter host densities (e.g., climate, especially temperature and precipitation, vegetation index), host population variables that may influence transmission and exposure (e.g., HY:AHY ratio in the population, waterfowl population density, species diversity

data), while accounting for variables known to be important such as age, which has the potential to drive spatiotemporal patterns and cycles.

Further studies are required to understand the role of AIV immunity in free-ranging populations over multiple years. To reach this goal, more samples from birds during winter and spring seasons as well as more samples of birds collected in the southern range of BWTE (southern United States, Mexico, Central and South America) would be required to further examine spatiotemporal trends. Molecular characterization of pathogens is helpful in understanding possible pathways of exposure and transmission across species and geographic areas, and thus provides insight into possible movement pathways of pathogens. Further studies are needed to understand movement and spread of infectious pathogens via migratory hosts by characterizing and tracking the movement of hosts themselves, using a variety of tools in conjunction with surveillance, including mark-recapture techniques using band recovery data, stable isotopes techniques in feathers to identify origins of hosts (e.g., using feather deuterium, e.g. see Gunnarsson et al 2012 and Hill et al 2012), and satellite telemetry studies. These interdisciplinary approaches will ultimately allow us to develop models that will predict the spread and movement of new emerging diseases of concern, and identify pathways of entry or potential hotspots in Canada or the US, if they were to enter our migratory bird populations.

LIST OF REFERENCES

Alexander, D.J. 2000. Newcastle disease and other avian paramyxoviruses. *Sci and Tech Rev* (OIE) 19:443-462.

Alexander, D.J. 2009. Ecology and epidemiology of Newcastle disease. Pages 19-26 in Capua, I., Alexander, D.J. editors. Avian Influenza and Newcastle Disease. Springer Milan.

Alexander, D.J., 2000. A review of avian influenza in different bird species. *Veterinary Microbiology* 74:3-13.

Altizer, S., Bartel, R., Han, B.A., 2011. Animal migration and infectious disease risk. *Science* 331:296-302.

Anderson, D.R., 2007. *Model based inference in the life sciences: A primer on evidence*. Springer, New York, NY. 184 pp.

Anderson, R. M. and R. M. May, 1979: Population biology of infectious diseases: Part I. *Nature*, 280, 361-367.

Arnold, T.W., 2010. Uninformative parameters and model selection using Akaike's information criterion. *J Wildl Manage* 74:1175-1178

Artois, M., Bicout, D., Doctrinal, D., Fouchier, R., Gavier-Widen, D., Globig, A., Hagemeijer, W., Mundkur, T., Munster, V., Olsen, B., 2009. Outbreaks of highly pathogenic avian influenza in Europe: the risks associated with wild birds. *Sci and Tech Rev* (International Office of Epizootics) 28:69-92.

Bailey, R.O., Seymour, N.R., Stewart, G.R., 1978. Rape behavior in blue-winged teal. *Auk* 95:188-190.

Beldomenico, P.M., Uhart, M., 2008. Ecoepidemiología de los virus de influenza aviar. *Revista FAVE* - Ciencias Veterinarias 7:23-40.

Berhane, Y., Leith, M., Embury-Hyatt, C., Neufeld, J., Babiuk, S., Hisanaga, T., Kehler, H., Hooper-McGrevy, K., Pasick, J., 2010. Studying possible cross-protection of Canada geese preexposed to North American low pathogenicity avian influenza virus strains (H3N8, H4N6, and H5N2) against an H5N1 highly pathogenic avian influenza challenge. *Avian Diseases* 54:548-554.

Blitvich, B.J., Marlenee, N.L., Hall, R.A., Calisher, C.H., Bowen, R.A., Roehrig, J.T., Komar, N., Langevin, S.A., Beaty, B.J., 2003. Epitope-blocking enzyme-linked immunosorbent assays for the detection of serum antibodies to West Nile virus in multiple avian species. *J Clin Microbiol* 41:1041-1047.

Botero, J.E., Rusch, D.H., 1988. Recoveries of North American waterfowl in the neotropics. In: *Weller MW (Ed.).Waterfowl in winter*. University of Minnesota Press: Minneapolis, Minnesota, 7-10 January, Maps 469-482.

Botero, J.E., 1992. Ecology of Blue-winged teal wintering in the Neotropics. 160 pages. PhD Thesis. Wildlife Ecology, University of Wisconsin-Madison,

Chen, C.C., Epp, T., Jenkins, E., Waldner, C., Curry, P.S., Soos, C., 2012. Predicting weekly variation of *Culex tarsalis* (Diptera: Culicidae) West Nile virus infection in a newly endemic region, the Canadian Prairies. *J Med Entomol* 49:1144-1153.

Chuang, T.W., Hildreth, M.B., Vanroekel, D.L., Wimberly M.C., 2011. Weather and land cover influences on mosquito populations in Sioux Falls, South Dakota. *J Med Entomol* 48:669-679.

Connelly JW, Ball IJ. 1984. Comparisons of aspects of breeding blue-winged Teal and Cinnamon Teal in eastern Washington. *Wilson Bull.*, 96, 626–633.

Deliberto, T.J., Swafford, S.R., Nolte, D.L., Pedersen, K., Lutman, M.W., Schmit, B.B., Baroch, J.A., Kohler, D.J., Franklin, A., 2009. Surveillance for highly pathogenic avian influenza in wild birds in the USA. *Integr Zool* 4: 426-439.

Doherty, P., Davis, A., Farnsworth, M., Gilbert, M., Hoeting, J.A., McLean, R., Merton, A., Piaggio, T., Miller, R., Reese, G., Webb, C., Wilson, K., Avian influenza risk assessment for the United States: Modeling pathways of disease spread by wild birds. Doherty, P. and Wilson, K. 1-107. 2010. USDA-WS-APHIS.

Drebot, M.A., Lindsay, R., Barker, I.K., Buck, P.A., Fearon, M., Hunter, F., Sockett, P., Harvey, A., 2003. West Nile virus surveillance and diagnostic: A Canadian perspective. *The Can J Infec Dis & Med Microbiol* 14:105-114.

Duffy D.L., Bentley G.E., Drazen D.L., Ball G.F., 2000. Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult European starlings. *Behav Ecol* 11:654–662.

Epp, T., Waldner, C., West, K., Townsend, H., 2007. Factors associated with West Nile virus disease fatalities in horses. *Canadian Veterinary Journal* 48:1137-1145.

Farnsworth, M.L., Miller, R.S., Pedersen, K., Lutman, M.W., Swafford, S.R., Riggs, P.D., Webb, T.B., 2012. Environmental and demographic determinants of avian influenza viruses in waterfowl across the contiguous United States. *Plos One*, 7, e32729.

Fereidouni, S.R., Grund, C., Häuslaigner, R., Lange, E., Wilking, H., Harder, T.C., Beer, M., Starick, E., 2010. Dynamics of specific antibody responses induced in mallards after infection by or immunization with low pathogenicity avian influenza viruses. *Avian Dis* 54:79-85.

Ferro, P.J, Budke, C.M., Peterson, M.J., Cox, D., Roltsch, E., Merendino, T., Nelson, M., Lupiani, B., 2010. Multiyear surveillance for avian influenza virus in waterfowl from wintering grounds, Texas Coast, USA. *Emerg Infect Dis*, 16, 1224-1230.

Flyways.us. 2008. General Flyways Info. 2011; http://www.flyways.us/flyways/info

Foppa, I., Beard, R., Mendenhall, I., 2011. The impact of West Nile virus on the abundance of selected North American birds. *BMC Veterinary Research* 7:43.

Gaidet, N., Cappelle, J., Takekawa, J.Y., Prosser, D.J., Iverson, S.A., Douglas, D.C., Perry, W.M., Mundkur, T., Newman, S.H., 2010. Potential spread of highly pathogenic avian influenza H5N1 by wildfowl: Dispersal ranges and rates determined from large-scale satellite telemetry. *J Appl Ecol* 47:1147-1157.

Gammonley, J., Fredrickson, L., 1995. Life history and management of the blue-winged teal. In: Waterfowl management handbook. U.S. Department of the Interior, National Biological Service.

Garamszegi, L.Z., Moller, A.P., 2007. Prevalence of avian influenza and host ecology. *Proceedings of the Royal Society Biological Sciences* 274:2003-2012.

Georgiev, V.S. 2009. West Nile virus. Pages 131-134 in Georgiev, V.S. editor. National Institute of Allergy and Infectious Diseases, NIH: Impact on Global Health. Humana Press, New York, NY.

Goekjian, V.H., Smith, J.T., Howell, D.L., Senne, D.A., Swayne, D.E., Stallknecht, D.E., 2011. Avian influenza viruses and avian paramyxoviruses in wintering and breeding waterfowl populations in North Carolina, USA. *J Wildl Dis* 47:240-245.

Guan, Y., Poon, L.L.M., Cheung, C.Y., Ellis, T.M., Lim, W., Lipatov, A.S., Chan, K.H., Sturm-Ramirez, K.M., Cheung, C.L., Leung, Y.H.C., Yuen, K.Y., Webster, R.G., Peiris, J.S.M.,

2004. H5N1 influenza: A protean pandemic threat. *Proceedings of the National Academy of Sciences of the United States of America* 101:8156-8161.

Gunnarsson, G., N. Latorre-Margalef, K.A. Hobson, S. L. Van Wilgenburg, J. Elmberg, B. Olsen, J. Waldenström. 2012. Disease dynamics and bird migration – linking mallards *Anas platyrhynchos* and influenza A virus in time and space. PLoS ONE 7(4): e35679

Hanson, B.A., Swayne, D.E., Senne, D.A., Lobpries, D.S., Hurst, J., Stallknecht, D.E., 2005. Avian influenza viruses and paramyxoviruses in wintering and resident ducks in Texas. *Journal of Wildlife Diseases* 41:624-628.

Hayes, E.B., Gubler, D.J., 2006. West Nile Virus: Epidemiology and Clinical Features of an Emerging Epidemic in the United States. *Annual Review of Medicine* 57:181-194.

Hegyi, G., Moller, A.P., Eens, M., Garamszegi, L.Z., 2009. Prevalence of avian influenza and sexual selection in ducks. *Behavioral Ecology* 20:1289-1294.

Henaux, V., Samuel, M.D., 2011. Avian influenza shedding patterns in waterfowl: implications for surveillance, environmental transmission, and disease spread. *Journal of Wildlife Diseases* 47:566-578.

Hill, N.J., J.Y. Takekawa, J.T. Ackerman, K.A. Hobson, G. Herring, C.J. Cardona, J.A. Runstadler and W.M. Boyce. 2012. Migration strategy affects avian influenza dynamics in mallards (*Anas platyrynchos*). *Molecular Ecology* 21: 5986–5999

Hill, N.J., Takekawa, J.Y., Cardona, C.J., Ackerman, J.T., Schultz, A.K., Spragens, K.A., Boyce, W.M., 2010. Waterfowl ecology and avian influenza in California: Do host traits inform us about viral occurrence? *Avian Diseases Digest* 5:e87-e88.

Himsworth, C.G., Gurney, K.E.B., Neimanis, A.S., Wobeser, G.A., Leighton F.A., 2009. An outbreak of West Nile virus infection in captive lesser scaup (*Aythya affinis*) ducklings. *Avian Diseases* 53:129-134.

Hinshaw, V.S., Webster, R.G., Turner, B., 1980. The perpetuation of orthomyxoviruses and paramyxoviruses in Canadian waterfowl. *Can J Microbiol* 26:622-629.

Hinshaw, V.S., Wood, J.M., Webster, R.G., Deibel, R., Turner, B., 1985. Circulation of influenza viruses and paramyxoviruses in waterfowl originating from two different areas of North America. *Bull World Health Organ* 63:711-719.

Horimoto, T., Kawaoka, Y., 2001. Pandemic threat posed by avian influenza A viruses. *Clinical Microbiology Reviews* 14:129-149.

Hoye, B.J., Munster, V.J., Nishiura, H., Fouchier, R.A.M., Madsen, J., Klaassen, M., 2011. Reconstructing an annual cycle of interaction: natural infection and antibody dynamics to avian influenza along a migratory flyway. *Oikos* 120:748-755.

Hoye, B.J., Munster, V.J., Nishiura, H., Klaassen, M., Fouchier, R.A.M., 2010. Surveillance of wild birds for avian influenza virus. *Emerging Infectious Diseases* 16:1827-1834.

Ip, H.S., Flint, P.L., Franson, J.C., Dusek, R.J., Derksen, D.V., Gill, R.E., Ely, C.R., Pearce, J.M., Lanctot, R.B., Matsuoka, S.M., Irons, D.B., Fischer, J.B., Oates, R.M., Petersen, M.R., Fondell, T.F., Rocque, D.A., Pedersen, J.C., Rothe, T.C., 2008. Prevalence of influenza A viruses in wild migratory birds in Alaska: Patterns of variation in detection at a crossroads of intercontinental flyways. *Virology Journal*, 5:71.

Jourdain, E., Gunnarsson, G., Wahlgren, J., Latorre-Margalef, N., Brojer, C., Sahlin, S., Svensson, L., Waldenström, J., Lundkvist A, Olsen B., 2010. Influenza virus in a natural host, the mallard: experimental infection data. *Plos One*, 5, e8935.

Kent, R., Juliusson, L., Weissmann, M., Evans, S., Komar, N. 2009. Seasonal blood-feeding behavior of *Culex tarsalis* (Diptera: Culicidae) in Weld county, Colorado, 2007. *J Med Entomol* 46: 380-390.

Komar, N., Langevin, S., Hinten, S., Nemeth, N., Edwards, E., Hettler, D., Davis, B., Bowen, R., Bunning, M., 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerging Infectious Diseases* 9:311-322.

Komar, N., 2003. West Nile virus: Epidemiology and ecology in North America. *Advances in Virus Research* 185-234.

Krauss, S., Walker, D., Pryor, S.P., Niles, L., Li, C.H., Hinshaw, V.S., Webster, R.G., 2004. Influenza A viruses of migrating wild aquatic birds in North America. *Vector-Borne and Zoonotic Diseases* 4:177-189.

Kuiken, T., Leighton, F.A., Wobeser, G., Danesik, K.L., Riva, J., Heckert, R.A., 1998. An epidemic of Newcastle disease in double-crested cormorants from Saskatchewan. *Journal of Wildlife Diseases* 34:457-471.

LaDeau, S.L., Kilpatrick, A.M., Marra, P.P., 2007. West Nile virus emergence and large-scale declines of North American bird populations. *Nature* 447:710-713.

Ledoux, H., Gold, C., 2005. An efficient natural neighbour interpolation algorithm for geoscientific modelling. Pages 97-108. Developments in Spatial Data Handling, Springer Berlin Heidelberg.

Lindh, E., Huovilainen, A., Ratti, O., Ek-Kommonen, C., Sironen, T., Huhtamo, E., Poysa, H., Vaheri, A., Vapalahti, O., 2008. Orthomyxo-, paramyxo- and flavivirus infections in wild waterfowl in Finland. *Virology Journal* 5:35.

Maldonado, A., Arenas, A., Tarradas, M. C., Luque, I., Astorga, R., Perea, J. A., Miranda, A., 1995: Serological survey for avian paramyxoviruses from wildfowl in aquatic habitats in Andalusia. Journal of Wildlife Diseases, 31, 66-69.

Meece, J.K., Kronenwetter-Koepel, T.A., Vandermause, M.F., Reed, K.D., 2006. West Nile virus infection in commercial waterfowl operation, Wisconsin. *Emerg Infect Dis* 12:1451-1453.

Morales, M.A., Barrandeguy, M., Fabbri, C., Garcia, J.B., Vissani, A., Trono, K., Gutierrez, G., Pigretti, S., Menchaca, H., Garrido, N., Taylor, N., Fernandez, F., Levis, S., Enia, D., 2006. West Nile virus isolation from equines in Argentina, 2006. *Emerging Infectious Diseases* 12:1559-1561.

Munster, V.J., Baas, C., Lexmond, P., Waldenstrom, J., Wallensten, A., Fransson, T., Rimmelzwaan, G.F., Beyer, W.E.P., Schutten, M., Olsen, B., Osterhaus, A.D.M.E., Fouchier, R.A.M., 2007. Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. *PLoS* Pathogens 3:630-638.

Murray, K., Conner, M.M., 2009. Methods to quantify variable importance: implications for the analysis of noisy ecological data. *Ecology*, 90, 348-355.

Nash, D., Mostashari, F., Fine, A., Miller, J., O'Leary, D., Murray, K., Huang, A., Rosenberg, A., Greenberg, A., Sherman, M., Wong, S., Campbell, G.L., Roehrig, J.T., Gubler, D.J., Shieh, W.J., Zaki, S., Smith, P., Layton, M., 2001. The outbreak of West Nile virus infection in the New York City area in 1999. *New England Journal of Medicine* 344:1807-1814.

National Wildlife Health Center/USGS, Jun 2011. West Nile Virus. Retrieved online 12 December 2011, from http://www.nwhc.usgs.gov/disease_information/west_nile_virus/.

North American Waterfowl Management Plan, Plan Committee. 2004. North American waterfowl management plan 2004. Implementation framework: Strengthening the biological foundation.

Canadian Wildlife Service, U.S. Fish and Wildlife Service, Secretaria de Medio Ambiente y Recursos Naturales, 106 pp.

OIE, 2005. Disease Notifiable to the OIE. Retrieved online 4 Jan 2010, from http://www.oie.int/eng/maladies/en_classification2009.htm?e1d7.

Olsen, B., Munster, V.J., Wallensten, A., Waldenstrom, J., Osterhaus, A.D., Fouchier, R.A., 2006. Global patterns of influenza A virus in wild birds. *Science* 312:384-388.

Owen, J., Moore, F., Panella, N., Edwards, E., Bru, R., Hughes, M., Komar, N., 2006. Migrating birds as dispersal vehicles for West Nile virus. *Ecohealth* 3:79-85.

Palmer, R., 1976. Handbook of North American birds: Waterfowl (Part 1). Volume 2. Yale University Press, Ltd., London.

Parmley, E.J., Bastien, N., Booth, T.F., Bowes, V., Buck, P.A., Breault, A., Caswell, D., Daoust, P.Y., Davies, J.C., Elahi, S.M., Fortin, M., Kibenge, F., King, R., Li, Y., North, N., Ojkic, D., Pasick, J., Pryor, S.P., Robinson, J., Rodrigue, J., Whitney, H., Zimmer, P., Leighton, F.A., 2008. Wild bird influenza survey, Canada, 2005. *Emerging Infectious Diseases* 14:84-87.

Pasick, J., Berhane, Y., Kehler, H., Hisanaga, T., Handel, K., Robinson, J., Ojkic, D., Kibenge, F., Fortin, M., King, R., Hamel, A., Spiro, D., Parmley, J., Soos, C., Jenkins, E., Breault, A., Caswell, D., Davies, C., Rodrigue, J., McAloney, K., Leighton, F., 2010. Survey of influenza A viruses circulating in wild birds in Canada 2005 to 2007. *Avian Diseases* 54:440-445.

Peig, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* 118: 1883–1891.

Public Health Agency of Canada. 2011. *West Nile virus National Surveillance Report, summary of 2010 WNV season*, http://www.phac-aspc.gc.ca/wnv-vwn/index-eng.php. Accessed February 2012.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org/.

Raftovich, R.V., Wilkins, K.D., Richkus, S.S., Spriggs, H.L., 2010. Migratory bird hunting activity and harvest during the 2008 and 2009 hunting seasons. Laurel, MD, USA, U.S. Fish and Wildlife Service. Retrieved online 27 June 2010, from http://www.flyways.us/sites/default/files/images/pdf/migratory-bird-hunting-activity-and-harvest-2008-2009-hunting-seasons-estimates.pdf.

Reed, K.D., Meece, J.K., Henkel, J.S., Shukla, S.K., 2003. Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, influenza A and enteropathogens. *Clinical Medicine Research* 1:5-12.

Rhyan, J.C., Spraker, T.R., 2010. Emergence of diseases from wildlife reservoirs. *Veterinary Pathology Online* 47:34-39.

Rohani, P., R. Breban, D. E. Stallknecht, and J. M. Drake. 2009. Environmental transmission of low pathogenicity avian influenza viruses and its implications for pathogen invasion. Proceedings of the National Academy of Sciences 106:10365-10369.

Rohwer, F.C., Jonnson, W.P., Loos, E.R., 2002. Blue-winged teal (*Anas discors*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology, http://bna.birds.cornell.edu/bna/species/625. Accessed October 2012

Runstadler, J.A., Happ, G.M., Slemons, R.D., Sheng, Z.M., Gundlach, N., Petrula, M., 2007. Using RRT-PCR analysis and virus isolation to determine the prevalence of avian influenza virus infections in ducks at Minto Flats State Game Refuge, Alaska, during August 2005. *Arch Virol* 152, 1901-1910.

Saito, M., Osa, Y., Asakawa, M., 2009. Antibodies to flaviviruses in wild ducks captured in Hokkaido, Japan: Risk assessment of invasive Flaviviruses. *Vector-Borne and Zoonotic Diseases* 9:253-258.

Sharp, G.B., Kawaoka, Y., Wright, S.M., Turner, B., Hinshaw, V., Webster, R.G., 1993. Wild ducks are the reservoir for only a limited number of influenza A subtypes. *Epidemiology and Infection* 110:161-176.

Shengqing, Y., Kishida, N., Ito, H., Kida, H., Otsuki, K., Kawaoka, Y., Ito, T., 2002. Generation of velogenic Newcastle Disease viruses from a nonpathogenic waterfowl isolate by passaging in chickens. *Virology* 301:206-211.

Shirafuji, H., Kanehira, K., Kubo, M., Shibahara, T., Kamio, T., 2009. Experimental West Nile virus infection in aigamo ducks, a cross between wild ducks (*Anas platyrhynchos*) and domestic ducks (*Anas platyrhynchos* var. *domesticus*). *Avian Diseases* 53:239-244.

Soos, C., Parmley, E.J., McAloney, K., Pollard, B., Jenkins, E., Kibenge, F., Leighton, F.A., 2012. Bait trapping linked to higher avian influenza virus detection in wild ducks. *Journal of Wildlife Diseases* 48: 444-448.

Spackman, E., McCracken, K.G., Winker, K., Swayne, D.E., 2006. H7N3 avian influenza virus found in a South American wild duck is related to the Chilean 2002 poultry outbreak, contains genes from equine and North American wild bird lineages, and is adapted to domestic turkeys. Journal of Virology 80:7760-7764.

Spackman, E., Senne, D.A., Myers, T.J., Bulaga, L.L., Garber, L.P., Perdue, M.L., Lohman, K., Daum, L.T., Suarez, D.L., 2002. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. Journal of Clinical Microbiology 40:3256-3260.

Stallknecht, D.E., Shane, S.M., Zwank, P.J., Senne, D.A., Kearney, M.T., 1990. Avian influenza viruses from migratory and resident ducks of Coastal Louisiana. *Avian Diseases* 34: 398-405.

Stallknecht, D., Brown, J., 2009. Tenacity of avian influenza viruses. *Scientific and Technical Review* (International Office of Epizootics) 28:59-67.

Stallknecht, D.E., Senne, D.A., Zwank, P.J., Shane, S.M., Kearney, M.T., 1991. Avian paramyxoviruses from migrating and resident ducks in Coastal Louisiana. *Journal of Wildlife Diseases* 27:123-128.

Stallknecht, D.E., Shane, S.M., 1988. Host range of avian influenza virus in free-living birds. *Veterinary Research Communications* 12:125-141.

Stallknecht, D.E., Shane, S.M., Kearney, M.T., Zwank, P.J., 1990. Persistence of avian influenza viruses in water. *Avian Diseases* 34:406-411.

Stewart, G.R., Titman, R.D., 1980. Territorial behaviour by prairie pothole blue-winged teal. *Canadian Journal of Zoology* 58:639-649.

Szymanski, M.L., Dubovsky, J.A., 2013. Distribution and derivation of the blue-winged teal (*Anas discors*) harvest, 1970–2003. U.S. Department of Interior, Fish and Wildlife Service, Biological Technical Publication FWS/BTP-R601x-201x, Washington, D.C.

Takekawa, J.Y., Prosser, D.J., Newman, S.H., Muzaffar, S.B., Hill, N.J., Yan,B., Xiao, X., Lei, F., Li, T., Schwarzbach, S.E., Howell, J.A., 2010.Victims and vectors: highly pathogenic avian influenza H5N1 and the ecology of wild birds. *Avian Biology Research* 3[2], 51-73.

Thiemann TC, Lemenager DA, Kluh S, Carroll BD, Lothrop HD, Reisen WK. 2012. Spatial variation in host feeding patterns of *Culex tarsalis* and the *Culex pipiens* complex (Diptera: Culicidae) in California. *J Med Entomol*. 49: 903-916.

U.S. Fish and Wildlife Service. 2007. *Early Detection and Response Plan for Occurrence of Highly Pathogenic Avian Influenza in Wild Birds,* 2007 edition.

U.S. Fish and Wildlife Service. 2010. *Waterfowl population status, 2010*. https://migbirdapps.fws.gov/mbdc/databases/mas/aboutmas.htm. Accessed October 2010.

Vickers, M.L., Hanson, R.P., 1982. Newcastle disease virus in waterfowl in Wisconsin. *J Wildl Dis* 18(2):149-158.

Webster, R.G., Krauss,S., Hulse-Post, D., Sturm-Ramirez, K., 2007. Evolution of influenza A viruses in wild birds. *Journal of Wildlife Diseases* 43:S1-S6.

Webster,R.G., Bean,W.J., Gorman,O.T., Chambers,T.M., Kawaoka,Y., 1992. Evolution and ecology of influenza-A viruses. *Microbiological Reviews* 56:152-179.

Wilcox, B.R., Knutsen, G.A., Berdeen, J., Goekjian,V., Poulson, R., Goyal, S., Sreevatsan, S., Cardona, C., Berghaus, R.D., Swayne, D.E., Yabsley, M.J., Stallknecht, D.E., 2011. Influenza-A viruses in ducks in Northwestern Minnesota: Fine scale spatial and temporal variation in prevalence and subtype diversity. *PLoS ONE* 6(9): e24010. doi:10.1371/journal.pone.0024010.

Wobeser, G. A. 1997. Diseases of wild waterfowl, 2nd Edition. Plenum Press, New York, New York, 324 pp.

Wobeser, G., Leighton, F.A., Norman, R., Myers, D.J., Onderka, D., Pybus, M.J., Neufeld, J.L., Fox, G.A., Alexander, D.J., 1993. Newcastle-Disease in wild water birds in Western Canada, 1990. *Canadian Veterinary Journal* 34:353-359.

Wojnarowicz, C., Olkowski, A., Schwean-Lardner, K., 2007. First Canadian outbreak of West Nile virus disease in farmed domestic ducks in Saskatchewan. *Can Vet J* 48: 1270-1271.

Yang, M., Berhane, Y., Salo, T., Li, M., Hole, K., Clavijo, A., 2008. Development and application of monoclonal antibodies against avian influenza virus nucleoprotein. *J Virol Methods* 147: 265-274.

Yeh, J.Y., Park, J.Y., Ostlund, E.N., 2011. Serologic evidence of West Nile Virus in wild ducks captured in major inland resting sites for migratory waterfowl in South Korea. *Veterinary Microbiology* 154:96-103.

Zimper, N.L., Rhodes, W.E., Silverman, E.D., 2010. Zimmerman, G.S., Koneff, M.D. Trends in duck breeding populations, 1955-2010. Laurel, MD, USA, U.S. Fish and Wildlife Service.