DOI: 10.7589/2013-07-191

Journal of Wildlife Diseases, 52(1), 2016, pp. 33–46

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

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ABSTRACT: The Canadian prairies are one of the most important breeding and staging areas for migratory waterfowl in North America. Hundreds of thousands of waterfowl of numerous species from multiple flyways converge in and disperse from this region annually; therefore this region may be a key area for potential intra- and interspecific spread of infectious pathogens among migratory waterfowl in the Americas. Using Blue-winged Teal (*Anas discors*, BWTE), which have the most extensive migratory range among waterfowl species, we investigated ecologic risk factors for infection and antibody status to avian influenza virus (AIV), West Nile virus (WNV), and avian paramyxovirus-1 (APMV-1) in the three prairie provinces (Alberta, Saskatchewan, and Manitoba) prior to fall migration. We used generalized linear models to examine infection or evidence of exposure in relation to host (age, sex, body condition, exposure to other infections), spatiotemporal (year, province), population-level (local population densities of BWTE, total waterfowl densities), and environmental (local pond densities) factors. The probability of AIV infection in BWTE was associated with host factors (e.g., age and antibody status), population-level factors (e.g., local BWTE population density), and year. An interaction between age and AIV antibody status showed that hatch year birds with antibodies to AIV were more likely to be infected, suggesting an antibody response to an active infection. Infection with AIV was positively associated with local BWTE density, supporting the hypothesis of density-dependent transmission. The presence of antibodies to WNV and APMV-1 was positively associated with age and varied among years. Furthermore, the probability of being WNV antibody positive was positively associated with 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 influence dynamics of these and other viruses in wild waterfowl populations.

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

INTRODUCTION

The Canadian prairies in Alberta, Saskatchewan, and Manitoba form one of the most important hubs for migratory waterfowl in North America with hundreds of thousands of waterfowl of numerous species from different flyways converging annually for breeding or staging (North American Waterfowl Management Plan

2004). Subsequently, birds from this region migrate to numerous wintering sites, via multiple flyways, ranging from the southern US to northern South America (Rohwer et al. 2002). Hence, the Canadian prairies are potentially a key area for intra- and interspecific transmission of pathogens among birds that have come from a variety of geographic locations and from which infectious agents can disperse across large distances.

Blue-winged Teal (*Anas discors*, BWTE) use the Canadian prairies extensively for breeding and staging. They have the most extensive migratory range among waterfowl, are gregarious, often forming large flocks that come in contact with shorebirds and other waterfowl (Botero and Rusch 1988; Szymanski and Dubovsky 2013), and have the second most abundant breeding population of ducks in North America (USFWS 2015). These factors may increase opportunities for exposure, transmission, and movement of a range of pathogens.

Given the importance of the Canadian prairies to North American waterfowl productivity and the potential for this region to be a hotspot for infectious diseases of wild waterfowl, we investigated ecologic risk factors for infection or antibody status to selected viruses in BWTE in the three prairie provinces. Viruses selected for our investigation included those significant to the Canadian prairies or to free-ranging waterfowl and included avian influenza virus (AIV), avian paramyxovirus-1 (APMV-1), and West Nile virus (WNV), which are also of broad significance to public and domestic animal health (Hinshaw et al. 1980; Webster et al. 1992; Hayes and Gubler 2006).

Wild waterfowl are natural reservoirs for low pathogenic AIVs (LPAIVs), which generally do not cause clinical disease in wild ducks. Transmission may occur directly or through the environment via the fecal-oral route. Prevalences of LPAIV infection in wild waterfowl are generally high during late summer prior to fall migration in Canada, when waterfowl densities and the proportion of juveniles in populations are high (Sharp et al. 1993; Wallensten et al. 2007; Wilcox et al. 2011).

The causative agent of Newcastle disease, APMV-1, is globally distributed, and most species of birds are likely susceptible to infection despite few reports of disease in wild birds (Wobeser et al. 1993; Kuiken et al. 1998; Alexander 2000). Previously considered exotic to North America, highly pathogenic APMV-1 was detected in Double-crested Cormorants (*Phalacrocorax auritus*) in Saskatchewan between 1990 and 2000, representing one of the most important incidents of wild bird mortality caused by this pathogen (Wobeser et al. 1993; Kuiken et al. 1998).

Since its emergence in North America in 1999, WNV has become endemic across southern Canada, particularly in the prairies, where the highest rates of human infection in Canada have occurred (Chen et al. 2013). A mosquito-borne flavivirus, WNV is primarily maintained and amplified in bird populations through transmission by mosquitoes (especially *Culex* species), with occasional spillover to horses and humans, which are dead end hosts (Hayes and Gubler 2006). Since its introduction into North America, WNV has been reported in >200 species, of which 31 were of the family Anatidae (USGS/NWHC 2005) and has caused declines in local populations of many wild bird species (LaDeau et al. 2007; Foppa et al. 2011). Although ducks are susceptible to infections with WNV (Komar et al. 2003; Himsworth et al. 2009; Shirafuji et al. 2009), little is known about its impacts on wild duck populations.

We evaluated demographic and ecologic factors associated with infection or antibody status to AIV, APMV-1, and WNV in BWTE in the Canadian prairies. For each virus, we examined the role of host factors (age, sex, body condition, evidence of exposure to other infections), spatiotemporal factors (year, province), population-level factors (local population densities of BWTE, total waterfowl densities), and environmental factors (local pond densities)

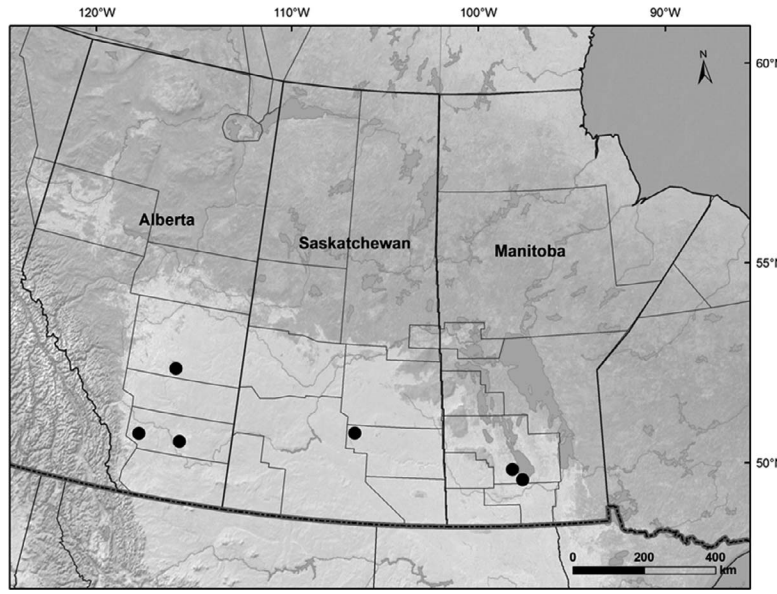

FIGURE 1. Capture sites (black dots) for Blue-winged Teal (*Anas discors*) sampled in the Canadian prairies, 2007–10. Thick, black, solid lines delineate provincial/territorial boundaries. Thin lines indicate strata used for estimating pond density and waterfowl breeding population densities by the Waterfowl Breeding Population and Habitat Survey. The thick dashed line indicates the Canada-US border.

as potential variables affecting antibody status or probability of infection. Understanding demographic and ecologic risk factors associated with infection and antibody prevalence in migratory waterfowl is useful for informing wildlife disease surveillance or management programs for these or other viruses that can affect or be carried by migratory birds.

MATERIALS AND METHODS

Field methods

From 2007 to 2010, BWTE were sampled in August, prior to fall migration, at several sites within the Canadian Prairies, including Frank Lake and regions around Brooks, Alberta; Last Mountain Lake, Saskatchewan; and Delta Marsh, Manitoba (Fig. 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, location, date, band number, age, sex, mass, and head-bill length were recorded. Oral and cloacal swabs were placed together in one vial containing transport medium and stored as described by Parmley et al. (2011). Blood samples were collected by jugular venipuncture, placed in tubes containing no additive, and stored on frozen gel packs. At the end of each day, blood samples were centrifuged, and serum was harvested and frozen at −20 C until further testing.

Bird capture, handling, and sampling procedures were approved by the University of Saskatchewan's Animal Research Ethics Board (protocol 20070039) and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

Laboratory analyses

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 influenza A virus nucleic acid using reverse transcriptase real-time PCR targeting the matrix 1 gene (Spackman et al. 2002). Samples with cycle threshold values ≤35 were considered positive. Serum antibodies to AIV nucleoprotein were tested using competitive enzyme-linked immunosorbent assays (cELISA) at the Canadian Food Inspection Agency, National Centre for Foreign Animal Disease (Winnipeg, Manitoba, Canada), as per Yang et al. (2008). Values >30% inhibition in relation to controls were considered positive (Yang et al. 2008).

TABLE 1. Outcome and explanatory variables used in models exploring risk factors associated with infection or exposure to avian influenza virus (AIV), West Nile virus (WNV), and avian paramyxovirus-1 (APMV-1), in Blue-winged Teal (*Anas discors*, BWTE) in the Canadian prairies, in the provinces of Manitoba (MB), Saskatchewan (SK), and Alberta (AB), 2007–10.

Variable	Type	Description	Categories/units
Year	Categorical, explanatory	Year of sampling	2007–10
Province	Categorical, explanatory	Province of Canada sample originates from	MB, SK, AB
Pond density	Continuous, explanatory	Suitable breeding pond density estimated by aerial surveys conducted in spring	No. ponds/km^2
Total duck density	Continuous, explanatory	Total duck breeding population density estimated by aerial surveys in spring	Total no. ducks/km^2
BWTE density	Continuous, explanatory	BWTE breeding population density estimated by aerial surveys in spring	Total no. BWTE/km^2
Residuals of BWTE density	Continuous, explanatory	Expressed as the residuals of BWTE density from its linear regression on pond density	Residual no. BWTE/km^2
Sex	Categorical, explanatory	Sex of bird	Female/male
Age	Categorical, explanatory	Age of bird at capture	Hatch year/after hatch year
Body condition index	Continuous, explanatory	An index, incorporating mass scaled according to body size to define the relative size of energy reserves accumulated in the body (Peig and Green 2009)	g
Head-bill length	Continuous, explanatory	Standard measure of length from back of the head to tip of bill	mm
AIV infection	Categorical, outcome and explanatory	Current infection status, as determined using PCR of matrix gene	Positive/negative
AIV antibody status	Categorical, outcome and explanatory	Avian influenza virus nucleoprotein–specific antibodies; positive is evidence of exposure to virus	Positive/negative
WNV antibody status	Categorical, outcome and explanatory	West Nile virus–specific antibodies; positive suggests previous exposure	Positive/negative
APMV antibody status	Categorical, outcome and explanatory	Avian paramyxovirus–specific antibodies; positive suggests previous exposure	Positive/negative

Samples were screened with a cELISA for flaviviruses using a mouse anti–West Nile/Kunjin virus monoclonal antibody MAB8152 (Millipore, Single Oak Drive, Temecula, California, USA) at the National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba. Samples with inhibition values >30% were considered positive (Blitvich et al. 2003) and subsequently confirmed using plaque-reduction neutralization specific for WNV. Samples that neutralized ≥90% of virus were considered positive (Blitvich et al. 2003; Drebot et al. 2003).

Serum antibodies against APMV-1 were measured using hemagglutination inhibition assays at the Poultry Diagnostic and Research Center, University of Georgia (Athens, Georgia, USA), as described by Alexander (2000), with the modification that each well contained 10 hemagglutinating units of antigen and 0.25% chicken red blood cells and plates were incubated for 45 min at room temperature. Samples with titers ≥10 were considered positive.

Bird population and pond densities

Since 1955 the USFWS and CWS have conducted annual spring Waterfowl Breeding Population and Habitat Surveys to estimate population sizes and pond densities across Canada (Smith 1995). We obtained data from this survey for 2007–10 (USFWS 2014) for each of the defined survey areas (strata; USFWS 2014) in which BWTE were sampled (Fig. 1). Total duck population density and BWTE density were estimated by aerial transects and defined as the number of dabbling ducks or BWTE per km^2 of transects, while pond density was defined as the number of standing water bodies estimated per km^2 of transects within each stratum surveyed during the breeding season (Smith 1995; USFWS

TABLE 2. Summary of apparent prevalence of avian influenza virus (AIV) infection and prevalence of antibodies (Ab) to AIV, West Nile virus, and avian paramyxovirus-1 in Blue-winged Teal (*Anas discors*) sampled in the Canadian prairies, 2007–10. Number positive/total (% positive).

Parameter	AIV[a]	AIV-Ab[b]	WNV-Ab[c]	APMV-1-Ab[d]
n	1,846	1,846	1,869	1,817
Overall positive (%)	346 (18.7)	817 (44.2)	78 (4.2)	115 (6.3)
No. positive by sex				
Male	205/1,194 (17.2)	644/1,194 (53.9)	53/1,209 (4.4)	74/1,169 (6.3)
Female	144/652 (22.1)	227/652 (34.8)	24/660 (3.6)	41/648 (6.3)
No. positive by age				
Hatch year	281/929 (30.2)	162/929 (17.4)	15/939 (1.6)	32/918 (3.5)
After hatch year	68/917 (7.4)	709/917 (77.3)	62/930 (6.7)	83/899 (9.2)
No. positive by province				
Alberta	23/646 (3.5)	412/646 (63.8)	23/655 (3.5)	37/655 (5.6)
Saskatchewan	177/777 (22.7)	348/777 (44.8)	46/785 (5.8)	9/785 (1.1)
Manitoba	149/423 (35.2)	111/423 (26.2)	7/429 (1.6)	6/429 (1.4)
No. positive by year				
2007	139/523 (26.6)	190/523 (36.3)	25/534 (4.7)	20/534 (3.7)
2008	2/429 (0.5)	292/429 (68.1)	27/436 (6.2)	30/436 (6.9)
2009	34/299 (11.4)	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)

[a] AIV = avian influenza virus infection status.

[b] AIV-Ab = avian influenza virus antibody status.

[c] WNV-Ab = West Nile virus antibody status.

[d] APMV-1-Ab = avian paramyxovirus-1 antibody status.

2015). We used spring breeding population density as a proxy for population density in August when sampling occurred, assuming a positive correlation between spring breeding density and August population density, in large part because spring population densities are positively correlated with spring pond densities (Johnson and Grier 1988; Supplementary Material Fig. S1), and reproductive effort and success are generally higher in years of abundant ponds and breeding populations (Howerter et al. 2014).

The residuals of BWTE density from its linear regression on pond density (Fig. S1) were used to create an alternate measure of BWTE population density (Table 1), which allowed us to combine it with pond density and province in models. This residual density variable could be interpreted as a measure of population density not explained by the number of ponds in a given area, but rather by other factors not measured in this study such as climate, pond size, and nesting habitat quality.

Statistical analyses

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 birds infected) or apparent antibody prevalence (proportion of birds with antibodies) for the selected viruses. Each outcome variable (AIV infection, AIV antibody status, WNV antibody status, and APMV-1 antibody status) was examined in relation to demographic and ecologic factors (Table 1). We constructed generalized linear models with a binomial distribution, using a logit link function based on maximum likelihood estimation, using R, version 2.14.1 (R Core Team 2012).

For each outcome, a set of models was built based on biologically meaningful combinations of predictors, using approaches described by Burnham and Anderson (2002) and Dohoo et al. (2009). Model selection was carried out using Akaike information criterion corrected for small sample size (AIC_c; Burnham and Anderson 2002) to rank competing models. Variables having no initial association with the outcome when examined alone were not included in models. Variables that were highly correlated (Pearson or phi correlation coefficient >0.7) were not combined in the same model (Murray and Conner 2009). The best supported model was defined as that with the lowest AIC_c. Models within 4 ΔAIC_c of the top model, excluding those containing noninformative variables, were used to construct a confidence set of models. Noninformative variables were those that did not decrease the AIC_c of a nested model when included (Anderson 2008; Arnold 2010). The confidence set of models was used to calculate parameter estimates by model averaging based on Akaike

TABLE 3. Summary of best-supported model to explain variation in avian influenza virus (AIV) infection in Blue-winged Teal (*Anas discors*, BWTE) sampled in the Canadian prairies in August, 2007–10. AIV infection was determined using reverse transcriptase real-time PCR on oral and cloacal swab samples (n=1,846).

Variables[a]	β[b]	SE	95% CI[c]
AIV-Ab (ref=negative)			
positive	0.422	0.215	0.002, 0.84
Age (ref=HY)			
AHY	−0.587	0.280	−1.13, −0.04
AIV-Ab*Age	−0.826	0.376	−1.56, −0.09
Year (ref=2007)			
2008	−4.415	0.730	−5.84, −2.98
2009	−0.658	0.233	−1.11, −0.20
2010	0.666	0.171	0.33, 1.00
BWTE density residuals on pond density	0.522	0.044	0.43, 0.61
Province (ref=SK)			
AB	−1.426	0.253	−1.92, −0.93
MB	0.470	0.173	0.13, 0.81
Intercept	−0.763	0.147	−1.05, −0.47

[a] AIV-Ab = avian influenza virus–specific antibody status; ref = reference category; HY = hatch year; AHY = after hatch year; AIV-Ab*Age = interaction term between AIV antibody status and age; SK = Saskatchewan; AB = Alberta; MB = Manitoba.

[b] β = coefficient estimate.

[c] CI = 95% confidence interval of β estimates.

weights (ω_i) of models (Burnham and Anderson 2002). Best supported models in each set passed Pearson's χ^2 goodness-of-fit test.

RESULTS

During 2007–10, we captured, banded, and sampled 1,971 BWTE in Alberta, Manitoba, and Saskatchewan. Due to missing information, 1,846, 1,869, and 1,817 samples were available for statistical analyses of AIV-, WNV-, and APMV-1-related outcomes, respectively (Supplementary Material Tables S1–S3).

Risk factors associated with AIV infection and exposure

Overall, 18.7% (95% confidence interval=17.1–20.6) of BWTE sampled in the Canadian prairies 2007–10 were infected with AIV, and 44.2% (42.0–46.5) had antibodies to AIV (Table 2). Among 871 AIV antibody-positive birds, 111 (12.7%, 10.7–15.1) were infected with AIV, 53 (6.1%, 4.7–7.9) were antibody positive for WNV, 71 (8.1%, 6.5–10.2) were antibody positive for APMV-1, and 6 (0.7%, 0.3–1.5) were antibody positive for all three viruses.

Candidate models to explain variation in AIV infection and antibody status are displayed in Supplementary Material Tables S4, S5. Based on the best-supported model, there was an interaction between age and AIV antibody status (AIV-Ab) (Table 3). Hatch-year (HY) birds with antibodies to AIV were 1.52 (1.00–2.32) times more likely to be infected with AIV compared to antibody-negative HY birds, while no difference was found between antibody-negative and antibody-positive, after-hatch-year (AHY) birds. Within antibody-negative BWTE, HY birds were 1.8 (1.04–3.11) times more likely to be infected than AHY birds while antibody-positive HY birds were 4.1 (2.42–6.95) times more likely to be infected compared to antibody-positive AHY birds.

Sex was noninformative in our final model; the initial positive association with females in a simple model was spurious, resulting from the large proportion of HY birds among females sampled (Table S4).

Our model showed annual variability in estimated prevalence of AIV infection, being lowest in 2008, then increasing over the following 2 yr, with the highest prevalence estimated in 2010 (Fig. 2). Birds sampled in Alberta had lower and birds sampled in Manitoba had higher probability of infection compared to those in Saskatchewan (Table 3). Probability of AIV infection was positively associated with residuals of BWTE density regressed on pond density (Table 3); this variable explained more of the variation in AIV status than did BWTE density (Table S4). For every unit increase in residual density, probability of AIV infection increased 1.68 times (1.54–1.83). Total duck density, pond density, head-bill length, body condition index (BCI, Table 1), and antibody status to WNV or APMV-1 did not have initial associations with AIV infection and therefore were excluded from model sets.

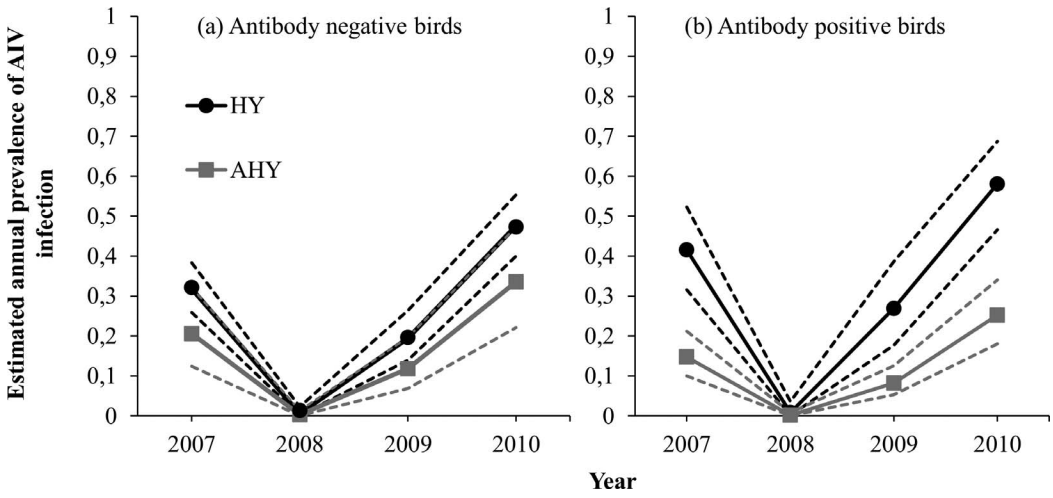

FIGURE 2. Estimated annual prevalence of avian influenza virus (AIV) infection in Blue-winged Teal (*Anas discors*, BWTE) sampled in the Canadian prairies prior to fall migration (August) in 2007–10, based on the best supported model (Table 3). Dashed lines represent 95% confidence intervals. For these estimates, residual BWTE density was set to its mean (0.0).

Model-averaged parameter estimates to explain variation in AIV antibody status were based on two models that merited consideration (Table S5; Tables 4, 5). The probability of a bird having antibodies to AIV was 17.46 (13.27–22.97) times higher in AHY birds compared to HY birds (Table 5). Birds with larger head-bill length were 1.04 (1.001–1.08) times more likely to have antibodies to AIV. As in models examining AIV infection status, there was annual variation in estimated AIV antibody prevalence. Lowest antibody prevalences in both age classes occurred in 2009, following a year (2008) of almost zero prevalence of infection (Fig. 3). Density variables and BCI were not informative in predicting AIV antibody status (Table S5). Although WNV and APMV-1 antibody status had initial associations with AIV antibody status, they were not informative once age was included (data not shown).

Risk factors affecting WNV antibody status

Overall prevalence of WNV antibody in BWTE sampled in the Canadian Prairies in 2007–10 was 4.2% (3.3–5.2; Table 2). Of 76 birds positive for WNV antibody, 12 (15.8%, 9.3–25.6) were infected with AIV, 53 (69.7%, 58.7–78.9) were positive for AIV antibody, and 9 (11.8%, 6.4–21.0) were positive for APMV-1 antibody. Model-averaged parameter estimates to explain variation in WNV antibody status were based on two models that merited

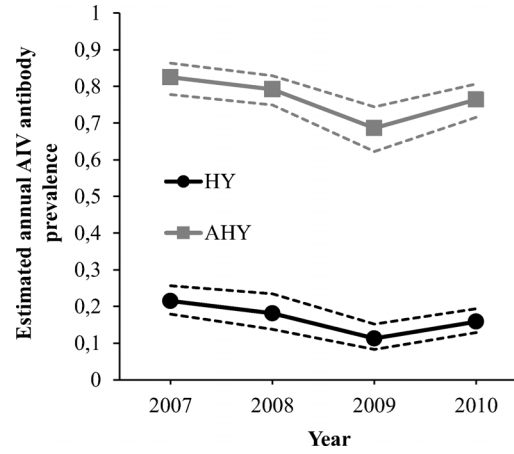

FIGURE 3. Estimated annual prevalence of avian influenza virus (AIV) antibody in Blue-winged Teal (*Anas discors*) sampled in the Canadian prairies prior to fall migration (August) in 2007–10, based on model-averaged estimates shown in Table 5. Dashed lines represent 95% confidence intervals. For these estimates, head-bill length was set to its mean value (83.6 mm). HY=hatch-year birds; AHY=after-hatch-year birds.

TABLE 4. Comparison of competing generalized linear models constituting the confidence set[a] of models to explain variation in antibody status for avian influenza virus, West Nile virus, and avian paramyxovirus-1 in Blue-winged Teal (*Anas discors*) in the Canadian prairies, 2007–10 (n=1,846, 1,869, and 1,817, respectively). Model averaging of coefficient estimates was based on these sets.

Outcome variable[b]	Model	K[c]	AIC_c[d]	ΔAIC_c[e]	ω_i[f]
AIV-Ab	Age+Year+Head-bill length	6	1,834.19	0.00	0.74
	Age+Year	5	1,836.32	2.12	0.26
WNV-Ab	Age+Year+Pond density	6	579.16	0.00	0.69
	Age+Year+Province	7	580.72	1.56	0.31
APMV-1-Ab	Age+Year	5	832.78	0.00	0.78
	Age	2	835.27	2.49	0.22

[a] Confidence set: models within 4 ΔAICc from top model not including models with noninformative variables (see Supplementary Material for full set of models).

[b] AIV-ab = avian influenza virus antibody status; WNV-ab = West Nile virus antibody status; APMV-1-ab = avian paramyxovirus-1 antibody status.

[c] K = number of parameters in the model.

[d] AIC_c = Akaike's Information Criterion adjusted for small sample size.

[e] ΔAIC_c = difference between AIC_c of the best model and each competing model.

[f] ωi = Akaike weight (the probability that the model is the best).

consideration (Supplementary Material Table S6; Tables 4, 6). The AHY birds were 6.82 times more likely to be WNV antibody positive compared to HY birds (3.43–13.54; Table 6). There was slight annual variation in WNV antibody prevalence, which was lowest in 2010. Blue-winged Teal were 1.15 times more likely to be WNV antibody positive for every unit increase (pond/km^2) in spring pond density (1.07–1.23; Table 6), which explained more variation in WNV antibody status compared to measures of population density (Table S6). In the second ranked model which included province instead of pond density (Table 4), birds sampled in Saskatchewan were 2.75 times more likely to have antibodies to WNV compared to those in Alberta (1.61–4.69), likely because Saskatchewan had higher pond densities than the other provinces (ANOVA, $F_{2,9}$=14.11, P=0.002). Province and pond density could not be entered into the same model given this association. Sex and head-bill length had no initial association with WNV antibody status (results not shown). Although AIV and APMV-1 antibody status had initial associations with WNV antibody status, once age was included, these effects were no longer present (data not shown).

Risk factors affecting APMV-1 antibody status

Overall prevalence of APMV-1 antibody was 6.3% (5.3–7.5) (Table 2). Of 115 birds with APMV-1 antibody, 16 (13.9%, 8.7–21.4) were infected with AIV, 71 (61.7%, 52.6–70.1) were positive for AIV antibody, and 9 (7.8%, 4.2–14.2) were WNV antibody positive. Model averaged parameter estimates to explain variation in APMV-1 antibody status were based on two models that merited consideration (Supplementary Material Table S7; Tables 4, 7). The probability that a bird had antibodies to APMV-1 was 2.64 (1.68–4.14) times higher in AHY birds compared to HY birds and influenced by year of collection, with 2009 having the highest apparent antibody prevalence (Table 7). Density variables, sex, head-bill length, and BCI were not associated with APMV-1 antibody status (results not shown). Although presence of WNV and AIV antibodies had initial associations with APMV-1 antibody status, once age was included, this effect was no longer present (data not shown).

DISCUSSION

We identified demographic and ecologic factors that may explain infection or

TABLE 5. Model averaged maximum likelihood estimates of variables explaining variation in avian influenza virus antibody status (as measured by competitive enzyme-linked immunosorbent assays) in Blue-winged Teal (*Anas discors*) sampled in the Canadian prairies, 2007–10 (n=1,846).

Variables[a]	β[b]	SE	95% CI[c]
Age (ref=HY)			
AHY	2.86	0.14	2.58, 3.13
Year (ref=2007)			
2008	−0.21	0.18	−0.56, 0.14
2009	−0.75	0.20	−1.14, −0.36
2010	−0.36	0.16	−0.67, −0.05
Head-bill length	0.04	0.02	0.001, 0.08
Intercept	−3.71	1.94	−7.51, 0.09

[a] ref = reference category; HY = hatch year; AHY = after hatch year.

[b] β = coefficient estimate.

[c] CI = 95% confidence interval of β estimates.

TABLE 6. Model averaged maximum likelihood estimates of variables explaining variation in West Nile virus antibody status (based on competitive enzyme-linked immunosorbent assays confirmed by plaque reduction neutralization) in Blue-winged Teal (*Anas discors*) sampled in the Canadian prairies, 2007–10 (n=1,869).[a]

Variables[b]	β[c]	SE	95% CI[d]
Age (ref=HY)			
AHY	1.92	0.35	1.23, 2.6
Year (ref=2007)			
2008	−0.36	0.34	−1.03, 0.31
2009	−0.35	0.37	−1.07, 0.37
2010	−1.43	0.44	−2.29, −0.56
Pond density	0.138	0.037	0.06, 0.21
Province (ref=SK)			
AB	−1.013	0.272	−1.54, −0.48
MB	−0.412	0.460	−1.31, 0.849
Intercept	−4.80	1.00	−6.76, −2.84

[a] Model averaging was based on the two best supported models listed in Table 4. Pond density and province were not entered in the same model.

[b] ref = reference category; HY = hatch year; AHY = after hatch year; SK = Saskatchewan; AB = Alberta; MB = Manitoba.

[c] β = coefficient estimate.

[d] CI = 95% confidence interval of β estimates.

exposure to AIV, WNV, and APMV-1 in BWTE in the three Prairie Provinces of Canada. Avian influenza virus infection in BWTE was associated with age and antibody status, BWTE density, and year. Similar to AIV, the presence of antibodies to WNV and APMV-1 was associated with age and year, and WNV was positively associated with pond density rather than host population density.

Avian Influenza Virus

Numerous studies have investigated AIV infection in wild migratory birds, and recent studies have examined both infection and serologic data (Hoye et al. 2011; Verhagen et al. 2012; Latorre-Margalef et al. 2013; Tolf et al. 2013). Similar to another study in BWTE (Nallar et al. 2015) and to studies in other species (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 lack of prior exposure. The latter is supported by our finding that adults were >17 times more likely to have antibodies to AIV nucleoprotein compared to HY birds. A unique finding in our study was the interactive effects of age and antibody status on AIV 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 current infection. This is plausible because 3-mo-old Mallard ducks experimentally challenged with LPAIV shed virus continuously for 12 d postinoculation (±1 d) and intermittently for an additional 3.7 d (±3.1 d), while producing detectable antibodies 1 wk postinoculation (Jourdain et al. 2010). Although AHY birds were more likely to have antibodies to AIV compared to HY birds, and were less likely to be infected compared to HY birds, there was no difference in AIV infection status between antibody-positive and antibody-negative AHY birds. Even among antibody-negative birds, AHY birds were less likely to be infected than HY birds. It is unclear whether this apparently higher resistance to infection in antibody-negative adults was due to humoral or other forms of immunity (e.g., cell-mediated immunity) important in protection against influenza viruses (Haussmann et al. 2005; Thomas

TABLE 7. Model-averaged maximum likelihood estimates of factors explaining variation in avian paramyxovirus-1 antibody status (as measured by hemagglutination inhibition) in Blue-winged Teal (*Anas discors*) sampled in the Canadian prairies, 2007–10 (n=1,817).

Variables[a]	β[b]	SE	95% CI[c]
Age (ref=HY)			
AHY	0.97	0.23	0.52, 1.42
Year (ref=2007)			
2008	0.14	0.32	−0.47, 0.76
2009	0.79	0.31	0.19, 1.38
2010	0.33	0.30	−0.24, 0.91
Intercept	−3.52	0.26	−4.03, −3.01

[a] ref = reference category; HY = hatch year; AHY = after hatch year.

[b] β = coefficient estimate.

[c] CI = 95% confidence interval of β estimates.

et al. 2006), and further studies are required to understand immunity to AIVs.

We did not find an association between sex and AIV infection. The role attributed to sex varies among studies, with some showing either males (Parmley et al. 2008; Farnsworth et al. 2012) or females (Runstadler et al. 2007) to have a higher probability of infection, and others, like ours, showing no effect of sex (Ferro et al. 2010; Soos et al. 2012). We found an association between head-bill length and AIV antibody status (Table 5), which may have been related in part to sex, given that males are larger than females. Sex was, however, a noninformative variable when combined with year and age in a candidate model. Further studies of larger sample sizes of both sex and age classes or experimental studies would be needed to clarify the importance of size or sex in relation to AIV antibody status.

Higher apparent prevalences were observed in 2007 and 2010 than in 2008 and 2009, which may suggest 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 2–3 yr (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 antibody prevalence (2009), possibly due to low exposure in the previous year (Figs. 2, 3). This was followed by the year with the highest apparent prevalence of infection in our study (2010), possibly resulting from the comparatively low proportion of individuals with evidence of AIV-specific immunity in the previous year (Fig. 3). Longer-term studies would be required to determine whether this cyclical pattern is similar in subsequent years.

Avian influenza virus 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. (2012) found a similar association in Africa, we believe this is the first report of a link between AIV infection and regional waterfowl breeding population density in North America. Higher waterfowl densities in a given wetland will result in higher concentrations of fecal material and higher contact rates among birds, increasing frequency of contacts between susceptible birds and virus. Total duck density was not associated with AIV infection, suggesting that BWTE are more likely to be exposed from conspecifics, possibly through more frequent interactions with each other compared with other species.

West Nile Virus

The ecology of WNV in migratory waterfowl is poorly understood. Numerous species of waterfowl 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). To our knowledge, this is the first study examining prevalence of WNV antibody in free-ranging waterfowl in Canada. The probability of detecting antibodies to WNV was influenced by age, pond density, and year (Table 6).

After-hatch-year birds were 6.8 times more likely to be WNV antibody positive than to HY birds, reflecting the increased probability of exposure to WNV with age, and providing evidence that at least some BWTE can survive WNV infection. The lowest prevalence of WNV antibody in BWTE was in 2010, 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 predictor for WNV antibody status in BWTE. Furthermore, the highest antibody prevalence was in Saskatchewan, likely because it had the highest pond density. Larger numbers of ponds in a given area may increase the amount of suitable developmental habitat for mosquitoes, the primary vectors. Not all wetlands are suitable for mosquito reproduction and development. For example, in South Dakota, a positive association was found between wetland density and *Aedes vexans*, although a similar relationship was not found for *Culex tarsalis* (Chuang et al. 2011). Temporary or semipermanent wetlands are likely more important as developmental sites for 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 needs to incorporate other parameters such as temperature, timing and patterns of rainfall, and seasonal patterns of mosquito population dynamics (Chen et al. 2013).

APMV-1

There is limited information on the ecology of APMV-1 in BWTE. Overall antibody prevalence appeared low each year, similar to other studies that showed low infection rates for APMV-1 in BWTE and low infection rates or antibody prevalence in other dabbling duck species in North America and Europe (Vickers and Hanson 1982; Stallknecht et al. 1991; Goekjian et al. 2011). Similar to our AIV and WNV serology results, APMV-1 antibody prevalence was significantly higher in AHY birds compared to HY birds, probably due to adults having had more opportunities for exposure to APMV-1. Antibodies are likely 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 APMV-1 antibody prevalence observed in 2009 and little difference between 2007, 2008, and 2010. Though this temporal pattern appeared opposite to that observed with AIV antibody prevalence, which was lowest in 2009, there was no relationship between AIV-specific antibody status and APMV-1-specific antibody status in our best-supported models.

Our results provide new insight into ecologic risk factors associated with infection with and exposure to infectious agents 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 and population levels, all of which may impact dynamics of infectious diseases. This information can be used to evaluate risks to wildlife, domestic animal, and human health and to inform wildlife disease surveillance or management programs for these or other viruses carried by migratory waterfowl.

ACKNOWLEDGMENTS

We thank Environment Canada (J. Leafloor, D. Walker, F. Baldwin) and US Fish and Wildlife Service crews (P. Thorpe, W. Rhodes, J. Wortham, J. Goldberg) for assistance with waterfowl capture and banding. We are grateful to H. Remenda, A. Allbright, A. Murray, J. Verhagen, M. Lux, M. Domoslai, K. Halford, W. van Dijk, T. Santander, and many volunteers who helped in the field and lab. We thank A. Dibernardo and M. Leith for technical assistance for WNV and AIV serology, respectively. Canadian Wildlife Health Cooperative provided AIV

Survey protocols, sampling materials for AIV PCR analyses, and coordination of RT-PCR sample analyses. R. Clark and C. Waldner provided statistical advice, and K. Meeres provided the map for Figure 1. We thank T. Bollinger, K. Hobson, M. Samuel, and anonymous reviewers for their valuable comments on previous versions of the manuscript. We acknowledge the Waterfowl Breeding Population and Habitat Survey for use of data. This research was funded by (in alphabetic order) Alberta Conservation Association, Canada's Inter-agency Wild Bird Influenza Survey, Canadian Food Inspection Agency, Ducks
Unlimited Canada's Institute for Wetland and Waterfowl Research, Environment Canada, Public Health Agency of Canada, University of Saskatchewan, US Department of Agriculture-Animal and Plant Health Inspection Service, and Wildlife Conservation Society (Graduate Fellowship Program).

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2013-07-191.

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Submitted for publication 25 April 2013.
Accepted 9 July 2015.