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RISK FACTORS ASSOCIATED WITH WEST NILE VIRUS MORTALITY IN AMERICAN CROW POPULATIONS IN SOUTHERN QUEBEC

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ABSTRACT: Soon after the appearance of West Nile virus (WNV) in North America, a number of public health authorities designated the American Crow (*Corvus brachyrhynchos*) a sentinel for WNV detection. Although preliminary studies have suggested a positive association between American Crow mortality and increased risk of WNV infection in humans, we still know little about dynamic variation in American Crow mortality, both baseline levels and mortality associated with WNV. We hypothesized that the complex social behavior of American Crows, which is shaped by age and seasonal factors, influences both baseline mortality and WNV mortality in American Crow populations. We examined American Crow mortality data from Quebec for the 2005 WNV surveillance year, which lasted from 5 June to 17 September 2005. The variables of interest were age, gender, body condition index, time of year, and land cover. We used a log-linear model to examine baseline mortality. Logistic regression and general linear regression models were constructed to examine variables associated with mortality due to WNV. We found that both age and time of year were key variables in explaining baseline mortality. These two variables were also risk factors for WNV mortality. The probability that a carcass tested positive for WNV increased with the age of the dead bird and as summer progressed. WNV-positive carcasses also had a lower body condition index than WNV-negative carcasses. We believe that the first major wave of American Crow mortality observed in the early summer of 2005 was the result of natural mortality among young American Crows. Because this mortality was not linked to WNV, it appears that American Crow may not be a good species for early detection of WNV activity. Our data also suggest that second-year American Crows play a major role in propagating WNV during their movements to urban land covers during midsummer.

Key words: American Crow, baseline mortality, population dynamics, risk factors, West Nile virus mortality.

INTRODUCTION

In 1999, West Nile virus (WNV) made its first appearance in North America in New York State (Marfin et al., 2001; Ludwig et al., 2002; McLean et al., 2002). It struck immunologically naïve populations, resulting in widespread mortality in birds and mammals (Kulasekera et al., 2001). The epidemiologic cycle of the virus requires an arthropod vector, generally a mosquito, and a reservoir. Birds are the most commonly suspected reservoir; however, mammals could also play this role (Root et al., 2006). Research to date suggests that the most sensitive bird species are passerines, as illustrated by their high mortality rate when infected experimentally (Komar et al., 2003; Meulen et al., 2005). This means that passerines could potentially act as sentinels for WNV in North America.

The American Crow (*Corvus brachyrhynchos*) is highly susceptible to WNV infection, and although this species has a lower index of reservoir competency for WNV transmission than does the Blue Jay (*Cyanocitta cristata*), Common Grackle (*Quiscalus quiscula*), or House Finch (*Carpodacus mexicanus;* Komar et al., 2003), it has been selected as the best sentinel for WNV detection for several reasons. First, American Crows are found in high densities throughout North America (Gauthier and Aubry, 1995; Verbeek and Caffrey, 2002), especially in urban land covers (Marzluff and Neatherlin, 2006). Second, they are large in size so that their carcasses are more easily located (Caffrey et al., 2003). Finally, the mortality

rate associated with infection is very high in this species (Komar, 2001; McLean et al., 2001; Julian et al., 2002; Komar et al., 2003; Watson et al., 2004).

Despite the proximity of Ontario to New York State, the virus was only first detected in dead birds in Ontario in 2001 (Public Health Agency of Canada, 2001). The arrival of WNV in Canada resulted in high mortality in both passerines (Barker and Campbell, 2003) and nonpasserines, including owls (Gangz et al., 2004). It also caused 394 cases of morbidity in humans in 2002 (Public Health Agency of Canada, 2004). The virus arrived in Quebec in 2002, causing encephalitis in 20 people (Brown and Dallaire, 2002; Public Health Agency of Canada, 2007). The government implemented a surveillance program for WNV detection in Quebec in the summers of 2003, 2004, and 2005. This program encompassed human, mosquito, and corvid surveillance (Vincent et al., 2003; Vincent and Brown, 2004; Brown et al., 2005).

For this study we analyzed American Crow carcasses recovered by the surveillance program in 2005. The behavior of the American Crow is complex, involving many migratory movements both within and between seasons (Marzluff et al., 2001b; McGowan, 2001; Marzluff and Neatherlin, 2006). We hypothesized that WNV circulation among American Crows is influenced by the social behavior and migratory movements of American Crows and that this would be reflected in a risk factor analysis of mortality due to WNV.

The objectives of this study were to 1) characterize the dynamics of baseline mortality in American Crow populations based on the recovery of WNV-negative American Crow carcasses; 2) characterize the dynamics of WNV mortality based on the recovery of WNV-positive American Crow carcasses; and 3) analyze the factors associated with WNV mortality in American Crow populations in southern Quebec, Canada.

MATERIALS AND METHODS

Sample collection and characterization

American Crow carcasses were collected as part of the provincially run WNV surveillance program in Quebec (Brown et al., 2005). The sample consisted of 332 Crow carcasses collected between 5 June and 17 September 2005. These carcasses had been found by the public and then picked up by wildlife conservation officers, packed in an icebox, and sent to the laboratory of the Canadian Cooperative Wildlife Health Centre in Saint-Hyacinthe, Quebec. Only birds in reasonable condition were sent to the laboratory.

The WNV status of each of the 332 carcasses was determined by taking an oral swab (Brown et al., 2005) and then testing it using VecTest® (Medical Analysis Systems Inc., Fremont, California). This test has been shown to have relatively good specificity (between 79% and 100%) and sensitivity (between 70.4% and 92.8%) (Lindsay et al., 2003; Stone et al., 2004, 2005; Padgett et al., 2006).

We assumed that the discovery date of the carcass was a reasonable approximation of the actual date of death because American Crow carcasses remain visible on the ground for only a very short period: 1.6 days in rural land covers and 2.1 days in urban land covers (Ward et al., 2006). The time of year was defined for all the 332 carcasses (Fig. 1).

Four times of the year (T) were used to examine the association between mortality and time of year: T1 (5 June to 2 July), T2 (3 July to 23 July), T3 (24 July to 27 August), and T4 (28 August to 17 September). We selected these times of the year based on American Crow biology (Verbeek and Caffrey, 2002; Withey and Marzluff, 2005) and on visual observation of the temporal variations of the age-specific mortality during the 2005 surveillance season (Fig. 2). T1 corresponds to when fledglings make their first flight, and T2 coincides with the period during which hatch year birds and auxiliaries move to urban land covers (Kilham, 1984). Auxiliaries are birds that forego breeding but are associated with a breeding territory in either a helping or nonhelping capacity; they are usually second-year birds (Verbeek and Caffrey, 2002). During T3 there is a spatial division of the population: adult birds stay on their breeding territories, which are often in rural land covers, and hatch-year birds and auxiliaries move to urban land covers where food is easier to obtain (Caffrey, 1992; Marzluff and Neatherlin, 2006). During T4 American Crows of all ages flock together in

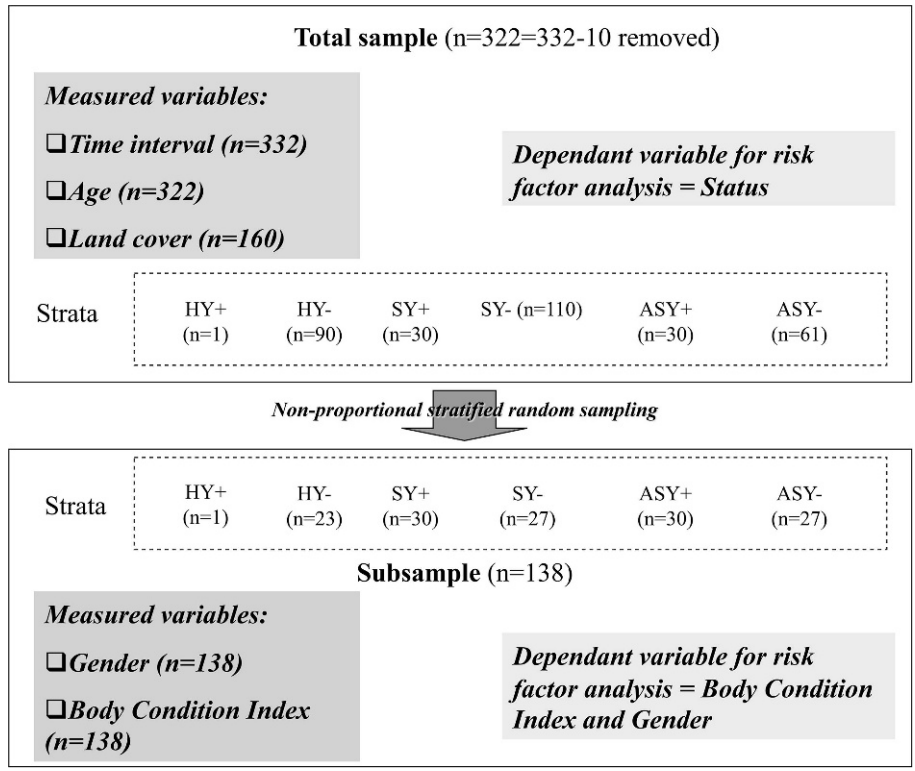

FIGURE 1. Sampling protocol for risk factors analysis of the crow carcasses collected in southern Quebec in summer 2005. The targeted risk analysis concerns the association of WNV-positive carcasses with the measured variables.

rural or suburban land covers in preparation for autumn migration.

We classified the carcasses according to three age classes. Ten of the 332 carcasses could not be classified because of their poor condition (Fig. 1). The classification was based on oral mucous membrane coloration, which changes from pink to black as the bird ages (Pyle, 1997). The three age classes were hatch year (HY), i.e., American Crows that hatched in 2005; second year (SY), i.e., American Crows that hatched in 2004; and after second year (ASY), i.e., American Crows that hatched in 2003 or earlier. To simplify our model and the expected behavior for each age class, we assumed that all SY birds are auxiliaries and all ASY birds are reproductive adults.

It was not possible to determine gender and take morphologic measurements for all 322 carcasses because of the limited availability of bio-safety level 3 facilities. We therefore selected 138 carcasses from the total of 322 carcasses using stratified nonproportional random sampling (Fig. 1). The stratification generated six combinations based on age and WNV infection status: HY positive, HY negative, SY positive, SY negative, ASY positive, and ASY negative. To ensure reasonable statistical precision in further analyses, we used all WNV-positive carcasses: one for HY, 30 for SY, and 30 for ASY. We randomly selected carcasses for the HY, SY, and ASY negative groups, yielding final sample sizes for negative birds of 23 for HY, 27 for SY, and 27 for ASY.

We determined the gender of each bird using abdominal cavity exploration during necropsy. We also took a variety of morphologic measurements: wing length, tail length, tarsus length, head-to-bill length, bill length, bill depth, bill width, and weight. Details on the measurement techniques can be found in the literature (Pyle, 1997).

Using the morphologic measurements of the 138 sample carcasses (Fig. 1), we calculated the body condition index (BCI) using a proposed method in which BCI is the body mass relative to the structural size of the carcass (Brown, 1997). This method enabled us to compare the body condition of American Crows of different sizes. BCI values are the raw residuals (BCI=predicted value−ob-

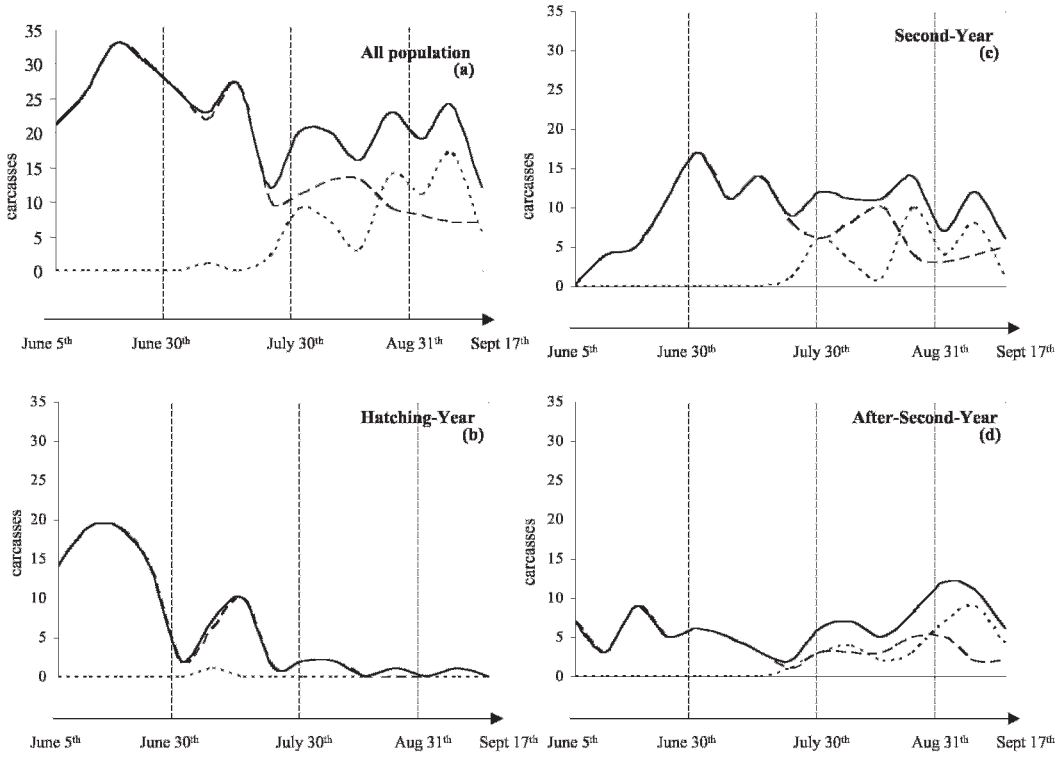

FIGURE 2. Temporal fluctuations in age-specific American Crow mortality in summer 2005 based on data from the West Nile virus surveillance program. The dotted line shows WNV-positive carcasses, the dashed line WNV-negative carcasses, and the solid line both WNV-positive and WNV-negative carcasses. The curves correspond to the weekly sum of negative and/or positive carcasses recovered. (a) All carcasses ($n=332$), (b) hatching-year carcasses ($n=144$), (c) second-year carcasses ($n=96$), and (d) after-second-year carcasses ($n=92$). The smooth curve has been built using the "by-default" smoothing algorithm used in Excel (Microsoft, Redmond, Washington, USA).

served value) of a general linear model predicting weight using a structural dimension measure, which in this case was head-to-bill length. We created the general linear model in SAS using Proc GLM (SAS version 9.1, SAS Institute Inc., Cary, North Carolina, USA).

Each carcass of the 332 carcasses was assigned the geographic coordinates of the civic address of the location where it was discovered. We assumed that these coordinates were a reasonable approximation of the geographic area in which the bird lived.

Because the land use data available covered an area smaller than the total study area, we determined land cover attribute for 160 of the 332 carcasses located in an area around Montreal (Quebec) within the following coordinates: $45°0' < \text{latitude} < 46°6'$ and $-74°2' < \text{longitude} < -72°17'$ (Fig. 1). Only 10% of the study area corresponded to urban land cover. The land cover classification in two categories (urban and nonurban) was derived from a methodology employed in a previous study (Marzluff et al., 2001a).

We used two different sources of spatial data for classifying land covers as urban or nonurban: CanMap Streetfiles (DMTI_Spatial, 2005; vectorial data) and LandSat (Savoie et al., 2005; pixel size 30 m×30 m). Land covers designated commercial, government/institutional, residential, or resource/industrial in CanMap were classified as urban, whereas open area, park and recreational location, or water body were classified as nonurban. Where CanMap data were not available, we used LandSat. Land covers designated as annual culture, permanent culture, forest, regeneration, recently cut forest, water, wetland, or peat bog in LandSat were classified as nonurban in our scheme, and land covers designated as urban in LandSat were likewise classified as urban in our scheme. The spatial

analyses for classification of land cover were performed using ArcGIS (ArcGIS Desktop 9.2, ESRI 2006; ESRI, Redlands, California).

We calculated the frequency of carcasses recovered (out of a total of 160 carcasses) for each age class, WNV status, and land cover to determine if some stratified groups had few or no observations.

Data analysis

We first analyzed weekly frequencies for positive, negative, and total collected American Crow carcasses over the surveillance period ($n=332$). We then examined frequencies for total collected carcasses and for positive carcasses by gender, age, and time of year ($n=138$). The variables were selected to explore the risk of quasi-complete separation of the data in our further analysis (stratum with very few or no observation).

In this analysis baseline mortality corresponds to non WNV mortality. A log-linear model was used to explore the effects of time of year and age (categorical variables) on the number of WNV-negative carcasses recovered during the surveillance season ($n=262$). The model included both main effects as well as their interaction. Some specific comparisons were made (HY versus ASY for T1, HY versus SY for T1, and HY for T1 versus ASY for T4). We generated 95% confidence intervals for the odds ratio (OR) of these estimates using Wald's method (Dohoo et al., 2003b). The effect of land cover (urban versus nonurban) on number of WNV-negative carcasses could not be examined because of the absence of observations for land cover. The effects of gender and BCI could not be examined because of the stratified nonproportional sampling strategy.

Using all recovered WNV-positive and WNV-negative carcasses, we constructed two simple logistic regression models to evaluate the unconditional association between age ($n=322$) and land cover ($n=160$) and the probability of a carcass testing positive for WNV (Fig. 1). We have called this association WNV-positive carcass probability. Confidence intervals of 95% for the odds ratio were generated using Wald's method (Dohoo et al., 2003b). Time-of-year effects could not be evaluated for the whole data set because of quasi-complete separation of the data with no WNV-positive carcasses being identified for T1.

A multiple logistic regression was also constructed to model WNV-positive carcass probability based on age and time of year ($n=139$). Land cover was not tested in the model because the preceding simple logistic regression found no significant relationship between land cover and WNV status ($P=0.1576$). The quasi-complete separation observed in the HY data, as well as in T1 and T2 data, caused a lack of convergence for parameter estimation using a traditional maximum likelihood estimation method (Dohoo et al., 2003a; Mather et al., 2007). Consequently, the analysis was restricted to the SY and ASY age classes and to times of the year T3 and T4 to obtain meaningful coefficients. The model began with age, time of year, and the interaction between age and time of year. A backward selection of the variables was then performed, which removed variables with p of greater than 5%. We generated 95% confidence intervals for the odds ratio using Wald's method (Dohoo et al., 2003b).

A multiple logistic regression model for predicting gender based on age, WNV status, and the interaction between age and WNV status was constructed. For this model it was not possible to retain the HY age class in the analysis because of the quasi-complete separation of the data, because there were no WNV-positive HY carcasses in the subsample where gender was estimated. In this analysis ($n=138$), the proportions of carcasses by WNV status groups are determined by the sampling protocol, and therefore the status cannot be put as the dependant variable (Fig. 1). We also evaluated variation in mean BCI values between all age, WNV status groups, and the interaction between age and WNV status using a general linear model ($n=138$).

We built the general linear model using Proc GLM in SAS and performed the log-linear analysis using Proc GENMOD in SAS. The simple logistic regressions were performed using Proc LOGISTIC in SAS, and the multiple logistic regressions using Proc GENMOD in SAS.

RESULTS

Data description

Fluctuations in occurrence of positive and negative carcasses are shown in Figure 2. A total of 69 WNV-positive and 263 WNV-negative carcasses were collected during the surveillance period. At the launch of the surveillance program, the frequency of carcass recovery was high: approximately 25 carcasses/wk. The first wave of carcasses found (during T1 and T2) were almost exclusively WNV negative

(Fig. 2a). Only one was WNV positive; it was recovered on 11 July. During T2 and T3, the frequency of negative carcasses decreased, whereas the number of positive carcasses increased.

Figure 2 also includes temporal changes in the frequency of carcasses recovered for each age class. Figure 2b illustrates the frequent mortality among WNV-negative HY birds during T1 and T2. This early mortality wave had a bimodal shape. There was a considerable decrease in the number of HY-negative carcasses recovered after 24 July.

In Figure 2c the temporal changes in the frequency of WNV-negative SY carcasses recovered are represented by a bimodal curve, with the first wave ending at the end of T2. The bimodal shape of this curve is very similar to that found for the entire population (Fig. 2a). For SY birds the first wave of mortality for positive carcasses ended in the middle of T3, and the second wave with two peaks ended at the end of T4.

In Figure 2d the frequency curve for negative ASY carcasses is approximately bimodal in shape. The first wave occurred during T1 and T2, and the second wave took place during T3 and T4. The first positive ASY birds were recovered on 24 July, and the frequency curve for subsequent dates is very similar to that for positive SY birds, but with a 2 wk delay in the peak dates.

Table 1 displays the frequency distribution for both WNV-positive and total carcasses according to age, gender, and time of year. The frequencies correspond to the stratified nonproportional sample of carcasses from which gender and BCI were determined (Fig. 1).

TABLE 1. Frequency distribution of American Crow carcasses according to gender, age class, and time of year. Numbers are given for all carcasses and for WNV-positive carcasses.

Gender	Age group	Time of year	All carcasses	WNV-positive carcasses %
Times of the Year 1 and 2				
Male	HY	1	14	0
		2	4	0
	SY	1	3	0
		2	4	0
	ASY	1	5	0
		2	5	0
Female	HY	1	3	0
		2	1	0
	SY	1	1	0
		2	5	0
	ASY	1	4	0
		2	1	0
Times of the Year 3 and 4				
Male	HY	3	1	100
		4	0	0
	SY	3	22	63.6
		4	7	85.7
	ASY	3	9	55.6
		4	13	84.6
Female	HY	3	1	0
		4	0	0
	SY	3	10	60
		4	5	80
	ASY	3	8	62.5
		4	12	75

Factors associated with baseline mortality

In the log-linear analysis of number of WNV-negative carcasses, all of the variables examined (age, time of year, and the interaction between age and time of year) were very significant ($P<0.0001$).

Contrast estimates for T1 also show that negative HY carcasses were more numerous than negative ASY carcasses (OR=2.75 [1.72; 4.38]) or negative SY carcasses (OR=3.30 [2.00; 5.44]; Table 2). Negative HY carcasses during T1 were also more numerous than negative ASY carcasses during T4 (OR=7.33 [3.65; 14.71]; Table 2).

Risk factors for WNV-positive mortality

In the simple logistic regression, the age effect was found very significant. Young age may be a protective factor ($OR_{\text{HY versus ASY}}=0.042$ [0.010, 0.180] with $P<0.0001$, and $OR_{\text{SY versus ASY}}=0.581$ [0.328, 1.028] with $P=0.0622$). The variable land cover was not significant ($OR_{\text{nonurban versus urban}}=0.753$ [0.387, 1.467] with $P=0.4048$). Table 3 shows the frequency distribution for both positive and negative carcasses according to age and land cover.

TABLE 2. Evaluation of age class and time of year as variables associated with the baseline mortality of Crows. The loglinear model predicts the WNV-negative mortality frequencies.

Variable	β	Std. error	*P*-value
Age class (ref.: ASY)			
HY	−0.5121	0.1964	0.0091
SY	0.5160	0.1264	<0.0001
Time of year (ref.: T4)			
1	0.7700	0.1344	<0.0001
2	0.4046	0.1432	0.0047
3	−0.0508	0.1688	0.7634
Age class × time of year (ref.: ASY in T4)			
HY in T1	1.2473	0.2169	<0.0001
SY in T1	−0.9747	0.1739	<0.0001
HY in T2	0.3134	0.2371	0.1862
SY in T2	0.1327	0.1683	0.4306
HY in T3	−0.5121	0.2971	0.0848
SY in T3	0.4339	0.1923	0.0240

Table 4 presents the results of the multiple logistic regression model (T1, T2, and HY were not included in this analysis). The reduced model contains the two variables age class ($P=0.14$) and time of year ($P=0.04$). The interaction between age and time of year ($P=0.66$) was eliminated because not significant. The odds ratio associated with the reduced model indicates that the probability of being WNV positive is lower for a carcass reported during T3 than during T4. Belonging to the second-year age class versus the after-second year age-class does not appear to be a risk factor (95% OR Wald CI contains the value 1).

The effects of BCI and gender according to age class and WNV status

According to the general linear model, there were no differences in mean BCI between the three age classes ($P=0.58$). Mean BCI was lower for WNV-positive carcasses than for WNV-negative carcasses ($P<0.01$). The interaction between age and WNV status was not statistically significant ($P=0.27$), indicating that the differences in BCI between the WNV status categories were similar across the three age classes.

The logistic regression was built using WNV status and two age classes (SY and ASY) to predict the proportion of carcasses that were female. The results were not significant for age ($P=0.47$), WNV status ($P=0.92$), and the interaction term between age class and status (P ranging from 1.00 to 0.29). Therefore, these variables did not account for differences in the number of male versus female carcasses in the sample.

TABLE 3. Frequency distribution of American Crow carcasses according to age class, WNV status, and land cover (urban versus nonurban).

	Hatch year		Second year		After second year		
	WNV+	WNV−	WNV+	WNV−	WNV+	WNV−	Total
Urban land cover	0	18	11	20	16	12	77
Nonurban land cover	1	18	16	24	7	17	83
Total	1	36	27	44	23	29	160

TABLE 4. Evaluation of age class and time of year as a risk factor in the reduced logistic regression model for predicting positive WNV status. The full model included age (SY and ASY), time of year (3 and 4), and the interaction between age and time of year ($n=139$).

Variable	β	Std. error	*P*-value	95% Wald CI	OR
Second year age class	−0.5272	0.3600	0.1431	0.291,1.195	0.590
After second year age class	0.00				1.00
Time of year 3	−0.7608	0.3638	0.0365	0.229,0.953	0.467
Time of year 4 (ref.)	0.00				1.000
Intercept	0.7035	0.3324	0.0343		

DISCUSSION

Characteristics of baseline American Crow mortality

Understanding the baseline mortality variation in the American Crow population is a key knowledge for a better understanding of risk factors associated with WNV-positive status of the carcasses. Baseline mortality estimates are also important piece of information in the calculation of the proportion of carcasses positive to WNV.

The fledging period is a difficult time for HY birds because they often find themselves defenseless on the ground and at the mercy of predators because they have difficulty flying to safety in trees. According to many authors, the annual mortality rate for HY American Crows is in the range of 40% to 60% (Good, 1952; Chamberlain-Auger et al., 1990; Verbeek and Caffrey, 2002). A previous study has also found temporal variation in annual mortality rates toward the end of the breeding season in June and July (Good, 1952). In our study, high mortality was observed in the HY age class at the beginning of the surveillance season (Table 1 and Fig. 2). This WNV-negative mortality was likely associated with fledgling mortality, an annual occurrence during this period. Research has shown that if the first reproductive effort fails, an American Crow pair will breed again 3 to 4 wk after the first laying (Emlen, 1942; Good, 1952). In our study the peak of the second WNV-negative mortality for HY birds occurred between 17 July and 23 July (Fig. 2), about 3 to 4 wk after the first mortality wave. This is consistent with fledging mortality in second broods. It seems, therefore, that both of the mortality waves for negative HY birds seen in Figure 2 are due to natural annual phenomena.

Since the arrival of WNV in North America, many studies have been conducted to validate early American Crow mortality as an indicator of virus circulation (Eidson et al., 2001; Julian et al., 2002; Theophilides et al., 2003; Watson et al., 2004; Johnson et al., 2006). Several of these studies have concluded that nonspecific American Crow mortality at the beginning of or throughout the surveillance season is associated with an increased risk of WNV circulation among American Crows and humans living in the same region. This interpretation is not supported by our findings. Each year there is significant mortality of HY birds that is not associated with WNV circulation (Good, 1952). This finding also serves to highlight the importance of determining if there is a causal link between two associated variables, in this case American Crow mortality and increasing risk for WNV transmission to humans.

Intraseasonal variation in the mortality rates of SY and ASY age classes has previously been observed, but no explanations have been offered (Good, 1952). We also observed seasonal variation in mortality for SY birds, with the highest WNV-negative mortality frequencies occurring before 30 July (Fig. 2c). After this date, mortality decreased for the SY age

class. Unfortunately, carcass necropsy did not allow us to explore other causes of mortality, and it was not possible to associate this seasonal variation with any specific cause other than WNV.

It is interesting to note that the number of negative SY carcasses was generally twice as large as the number of negative ASY carcasses (Fig. 2). This suggests that baseline mortality is higher for SY American Crows than for ASY American Crows, a finding that is supported by the literature (Good, 1952; Verbeek and Caffrey, 2002).

For SY and ASY age classes, there were no gender differences in mortality rates associated with WNV-negative status. This is consistent with the literature, which does not report any gender differences in specific baseline mortality rates (Verbeek and Caffrey, 2002). However, the quasi separation of the data required us to exclude the HY age class from the analysis, and WNV-negative male carcasses were much more numerous than WNV-negative female carcasses (18 males versus five females). If this age class could have been included, perhaps the results would have indicated a larger number of male American Crows dying than females in the WNV-negative HY group.

Characteristics of WNV-positive American Crow mortality

Our data suggested that age and time of year are significant risk factors for WNV-positive carcass probability. It appears that WNV circulated in the American Crow population after 30 July, reaching SY birds before ASY birds. The simple logistic regression analysis showed that younger carcasses were significantly less likely to be WNV positive. Age was no more significant in the multiple logistic regression model, but this omission was likely due to the fact that data limitations prevented us from including the HY age class in the model. This reinforces our hypotheses that the protected age class against West Nile virus infection is the hatching-year age class, because, when HY are not considered in the risk analysis, the age class factor is no more significant.

Age can be considered a risk factor associated with individual characteristics. We had the expectation that the ASY population were more likely to have a higher proportion of resistant individuals compared with the other age classes because WNV had been around for a few years when the surveillance was conducted. However, in our study it appears that HY birds suffered much lower mortality due to WNV in comparison to SY and ASY birds, leading us to conclude that young birds are somehow protected against WNV infection. Previous studies have explored the impact of age on sensitivity to WNV. Two studies have shown that sensitivity to WNV increases with age: one on geese with an Israeli strain (McLean et al., 2002) and another on chickens with an Egyptian strain (Malkinson et al., 2001). Although both the Egyptian and Israeli strains closely resemble the NY99 strain present in North American, neither geese nor chickens are passerines (Ludwig et al., 2002; Meulen et al., 2005), which limits the comparability of these results to our findings. In 2002, during a WNV epidemic in an Ontario owl population, HY owls were more resistant to WNV than were adult owls (Gangz et al., 2004). Additionally, during the WNV surveillance program for corvids from 2004 to 2006 in Ontario, and for 2003 and 2004 in Quebec, the same phenomenon was observed: although HY carcasses were more numerous than SY and ASY carcasses, the proportion of HY carcasses testing positive for WNV was smaller than the proportion of combined SY and ASY carcasses testing positive (Vincent et al., 2003; Vincent and Brown, 2004; Barker, pers. comm.). Spatial segregation for age classes would not be a good explanation of this result because, in midsummer, both SY and HY American Crows are present in the same geographic area (Caffrey, 1992; Withey and Marzluff, 2005; Marzluff and

Neatherlin, 2006), and toward the end of the summer, ASY, SY, and HY American Crows gather together in rural or suburban land covers (Caffrey, 1992; Withey and Marzluff, 2005; Marzluff and Neatherlin, 2006). The behavior differences could lead the HY to be less exposed to the WNV than older age classes by occupying places less populated by mosquitoes. But as HY are supposed to have the same behavior as SY (Marzluff and Neatherlin, 2006) during the WNV season (summer), this last hypothesis seems questionable. This reinforces our conclusion that young American Crows are protected against WNV. This protection could be due to differences between the HY class and older age classes in physiology. The physiologic differences could come from maternally transmitted antibodies, but vertical transmission of WNV protection from mother to offspring has not been demonstrated to date. All the more so, the number of WNV-resistant female adult crows that could transmit antibodies to their nestlings would not be high enough to cause the large observed young protection effect. For that reason, the most reasonable hypothesis to explain the young protection effect would be a physiologic phenomenon as the reproductive trade-off hypothesis (young individuals are not sexually mature and can put more energy toward immunity, whereas older individuals must invest resources in sexual maturity).

Age can be considered a collective risk factor through the link between age and American Crow behavior. The wave pattern of temporal variations in mortality due to WNV is consistent with WNV spreading from one age class to another within the American Crow population (Fig. 2) with an amplification of the number of cases during the surveillance season (Table 4 shows that the risk for a carcass to be WNV positive is the highest at the end of the surveillance season). The gap of 1 to 2 wk between the WNV mortality peak in the SY and ASY age classes would suggest that the two age classes are spatially segregated. WNV appears to reach SY birds before ASY birds and during the time of year when SY are in urban land covers (Marzluff and Neatherlin, 2006). It can be speculated that the infection of SY birds occurs in urban land covers where American Crow densities are high, thereby facilitating circulation of the virus. The virus then propagates beyond urban land covers and to ASY populations because of the continuous movements between urban and nonurban land covers by vagrant American Crows (Stouffer and Caccamise, 1991; Caccamise et al., 1997). The proportion of vagrants in an American Crow population can be as high as four of 11 individuals and appears to change according to season and food source (Stouffer and Caccamise, 1991; Caccamise et al., 1997).

American Crow behavior during the summer season appears to be the result of a complex interaction between time of year, age, and land cover (urban versus nonurban). For this reason, it is impossible to discuss age or time of year without considering land cover. In Illinois a study conducted in 2002 demonstrated a strong association between SY birds and urban land covers (Yaremych et al., 2004b). It found that ASY birds were strongly associated with rural land covers during the summer and early autumn. In our study, we found that age and time of year, but not land cover, were significant factors. The chosen classification scheme of urban versus nonurban should reflect American Crow biology because it was directly inspired by a study of American Crows conducted in New York State (Marzluff et al., 2001a, 2001b). However, certain measurement errors may have been introduced and prevented us from detecting significant associations between age class and land cover. For example, there may have been measurement errors associated with our definition of land cover, the use of spatial data, or classification of carcasses by land cover. Perhaps

a land cover classification scheme based on the single coordinates of the civic address where a carcass is found should be replaced by one based on the spatial characteristics of a buffer zone around the carcass. Likewise, the existence of a high proportion of vagrant American Crows in the population from which our carcasses were sampled (Stouffer and Caccamise, 1991) could lead to errors in the land cover classification of American Crows that are not vagrant by dilution of the nonvagrant American Crow association with a certain type of land-cover effect.

According to our results, WNV-positive carcasses had lower BCI than WNV-negative carcasses. This lower BCI could have been caused by dehydration or poor body condition after WNV infection. The high sensitivity of American Crows to WNV, the high rate of mortality following WNV infection, and the swiftness of death-approximately seven days post-infection (Ludwig et al., 2002; Komar et al., 2003)—could cause major dehydration or a lowered BCI in the infected American Crow.

The low BCI could also have preceded WNV infection. A low BCI often signifies chronic disease and the resulting loss of efficient immune response, and/or a predisposition to develop another disease (in this case, disease from WNV infection).

Since 1999, the number of WNV-serologically positive live American Crows has risen from 10% to 50% (Ringia et al., 2004; Bell et al., 2006; Gibbs et al., 2006). This signifies nonfatal contact and the development of individual resistance to WNV, which would diminish mortality due to positive WNV status. An increasing resistance to the virus within the population should be associated with an increasing level of seropositivity for the virus. At present, the proportion of WNV-seropositive American Crows in Quebec is unknown.

Gender was not identified as a risk factor associated with WNV-positive status in American Crow carcasses, a finding that is consistent with the literature (Yaremych et al., 2004a).

Limits imposed by the surveillance system

There are various limitations associated with the data that we used and the accuracy with which WNV-positive carcass probability reflects the actual risk of a live American Crow dying from WNV. The mortality is age dependent (Good, 1952; Chamberlain-Auger et al., 1990; Verbeek and Caffrey, 2002). Therefore, the proportion of carcasses in the sample in each age group is not necessarily representative of the age classes in the living American Crow population. As an example, a lower baseline mortality rate in a specific age class could lead to a biased higher WNV-positive carcass probability. The second situation involves the fact that the surveillance system relies on voluntary phone calls (Vincent et al., 2003); consequently, the reporting probability is associated with human density that are obviously higher in urban than nonurban land covers. Therefore, the number of dead American Crows reported in urban land covers may be artificially higher than in nonurban land covers. However, this bias should not affect WNV-positive carcass probability as long as it affects both WNV positive and WNV-negative carcasses with the same strength. In the same way, that the reporting drops off as the season progresses should not affect the results of this study as long as it is not associated with WNV status group. The third situation involves the sensitivity of VecTest® that has been measured at between 78.4% and 92.8%, which is less than ideal (Lindsay et al., 2003; Stone et al., 2004; Stone et al., 2005; Padgett et al., 2006). This weak sensitivity leads to a general underestimation of the number of positive carcasses. But if the factors linked to variation in sensitivity are associated with age or time of year, the tested variables in our analysis, the underestimation of the true WNV-positive carcasses would lead to a confusing bias in comparing WNV-positive

carcass probabilities. However, the VecTest detects WNV antigens, and genetic variation of WNV is very slow (Grinev et al., 2008), so the test sensitivity should not change according to time of year in our data set covering one single surveillance season in a limited geographic area.

The above observations also apply to differences between the proportion of living American Crows that die and test negative for WNV under the surveillance system and the proportion of carcasses reported by the surveillance system that test negative for WNV.

In conclusion, this work has enabled us to further explore and validate some of the risk factors shaping baseline mortality and mortality associated with WNV infection in American Crow populations. Age and time of year appear to be fundamental variables associated with baseline mortality. Plots of fluctuations in the frequency of WNV-positive carcasses recovered and the statistical analysis revealed that age and time of year were also strongly associated with positive WNV status of carcasses. Age appears to be a behavioral risk factor with different behaviors associated with different age classes. It is also an individual risk factor: younger birds suffer greater mortality but mortality that is not associated with WNV. For those reasons American Crows may not be an ideal sentinel because early in the season, high American Crow mortality is due to natural mortality and not associated with WNV. More generally, when using an animal as a sentinel in a surveillance system, we need to have an in-depth understanding of its behavior to avoid introducing biases when interpreting surveillance data.

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