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Factors associated with the risk of West Nile Virus among crows in New York State

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Abstract

West Nile virus (WNV) is transmitted between avian hosts in enzootic cycles by a mosquito vector. The virus has significant disease effects on humans and equines when it bridges into an epizootic cycle. Since the initial epidemic of WNV in 1999, perennial outbreaks in New York State suggest the local establishment of natural foci with perpetuation of the virus among susceptible hosts rather than reintroduction of the virus. The factors that play a role in the perpetuation of the virus are not fully understood. American crows (*Corvus brachyrhynchos*) are known to be highly susceptible to infection with the virus. We investigate the factors that put crows at risk of infection in Tompkins County, New York during the period of 2000 through 2008 in a case-control study. Cases were crow carcasses that were found dead and tested positive for WNV using real time reverse transcription (RT-PCR) or VecTest[®]. Data on putative risk factors were collected and assessed for significance of association with the presence of WNV using logistic regression analysis to evaluate the significance of each factor while simultaneously controlling for the effect of others. The risk of a crow carcass testing WNV positive varied with age, season of the year, and ecological area where the carcass was found. Crows that were more than one year old were 4 times more likely to be WNV positive in comparison to birds that were less than one year of age. It was three times more likely to find WNV positive carcasses in residential areas in comparison to rural areas. The risk of testing WNV positive did not vary by sex of the crow carcasses.

Keywords

West Nile virus; epidemiology; case-control; American crow; risk factors

Introduction

West Nile virus (WNV) has been of public health concern in North America since it first appeared in the northeastern region of the United States (US) in 1999 (Komar *et al.*, 2003). The emergence of this zoonotic pathogen poses a great challenge because of its complex epidemiologic characteristics. WNV infects multiple host species and this ability to infect and cause viremia in a wide variety of host species coupled with the involvement of multiple arthropod vectors in the transmission of the disease may have hindered efforts to control the spread of the disease (Bowen & Nemeth, 2007, Andreadis *et al.*, 2004, Anderson *et al.*, 2006). The adaptation of a generalist strategy (i.e., WNV capacity to infect multiple hosts) is a likely process by which the pathogen persists in the environment, and could be affected by a combination of agent, host, and environmental factors.

The endemicity of WNV in the northeastern US is believed to be sustained by an urban cycle of transmission involving birds as reservoir hosts and mosquito species as vectors (Brown *et al.*, 2008, Brault, 2009). There is a wide range of susceptibilities of WNV infection among birds, and corvids (particularly American crows [*Corvus brachyrhynchos*]) have the highest mortality rates among North American birds (Salazar *et al.*, 2004). WNV-infected crows develop high viral titers in blood and other tissues (Komar *et al.*, 2003). Because of their high mortality and proximity to humans, American crows have been used as an indicator or sentinel for WNV activity (Eidson *et al.*, 2001, McLean *et al.*, 2001). Crows have been useful as sentinels because they are easier to spot than smaller birds. As the study of factors affecting the health and illness of populations, epidemiology can serve as the foundation of interventions made in the interest of public health and preventive medicine.

Transmission factors that exacerbate the risk of WNV-associated disease in humans in the Northeast remain a focus of investigation. Tompkins County in New York State has been the center of a long term study of crow behavior and ecology (McGowan, 1998, McGowan, 2001, Clark *et al.*, 2006). Looking at cases of crow mortality within the county could lead to identifying potential risk factors associated with WNV that strengthen disease surveillance and efforts to control future outbreaks. Our knowledge of the ecology of the disease remains limited. Therefore epidemiologic studies focusing on ecological factors which play a role in perpetuating the risk of WNV in ecological niches will contribute to our understanding of the disease and the implementation of cost-effective mitigation strategies.

Methods

Study concept

A case-control study was undertaken to identify factors that put crows at risk of WNV infection and might enhance or detract from the perpetuation of the virus among crows within an ecological niche.

Target and study populations

The target population consisted of sick and dying American crow in Tompkins County in central New York State during the period of January 2000 through December 2008. Crows were collected and submitted to either the Animal Health Diagnostic Laboratory (AHDL) at

Cornell University or the New York State Department of Health, Wadsworth Laboratory (NYSDH) to be tested for WNV.

Definition of a case—Cases consisted of dead birds that tested positive for WNV by VecTest[®] (Medical Analysis Systems, Inc., Fremont, CA) or real-time reverse-transcriptase polymerase chain reaction (RT-PCR) method targeting the envelope (E) coding region of the gene. Birds that tested positive by RT-PCR in the initial assay were confirmed using standard RT-PCR targeting the nucleocapsid region (Lanciotti *et al.*, 2000, Shi *et al.*, 2001).

Definition of controls—Controls were defined as all crow carcasses within the study areas that tested negative for WNV by real-time RT-PCR.

Virus detections

5[′] Nuclease real-time RT-PCR: The real-time and standard RT-PCR assays were run as previously described with some cycling modifications (Lanciotti *et al.*, 2000, Shi *et al.*, 2001). The SmartCycler (Cepheid, Sunnyvale, CA) thermal cycling conditions consisted of 42°C for 15 minutes, 95°C for 10 minutes, and 36 cycles of 95°C for 15 seconds, 50°C for 10 seconds, and 60°C for 100 seconds.

Standard RT-PCR: The Perkin Elmer Cetus (PerkinElmer, Waltham, MA) thermal cycling conditions consisted of 50°C for 30 min, 95°C for 15 min, and 50 cycles of 95°C for 45 sec, 62°C for 45 sec, and 70°C for 1 min.

Risk factors

Data collected for each crow carcass included date of carcass recovery, host factors (age and sex), and ecological factors (development and habitat). Sex determination was based on necropsy and age was a two-tier aging classification based on tail shape (Clark *et al.*, 1991, Yaremych *et al.*, 2004a). The age of the bird was classified as adult if older than one year and juvenile if less than one year. The location where the bird was found was recorded as GPS coordinates. This location was later used in the Arc Manifold 8.3 (Manifold Net, Ltd, Carson City, NV) to be translated into land cover classification using National Land Cover Data 2001 (Table 1). These ecological factors included intensity of development (no development, low, and medium), land coverage (forest, deciduous, and evergreen), and type of pasture (hay, cultivated crops). Medium density refers to apartments, townhomes, and condominiums and two-story single family homes on small lots. Low density is single family homes on at least a half acre of land. The geographical locations of the cases and control birds were then grouped into rural (no development) and residential areas (low and medium density development), and were analyzed according to location and population density.

Statistical Analysis

All putative risk factors were screened initially for their significance of association with the likelihood of cases using the chi-square test (all the factors were categorical). If the expected number of observations per a specific level of the putative factor and disease status was less than 5, Fisher Exact test was used to evaluate the significance of association. Factors that

were significant in this bivariate analysis were considered further in a multivariate analysis using the logistic regression approach to assess the effect of each factor while simultaneously controlling for the effect of other factors. In the multivariate analysis, season was grouped as either summer/spring or fall/winter. The magnitude of risk or the strength of the association was quantified using the odds ratio (odds of being a case if the animal had the factors). Confidence interval estimates were computed at α of 0.05. The statistical analysis was performed using Egret, Cytel Statistical Software, Cytel Software Corporation, Cambridge, MA.

Results

A total of 228 crow carcasses (61 cases and 167 controls) met the inclusion criteria and were included in the analysis. Figure 1 is an ecological map showing the location where WNV-positive crow carcasses were found. We were unable to pinpoint the coordinates for 20 cases and 27 control crow carcasses and hence, they were omitted from further analysis.

Table 1 shows the results of the analysis between different land cover and the likelihood of WNV. A WNV-positive crow was twice as likely to be located in a developed areas (residential) in comparison to non developed (rural) area [odds ratio (OR) = 2.4 and 95% CI (1.1, 5.3)] (Table 1). We further examined the association between the likelihood of WNV and the intensity of development. Type of development (medium versus low density of human population) was not significantly associated with the likelihood of WNV in dead crows [OR= 0.6 (0.3, 1.5)]. Furthermore, we examined the land coverage within no development areas. There was no significant association between pasture or forested land (regardless of tree type) and the likelihood of encountering a WNV case [OR = 0.34 (0.03, 2.91)].

Table 2 shows the results of the bivariate analysis of the intrinsic factors and season that were hypothesized to associate with the likelihood of WNV. Carcasses of crows that were less than a year old were less likely to be WNV positive in comparison to younger crow carcasses 95% CI (0.2, 0.6). It was more likely to find dead crows that tested positive for WNV in the summer than any other season of the year (OR for fall was 0.2, winter was 0.01, and for spring was 0.01). There was no association between the likelihood of testing WNV positive and the sex of the crow carcasses (Table 2).

The multivariate analysis showed that the risk of WNV infection in crows was associated with age of crow, and season and geographic location where the crow was found (Table 3). The results show the odds ratios, adjusted for the presence of other variables in the model. Crows that were more than one year of age were four times more likely to be WNV positive in comparison to crows that were less than one year old (95% CI was 1.8, 10). Crow carcasses that were found during spring and summer seasons were at greater risk of being WNV positive in comparison to carcasses that were discovered during fall or winter seasons (OR = 13.4; 95% CI 2.8, 64.6). Crow carcasses that were located in low-medium development (residential) areas were three times more likely to be WNV positive in comparison to carcasses that were located in rural areas (95% CI was 1.3, 7.8). The

likelihood that a crow carcass was positive for WNV was not influenced by the sex of the bird.

Discussion

We carried out a case-control epidemiologic study to address the stated objectives of our study. The case-definition included crows that were found dead and submitted to the AHDL at Cornell for WNV testing (Lanciotti *et al.*, 2000, Shi *et al.*, 2001). Although these birds had been submitted to the AHDL through an active surveillance program that included media awareness and bird watcher solicitation, there is a chance that we might have missed some of the birds that were scavenged by wild animals. Furthermore, while these crows are non-migratory, it is difficult to know if crows moved from where they were infected to other sites before death. There is also a possible bias to the location of where the birds were found as it is more likely for birds to cluster in urban areas and also for their carcasses to be found due to their size and increased foot traffic.

By virtue of their design, data on putative risk factors in case-control studies are collected retrospectively and hence there is a potential for confounding effects among these factors (Schlesselman 1982). We controlled for the potential confounding effect among the hypothesized risk factors by developing a systematic approach to data analysis. First, we screened variables for potential association with crow mortality from WNV, and second, consider only risk factors that were significant in the multivariate analysis. Variables that were considered to have a biological role in the risk of mortality but were not significant in the bivariate analysis were also included in the multivariate analysis.

Since WNV was first introduced to the US in 1999, season and specifically summer has been the greatest risk factor for crow morbidity and mortality (CDC, 2000, Caffrey *et al.*, 2005). The most obvious cause is that vector abundance and activity is greatest during the summer season in temperate latitudes, such as much of the US (Andreadis *et al.*, 2004). Therefore, the finding of crow carcasses corresponding to this time of known mosquito activity is not surprising.

The increased risk of WNV exposure linked to older age biologically makes sense since the first year of life for crows has many risk factors for death beyond disease exposure (McGowan & Caffrey, 1994, Ludwig *et al.*, 2010). The greater exposure risk in residential areas supports previously published data. Urbanization is one of the ecological risk factors associated with WNV transmission in the North East United States (Brown *et al.*, 2008). This may be due in part to the fact that crows, like the pigeon and the gull, are adapted to human habits and therefore often select peri-domestic habitats (Yaremych *et al.*, 2004b). A distinct feature of WNV in North America has been large numbers of corvids dying in peri-urban areas (Nielsen & Reisen, 2007). One outcome of high densities of crows and some other birds in peri-urban vs. rural habitats is the increased likelihood of being found by the public and submitted for testing.

In our study, carcasses of younger crows were less likely to test positive for WNV. This does not align with one study that had been previously observed and published (Clark *et al.*,

2006). However our study differs in several ways. First our study was of longer duration. Second, ours is a case-control study while the Clark et al., 2006 was a population based study. Also our controls and cases were gathered concurrently. A recent paper regarding a 2005 WNV study of crows in Quebec, Canada found that younger crow carcasses were less likely to be WNV positive (Ludwig et al., 2010). One hypothesis for higher WNV positive results in older animals is simply a greater likelihood of being exposed or infected over time.

The observed association between residence, pasture, and forest is that birds were more likely to be found along major roads in Tompkins County. There was no significance of association between the classes of ecological niches possibly because the areas are not mutually exclusive between either the crows inhabiting the niche or the vector species co-inhabiting the same area.

Impacts

- West Nile Virus (WNV) is a zoonotic disease that can be transmitted between animals and man through a vector.
- Since its introduction in 1999, West Nile Virus (WNV) has become endemic in North America and crows have been used as sentinels for the disease.
- Factors that put crows at risk of this disease are not fully known and it was the objective of this paper to shed light on some of them in hopes of understanding the mechanism of introduction and perpetuation of the disease.

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Figure 1. National Land Cover Data (NLCD) map of West Nile virus cases (N=61) in Tompkins County, NY (2000–2008). Black dots indicate location of where WNV positive crow carcasses were found.

Table 1

Crow carcasses found in various types of land cover in Tompkins County, New York, 2000–2008

Land Cover Classification (NLCD 2001)	Number of Cases	Number of Controls	Odds Ratio (95% Confidence interval)
Type of Development			
Development (rural)	10	61	
Low, Medium Intensity (residential)No	31	79	2.4 (1.1, 5.3)
Development/Type of Intensity			
Low Intensity	13	42	
Medium Intensity	18	37	0.63 (0.3, 1.5)
No Development/Land Coverage			
Deciduous, Evergreen, Forest	1	15	
Pasture Hay, Cultivated Crops	9	46	0.34 (0.03, 2.91)

*
20 cases and 27 controls were not included because of missing coordinates

Table 2

Risk factors examined for associations with WNV in crow carcasses tested in Tompkins County, New York, 2000–2008

Factor	Cases	Controls	Odds ratio (95% confidence interval)
Age			
< 1 year	24	91	1.0
1 year	34	46	0.3 (0.2, 0.6)
Season of the year *			
Summer	57	25	1.0
Fall	3	11	0.2 (0.03, 0.5)
Winter	0	54	0.01 (0.001, 0.06)
Spring	1	77	0.01 (0.001, 0.04)
Sex			
Male	25	42	1.0
Female	23	47	0.8 (0.4, 1.7)

* Winter = January – April; spring = May–June; summer = July – September, fall = October – December

Table 3

Results of logistic regression analysis of factors associated with the likelihood of WNV in American crows in Tompkins County, NY 2000–2008

Factors	Regression coefficient	Standard Error	Odds Ratio (95% CI Lower)
Age			
< 1 year	0		1.0
1 Year	1.446	0.437	4.3 (1.8, 10.0)
Season*			
Fall and Winter	0		1.0
Spring and Summer	2.604	0.798	13.5 (2.8, 64.6)
Geographical location			
Rural			1.0
Residential	1.146	04.60	3.4 (1.3, 7.8)
Constant	−4.666	0.955	

Fall and winter = October – April; Spring and Summer = May – September