

Bird species involved in West Nile virus epidemiological cycle in southern Quebec

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Abstract

There have been many studies of the ecology of West Nile Virus (WNV) in the US, including the role of different bird species as reservoirs and mid-late summer shifts of feeding of *Culex pipiens-restuans* (CPR) mosquitoes from birds to mammals. It is often assumed that these findings are generalizable to neighbouring regions of Canada where WNV is endemic; however there have been little equivalent studies in Canada to confirm that this is reasonable. Here we construct a priority list of bird species likely involved in the transmission of WNV in the greater Montreal area by combining three sources of data i) results of WNV surveillance in wild (dead) birds in the province (2002-2015); ii) evidence from molecular blood meal analysis that bird species are fed upon by CPR, the primary enzootic vectors of WNV in the region, collected in mosquito surveillance in the study area in 2008 and 2014; and iii) a literature review on evidence of sero-prevalence and host competence of resident bird species. Three lists of 18, 23 and 53 bird species, respectively, from the three data sources, and totalizing 67 bird species were identified as potential WNV reservoirs/amplifiers. Of the 23 species identified from CPR blood meals, Common starlings, American robins, Song and House sparrows ranked the highest as possible WNV reservoirs/amplifiers. In addition, we found using logistic regression that the proportion of blood meals taken from birds compared to mammals by CPR declined significantly with surveillance week. These findings indicate broad similarity in the ecology of WNV between the study region and northeastern US although the relative importance of some bird species as hosts of CPR and WNV in this area may be somewhat different, and field studies are needed to confirm this, and explore the consequences for the risk of WNV to the human population.

Main text

Introduction

First described in Uganda in 1937 (Smithburn et al. 1940), West Nile Virus (WNV) is an arbovirus of the *Flaviviridae* virus family, genus *Flavivirus*. Responsible for infections in humans and horses, the virus has a transmission cycle involving mosquitoes as vectors and birds as amplifying hosts or reservoirs (Work et al. 1953, Taylor et al. 1956). In North America, the first outbreak occurred in New York in 1999, and then spread rapidly across the continent causing the death of many bird species (Garmendia et al. 2001, Komar

et al. 2001). In less than four years, the virus spread throughout much of the continental United States, and activity was first reported in Canada in 2001 (Gancz et al. 2004). WNV is now endemic to much of southern Canada, and given the potential severity of the disease in humans and the lack of treatment or a specific vaccine, WNV infection is a major public health problem in Canada, including in the province of Quebec (INSPQ 2016). Most human cases of WNV are reported from Quebec, Ontario and the Prairie provinces and outbreaks of WNV infection occur sporadically (Figure 1) In Quebec, human cases of WNV are mainly reported from the Montérégie, Montréal and Laval regions of southern Quebec (INSPQ 2016). This study focuses on the Montreal area, one of the regions most affected by WNV in Quebec, with 28% of clinical cases reported between 2002 and 2014 (INSPQ 2016).

The epizootiological cycle of WNV transmission involves bird of several species that act as reservoirs/amplifying hosts and a range of mosquito species that act as enzootic and epizootic vectors of WNV. Mammals such as horses and humans are incidental or dead-end hosts that are not part of the virus transmission cycle because they do not produce viremia sufficient to infect mosquitoes (Dauphin et al. 2004). In southeastern Canada, including Quebec, the main WNV vectors are *Culex pipiens* and *Culex restuans* (Ripoche et al. 2017) and these species are thought responsible for transmitting the majority of WNV cases humans in neighbouring parts of the northeastern US (Kilpatrick et al. 2006, Andreadis 2012).

A range of bird species serve as hosts for WNV, and long-distance migration by many species may disperse WNV over long distances (Rappole et al. 2000, Owen et al. 2012, Jourdain et al. 2007, Peterson et al. 2003, Dusek et al. 2009). The importance of birds species as reservoir hosts depends on a combination of factors: i) their susceptibility to infection; ii) the duration of viraemia at levels greater than those needed to infect feeding mosquitoes; iii) the density of naïve individuals (a combination of the density of the species and rates of infection followed by protective immunity); iv) the ‘attractiveness’ of the species to vector mosquitoes and thus the proportion of mosquito bites per unit of space-time that occur on the species; and v) the rates of mortality, including WNV-specific mortality, of infective individuals.

Experimental studies have shown that several North American bird species are susceptible to WNV, are likely able to transmit the virus by virtue of the level and duration of viraemia, and die due to the infection (Komar et al. 2003). Mortality in wild bird populations, particularly corvids, was used as an early surveillance signal of WNV activity in a given locality as the virus first spread across the US and then Canada (Marra et al. 2004; Thomas-Belachi et al. 2015), and an index of the rates of expected human cases (Vekler et al. 2009). However retrospective analysis suggested that when it first invaded North America, WNV caused mortality in a wide range of bird species (LaDeau et al. 2007).

Studies in the US have taken account of the multiple factors described above, by combining field observations and laboratory experiment results, to conclude that the American Robin is a key reservoir species (Kilpatrick et al. 2006a). Furthermore, studies from the US suggest that the seasonal nature of human WNV cases (with most cases from late summer through to mid autumn) is associated with a shift in mosquito blood meals from birds to mammals during the high risk period, which may be driven by birds beginning their southwards migration at this time (Kilpatrick et al. 2006b). There is, however, little literature to confirm the degree to which studies in the US are directly relevant to Canada.

In this study, we aimed to develop a priority list of bird species likely involved in the circulation of WNV in the region of Montreal, in southern Quebec, Canada. To do so we identified species that breed in the region, and are known to be competent reservoirs, from the literature and dead bird surveillance, and then prioritised these according to field and laboratory data from field studies on the feeding preferences of *Culex pipiens-restuans* (CPR) mosquitoes, accounting for bird species abundance in the Montreal area. The blood meal analysis data also allowed us to explore the occurrence of seasonal shifts in host-feeding behaviour.

Materials & Methods

1. Study area

The greater Montreal area is located in the south of the province of Quebec (Figure 2) and in the central

part of the St. Lawrence Lowlands. The region is bordered to the north by the Canadian Shield and to the southeast by the Appalachians (Fougères et al. 2017). The region is characterized by a temperate continental climate, with cold winters, due to the influence of Arctic air, and hot summers (Fougères et al. 2017). The study area is 30,231.2 km², and includes the island of Montreal and was limited by the coordinates 46°13'48 N, 45°1'12 N, and 74°24'36 W and 72°23'24 W. In 2011, the greater Montreal area comprised 21% of buildings, 40% cultivated agricultural land and 39% natural or semi-natural areas, of which 66% was forest (StatisticsCanada 2016). With 2,900,000 inhabitants, about 45% of the Quebec province's population, the greater Montreal area is the most densely populated area in Quebec and the second most densely populated city in Canada.

2. Identification of priority list of wild bird reservoir species

To construct the list of possible WNV reservoir and amplifier bird species for the study area, we used an approach by successive augmentation. Starting with a reference list of regional breeding bird species L_0 , the priority list L_f of birds that could potentially play a role in the WNV transmission cycle was obtained as, $L_f = L_0 \cap [L_1 + L_2 + L_3 - L_1 \cap L_2 - L_1 \cap L_3 - L_2 \cap L_3 + L_1 \cap L_2 \cap L_3]$, where \cup indicates "union" of data from databases (bringing all species from the databases together), and \cap indicates "intersection" of data from the databases (i.e. where species are in the different databases). L_1 , L_2 and L_3 are respectively the "databases" of i) species identified as infected by WNV in dead bird surveillance; ii) species identified as being targets for *CPR* blood meals on the basis of blood meal analyses and iii) the species identified as WNV reservoirs by literature review. Clearly, L_1 is the first basic list that is augmented with species from L_2 and L_3 but not already in L_1 . The comparison with L_0 is a check insuring that selected bird species belong to study area. L_0 comprised 318 species extracted from the Avibase database (Lepage 2017), which includes all breeding species present in the Montreal area. Species reported as rare ($n=65$) in the Avibase were excluded from L_0 .

2.1 Wild bird mortality data: list L_1 : Mortality data were obtained from the Canadian Wildlife Health Cooperative (CWHC) Passive Mortality Monitoring Program (Drebot et al. 2003). The data came from the passive WNV wild bird surveillance program established in Canada since 2001, the first year the WNV circulation was detected in Canada (Lindsay et al. 2003). This "passive" surveillance work is carried out in collaboration with the local population, who are invited to report to the relevant authorities. Dead birds were retrieved and submitted to veterinary diagnostic laboratories (Drebot et al. 2003). In these laboratories, necropsies were performed and selected tissues were tested by PCR for WNV infection (Lindsay et al. 2003, CWHC 2016). The data cover the period 2002-2015 during which time two WNV epidemics occurred in Quebec. The locations where dead WNV infected birds were collected are reported in Figure 2. To rank birds species belonging to L_1 , the standardized mortality ratio, or relative ratio, RR_s , for each bird species "s" was calculated as, $RR_s = \frac{m_s}{(\lambda \times n_s)}$, where m_s is the number of dead birds of species "s" found positive to WNV, n_s the corresponding sample size and, $\lambda = \frac{\sum_s m_s}{\sum_s n_s}$, is the mortality rate under the homogeneous hypothesis; $(\lambda \times n_s)$ being the expected number of dead birds of species "s".

2.2 Blood meal data: list L_2 Mosquitoes were collected in our study region between 2008 and 2014 as part of a provincial mosquito surveillance program conducted in southern Quebec (INSPQ 2016). Blood meal analysis was conducted only on engorged females of either *Culex pipiens* or *Cx. restuans* (*CPR*) complex in this study. Capture sites of engorged females are shown in Figure 2. The list L_2 consists of bird species identified as blood meal sources for *CPR* complex mosquitoes.

Blood-fed mosquitoes were stored individually in 1.5 ml tubes at -80° C until processing. Extraction of DNA was carried out using a protocol described by Molaei et al. (Molaei et al. 2016). Briefly, 200 µl of DNAzol® BD (Molecular Research Center, Cincinnati, OH) was added to each tube. Mosquitoes were homogenized with a pestle, followed by the addition of another 200 µl of DNAzol BD and 15 µl proteinase-K. Tubes were vortexed briefly, incubated at 70° C for ten minutes, then centrifuged for ten minutes at 14000 rpm. The supernatants were transferred to new 1.5 ml tubes and 3 µl of polyacryl carrier (Molecular Research Center, Cincinnati, OH) was added to each tube. Tubes were incubated at room temperature for three minutes

and then 200 μ l 100% ethanol was added. Following mixing by gentle inversion, tubes were incubated on ice for ten minutes then centrifuged for six minutes at 6000 rpm. The supernatants were removed, and the remaining DNA pellets were washed twice by the addition of 750 μ l of 75% ethanol and two minutes centrifugation at 3000 rpm. After final removal of the ethanol, the tubes were left open to allow the DNA pellets to air dry. Once dry, the pellets were resuspended in 20 to 50 μ l 1X TE buffer. DNA extracts obtained from blood-fed mosquitoes were used as templates for amplification of the *cytochrome b* gene in avian and mammalian species using primers previously described by Molaei et al. (Molaei et al. 2016). Extracted DNA was amplified in 50 μ l reactions using the Platinum taq DNA polymerase system (Invitrogen, USA) with final concentrations of 1.5 mM $MgCl_2$, 200 nM dNTP and 200 nM per primer. Amplification was carried out in the Applied Biosystems GeneAmp PCR System 9700 using the following conditions: denaturation for two minutes at 94°C, 40 cycles of amplification consisting of 30 seconds at 94°C, 50 seconds at 55°C (mammalian) or 60°C (avian), 60 seconds at 72°C, extension for seven minutes at 72°C and then held at 4°C. The first 100 amplification products were visualized by gel electrophoresis on 1.9% agarose gel whereas the remaining samples will be run on QIAxcel (Qiagen, Toronto, Canada). Positive samples were purified using the Wizard SV Gel and PCR Clean-up system (Promega, USA) and Sanger sequencing was performed by the NML Genomics Core Facility using Applied Biosystems 3730 xl DNA Analyzer with Big Dye Terminator version 3.1 and pop7 chemistries. Sequence data were analyzed using DNASTAR Lasergene 9 Software and compared to those in the GenBank (NCBI). Sequence data from mosquitoes that have taken multiple host blood meals from different species would result in poor quality sequence data and host species would not be identifiable. However, we expected that these events would be rare because adult female *CPR* take mostly one single host before digesting the meal and laying eggs.

The species identified are characterized by the proportion (f_s) of all identified CPR blood meals taken from species s . The value of f_s will depend on the relative density of species s and on the degree to which a species may be particularly attractive to the mosquitoes – if so then mosquito bites on a particular species may be disproportionate to the density of the species. To explore this a feeding preference index can be obtained as (Rizzoli et al. 2015): $p_s = \frac{f_s}{a_s}$, where f_s is as defined above and a_s is the ratio of the abundance of species s over that of the total density of the birds in the area. Such a p_s can be regarded as a relative risk for a bird of being bitten by the mosquito in relation to its relative abundance (Rizzoli et al. 2015). Unfortunately, p_s gets higher and diverges when a_s tends to zero. Therefore, we use the following definition for the preference index (Balenghien et al. 2011, Bicout 2013), $p_s = \frac{a_s f_s}{\sum_{j=1}^n a_j f_j}$. Likewise, the relative risk for a bird of being bitten by the mosquito relative to the homogeneous abundance is, $RR_s = n \times p_s$. Over and under bitten bird species are characterized by $RR_s > 1$ and $RR_s < 1$, respectively.

The values for a_s were obtained from wild bird count data taken from the EPOQ-eBird database, managed in part by the Regroupement Québec Oiseaux (RQO) for the years 2001 to 2016 (<https://www.oiseauxqc.org/epoq.jsp>). This database contains more than six million observations made by ornithologists during their daily trips within Quebec. These data are obtained from opportunistic sampling: each observer lists the species observed on a leaflet, as well as the number of individuals of each species. Each leaflet corresponds to an observation site at a given date during a given period as well as to the number of individuals of each species. Locations at which bird counts were made are shown in Fig. 2.

Analysis of seasonal bird-to-mammal feeding shift of CPR mosquitoes

Culex mosquitoes are predominantly ornithophilic species, i.e. mainly feeding on birds. We investigated whether the feeding preference of the mosquitoes for birds versus mammals changed over the activity season. We considered the fraction (or probability π_{bird}) of blood meals taken on bird species (= number of blood meals on all bird species only/total number of blood meals over all species (birds and mammals) as a function of week of the year. Logistic regression was used to model the variation of the feeding preference over weeks as follows, $\text{logit}(\pi_{\text{bird}}) = \beta_0 + \beta_1 \text{week}$. Statistical analyses were performed using the glm (generalized linear model) function in R version 1.1.383 (R Foundation for Statistical Computing <http://www.R-project.org>).

2.3 Literature review: list L_3 : Literature research was conducted in August 2017 in five electronic databases: Scopus, Pubmed, CAB Abstract, Embase and Medline. The phrase used for searching in all databases was:

“(West Nile Fever OR West Nile Virus) AND (Bird* OR Avian) AND (Mortality OR Sero-prevalence OR prevalence OR competence OR capacity OR transmission) AND (USA OR Canada)”. All articles published from January 1, 1999 to August 16, 2017 (end of the search) were included in the selection process using criteria on the language, title, and abstract (Table 1). In short, selected studies had to be written either in English or French, dealing with an epidemiological content about bird sensitivity to WNV in Canada and/or the United States. Data on sero-prevalence and host competence were extracted from the retrieved publications. To rank birds species belonging to L_3 , the standardized WNV sero-positive ratio, or relative ratio, RR_s , for each bird species “s” was calculated as, $RR_s = \frac{m_s}{(\lambda \times n_s)}$, where m_s is the number of WNV sero-positive birds of species “s”, n_s the sample size of tested birds and, $\lambda = \frac{\sum_s m_s}{\sum_s n_s}$, is the sero-positive rate under the homogeneous hypothesis; $(\lambda \times n_s)$ being the expected number of WNV sero-positive birds of species “s”.

Migratory status (migratory M, resident R birds or both M, R), wintering and breeding areas were also added. Wintering/breeding areas have been defined as the three main regions of the East Atlantic migration corridor: region of Quebec QC, North USA and South USA, delimited by the northern border of the states of North Carolina and Tennessee (Peterson et al. 2004). The two US areas make it possible to take into account short and long distance migration. These “bird pathways” allow taking in consideration the viral infection origin (wintering zone vs. breeding zone) and the possible role of each bird species in the dispersal of WNV in North America.

Results

Wild bird mortality data: list L_1

From the passive WNV wild bird surveillance we found a list $L_1 = 18$ that is sorted based on the relative ratio of dead birds positive to WNV (Figure 3). Of all these birds, only 2 (Bald eagle and Blue jay) were found less represented (with the relative ratio of mortality < 1) in mortality data.

Blood meal analysis: list L_2

DNA from 273 engorged mosquito samples was extracted although only 263 were included in the study as ten did not have sufficient volume of DNA for amplification with both primer sets. Ninety-seven of 263 (36.9%) were positive using PCR and sequencing with avian primers, whereas 14/263 (5.3%) were positive with PCR and sequencing using mammalian primers. Ten of 263 extracts (four positives with avian primers and six positives with mammalian primers) were reported as indeterminate, as sequencing of the PCR product was not successful and there was insufficient volume remaining for repeat testing (Table 2).

Twenty-three ($L_3 = 23$) different bird species were identified as hosts for CPR mosquitoes (Figure 4). The most frequently bitten bird species (with relative feeding [?] 1) belong to the Passeriformes order, with the American robin 31% ($n = 30$) being the most commonly identified, followed by the Common starling 11.3% ($n = 11$), the Song sparrow 9.3% ($n = 9$) and the Cedar waxwing 8.2% ($n = 8$) (Fig. 4, left panel). A large proportion of the bird species (13 out of 23) were represented only by one or two blood meals. White-tailed deer were the most frequently bitten mammal species while two blood meals were from humans in weeks 27 (first week of July) and 32 (first week of August).

Likewise, the most abundant birds (with relative abundance [?] 1) were Common starling 21%, Red-winged blackbird 10% follow by Cedar waxwing, American goldfinch, Chipping sparrow, American robin and American crow all with 6% (Fig. 4, left panel).

To explore the feeding preference of CPR, the list L_3 is sorted based on the value of p_s (Fig. 4, right panel). Of these the highest ranked species (with relative feeding preference [?] 1) are in descending order, Common starling, American robin, Song sparrow and House sparrow. All other bird species are associated with a relative feeding preference lower than 1 suggesting that while very abundant, like Red-winged blackbirds, these species may be disfavoured by CPR mosquitoes (Fig. 4)

Analysis of seasonal bird-to-mammal feeding shift of CPR mosquitoes

There was a significant shift in the proportion of blood meals obtained from mammals compared to birds with advancing surveillance week (coefficient = - 0.27 [95% confidence interval = - 0.47; - 0.06], $p < 0.01$). The shift appeared approximately continuous from week 26, with an odds ratio of change in the proportion of blood meals from birds of 0.76 (95% confidence interval = 0.62 – 0.94) per week (Figure 5).

Literature review: list L_3

The literature search identified a total of 1,244 articles, 23 of which met the selection criteria (Figure 6). As a result, we found a list $L_1 = 53$ that is sorted based on the relative ratio of sero-positives (Figure 7) as follows: 22 birds were found most sero-prevalent (with the relative ratio of sero-positives [?] 1) to WNV of which the top five are Green heron, Cooper’s hawk, Red-shouldered hawk, Merlin and Eastern meadowlark (all with the same ratio of about 4). And 21 birds are less sero-prevalent with American redstart as the lowest sero-prevalent ones.

Final list L_f

The distribution of the species we identified among the lists is as follows: $L_1 = 18$ (mortality data of wild birds), $L_2 = 23$ (blood meal analysis of the *CPR*) and $L_3 = 53$ (literature review) with the number of common species, $L_1 \cap L_2 = 2$, $L_1 \cap L_3 = 7$ and $L_2 \cap L_3 = 20$, and $L_1 \cap L_2 \cap L_3 = 2$ species (American crow and Cooper’s hawk) belonging to all three lists. The final list of 67 bird species was obtained as, $67 = [L_1 + L_2 + L_3 - L_1 \cap L_2 - L_1 \cap L_3 - L_2 \cap L_3 + L_1 \cap L_2 \cap L_3] = 18 + 23 + 53 - 2 - 7 - 20 + 2$, representing 21% of $L_0 = 318$ species from the Avibase database (Lepage 2017), which includes all the species present in the Montreal area.

Table 3 presents the summary results of the characteristics of each of the 67 bird species of interest selected. Characteristic variables include data on bird mortality, bird abundance and *CPR* feeding preference, and sero-prevalence, host competence, migratory status, wintering and breeding areas extracted from the literature.

Discussion

In this study, we constructed a priority list of birds potentially involved in the transmission of WNV in the greater Montreal region using data from a literature search, data from wild bird surveillance for WNV and evidence from blood meal analysis of *CPR* mosquitoes that species are being selected by the mosquitoes as hosts. There were 67 breeding species identified by these data sources, which emphasises the possible complexity of WNV transmission cycles.

Out of the 67 bird species, host competences were documented for 22 species, including eight higher competent (competence > 1) species (from the most to the least competent): Blue jay, Common grackle, House finch, House sparrow, Song sparrow, American robin, American crow and Ring-billed gull. The American crow and Blue jay were considered as an indicator for WNV circulation at the beginning of the epidemics (Ludwig et al. 2010, Foppa et al. 2011, Thomas-Bachli 2020)). In addition to being a good indicator, both species exhibit high viraemia when infected (Komar et al. 2003) with a high mortality rate. The mortality of infected birds shortens the period of virus transmission while this period can last several days for infected and still alive species, during which time mosquitoes can continue to feed on these species. Usually, good amplifying hosts are medium-sensitive to the virus infection. This was demonstrated for WNV amplification in California, using House finch as the host species and *Culex tarsalis* as the main vector species (Worwa et al. 2019).

Blood meal analysis data identified 23 bird species as hosts for blood meals by *CPR* mosquitoes. The total sample size of mosquitoes was small, but there is no specific reason we are aware of as to why the lack of detection of a species by this method, and the proportions of blood meals identified to each species, are biased and not a reflection of the proportion of mosquito bites per species.

The most bitten species were, from the most preferred to the least, Common startling, American robins, cedar waxwing, song sparrow and house sparrow. American robin has been found in other studies (e.g. Hamer et al. 2009; Kilpatrick 2006b). All except cedar waxwings (to our knowledge) have been shown to be

competent reservoirs for WNV in at least one study (Table 2). The species other than robins likely play a role in WNV transmission, particularly as some, including some sparrow species, may have greater capacity to transmit WNV (via longer duration and higher viraemia) than robins.

The role of the species other than robins in the transmission of the West Nile virus could also be associated with the participation of vector mosquito species other than CPR which are competent for transmitting the West Nile virus. In eastern Canada, for example *Aedes vexans*, a widespread mosquito, has demonstrated a vector competence for the West Nile virus but with a rather mammophilic trophic preference and biting birds occasionally (Ripoche, 2019; Giordano 2018; Andreadis 2004).

Other studies have found that house sparrows are under-represented as mosquito blood meal hosts relative to their densities (Hamer et al. 2009 Kilpatrick et al. 2006b) while here they appeared over represented. To what extent these observations may be driven by regional factors such as climate affecting bird population densities, or are associated with the relatively highly urbanised nature of the study area requires further study. The mosquitoes were collected in routine entomological WNV surveillance in CO₂-baited CDC light traps (Ripoche et al. 2019), and marginally gravid traps that attract greater numbers of gorged mosquitoes (Silver, 2008) were not used. We could not rule out the possibility that this could affect comparisons with results of studies in which gravid traps were used, although different findings of gravid and light traps regarding blood meals has not been reported (Kilpatrick et al. 2006b).

There was a shift in feeding behaviour from birds to mammals as in other studies (Kilpatrick et al. 2006b, 2005 Levine et al. 2016). Almost 100% of blood meals taken from birds in early summer (week 23) while the bird/mammal ratio decreases between July and September towards mammals. However, as pointed out by others (Hamer et al. 2009) the main shift in mosquito feeding from birds to mammals occurred in parallel with the onset of reported human cases (which in Quebec usually happens during surveillance weeks 28-31: <https://www.canada.ca/en/public-health/services/diseases/west-nile-virus/west-nile-virus-other-mosquito-borne-disease.html>). The date of acquisition of cases reported in human case surveillance is likely up to weeks before the date of reporting in surveillance (Ogden et al. 2019), so while a shift of feeding behavior from birds to mammals (including humans) may contribute to the seasonal pattern of WNV infection in humans, it is unlikely to be the main cause.

Conclusion

These findings indicate broad similarity in the ecology of WNV in the study region and regions in the US where similar studies have occurred in terms of the range of key avian reservoir host species and seasonal change in host selection by mosquitoes. This work has shed lights on the involvement of American robin and other bird species in the circulation of the West Nile virus in southern Quebec. However, the relative importance of some bird species as hosts of CPR and WNV in the greater Montreal area may be somewhat different to that occurring in northeastern US, and field study is needed to confirm this, and explore the consequences for the risk of WNV to the human population. In addition, studies both in the field and in modeling are necessary to elucidate the roles of each bird, which would help to synthesize and consolidate knowledge in the eco-epidemiology of WNV in this area. Such of kind studies would allow to improve the surveillance, control and management of these vector-borne wildlife diseases, which are becoming increasingly important in North America (Mermel 2020, Shannon 2019, McDonald 2019).

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Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to. No ethical approval was required as this study used data from the literature and from

Canadian monitoring and surveillance programs.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

LT: data analysis; drafted and edited the manuscript,

AL: conception and design of the study; drafted and edited the manuscript, drafted the revised final version.

NHO: edited the final version of the manuscript

LRL: molecular analysis, manuscript edition

MI: molecular analysis, manuscript edition

CAG: manuscript edition

DJB: conception and design of the study; drafted and edited the manuscript, drafted the revised final version.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1. Search strategy for the literature review

Question	Description
Level 1: Language	Level 1: Language
Q1	Is the paper written in English or French? L1 = Q1; eligible for L1 = 1
Level 2: Title	Level 2: Title
Q1	Does the title mention West Nile terms?
Q2	Does the title mention bird terms?
Q3	Does the title mention a region of study that is concerned (East Coast
Q4	Do the title mention terms related to seroprevalence, prevalence, comp L2 = Q1 x Q2 (1 + Q3 + Q4); eligible for L2 [?] 1
Level 3: Abstract	Level 3: Abstract
Q1	Does the abstract describe search results rather than a method?
Q2	Do the abstract mention terms related to bird mortality?
Q3	Do the abstract mention terms related to prevalence or sero-prevalence
Q4	Does the abstract mention terms relating to host competence ¹ ?
Q5	Does the abstract mention terms relating to host capacity ² ?
Q6	Does the abstract specify the regions of study: states of the Eastern m L3 = Q1 x Q6 x (1 + Q2 + Q3 + Q4 + Q5); eligible for L3 [?] 1
Score = L1 x L2 x L3; eligible for score [?] 1	Score = L1 x L2 x L3; eligible for score [?] 1

¹ The host competence designates the capability of a host species, birds in this case, of being infected and developing a sufficient viremia for transmitting infectious agents to vectors (Komar et al. 1999). ² The concept of host capacity brings subtlety to the skill of the host. A competent bird can only be involved in

the transmission of the virus if it is sufficiently abundant and belongs to the species on which a mosquito competent for WNV feeds (Bicout 2013).

Table 2. Results of the molecular analysis of blood meals

Species	Family	Order	N	Birds (%) (n = 97)	Mammals (%) (n = 14)	Total (%) (n = 111)*	
Birds	Birds	Birds	Birds	Birds	Birds	Birds	Birds
American robin (<i>Turdus migratorius</i>)	Turdidae	Passeriformes	30	30.9	-	27.0	
Common starling (<i>Sturnus vulgaris</i>)	Sturnidae	Passeriformes	11	11.3	-	9.9	
Song sparrow (<i>Melospiza melodia</i>)	Emberizidae	Passeriformes	9	9.3	-	8.1	
Cedar waxwing (<i>Bombycilla cedrorum</i>)	Bombycillidae	Passeriformes	8	8.2	-	7.2	
House sparrow (<i>Passer domesticus</i>)	Passeridae	Passeriformes	7	7.2	-	6.3	
Savannah sparrow (<i>Passerculus sandwichensis</i>)	Emberizidae	Passeriformes	4	4.1	-	3.6	
Northern cardinal (<i>Cardinalis cardinalis</i>)	Cardinalidae	Passeriformes	3	3.1	-	2.7	
Gray catbird (<i>Dumetella carolinensis</i>)	Mimidae	Passeriformes	3	3.1	-	2.7	
House finch (<i>Haemorrhous mexicanus</i>)	Fringillidae	Passeriformes	3	3.1	-	2.7	

Species	Family	Order	N	Birds (%) (n = 97)	Mammals (%) (n = 14)	Total (%) (n = 111)*
Black-capped chickadee (Poecile atricapilla)	Paridae	Passeriformes	3	3.1	-	2.7
Red-winged blackbird (Agelaius phoeniceus)	Icteridae	Passeriformes	2	2.1	-	1.8
Common grackle (Quiscalus quiscula)	Icteridae	Passeriformes	2	2.1	-	1.8
Red-eyed vireo (Vireo olivaceus)	Vireonidae	Passeriformes	2	2.1	-	1.8
Cooper's hawk (Accipiter cooperii)	Accipitridae	Accipitriformes	1	1.0	-	0.9
Green heron (Butorides virescens)	Ardeidae	Pelecaniformes	1	1.0	-	0.9
Northern flicker (Colaptes auratus)	Picidae	Piciformes	1	1.0	-	0.9
American crow (Corvus brachyrhynchos)	Corvidae	Passeriformes	1	1.0	-	0.9
American yellow warbler (Dendroica petechia)	Parulidae	Passeriformes	1	1.0	-	0.9
Ovenbird (Seiurus aurocapilla)	Parulidae	Passeriformes	1	1.0	-	0.9
American goldfinch (Spinus tristis)	Fringillidae	Passeriformes	1	1.0	-	0.9

Species	Family	Order	N	Birds (%) (n = 97)	Mammals (%) (n = 14)	Total (%) (n = 111)*	
Chipping sparrow (Spizella passerina)	Emberizidae	Passeriformes	1	1.0	-	0.9	
Warbling vireo (Vireo gilvus)	Vireonidae	Passeriformes	1	1.0	-	0.9	
Mourning dove (Zenaida macroura)	Columbidae	Passeriformes	1	1.0	-	0.9	
Mammals	Mammals	Mammals	Mammals	Mammals	Mammals	Mammals	Mammals
White-tailed deer (Odocoileus virginianus)	Cervidae	Artiodactyla	7	-	50.0	6.3	
Bovine (Bos taurus)	Bovidae	Artiodactyla	2	-	14.3	1.8	
Cat (Felis catus)	Felidae	Carnivora	2	-	14.3	1.8	
Human (Homo sapiens)	Hominidae	Primates	2	-	14.3	1.8	
Mule deer (Odocoileus hemionus)	Cervidae	Artiodactyla	1	-	7.1	0.9	

* of the 273 blood meal samples analyzed, 111 resulted in interpretable sequencing while 162 did not.

Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Table 3. Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	
Family	Species (En- glish name)	Species (Latin name)	Literature Seroprevalence (%) ¹ Min (n) – Max (n)	Literature Host Competence Min- Max	Mortality, % (n) ³ 2001- 2008	Mortality, % (n) ³ 2009- 2017	Abundance, a_s	CPR f_s	Feeding Status ⁶	Wintering area ⁷	Breeding area ⁷	M c S M
Anatidae	Canada Goose	<i>Branta canaden- sis</i>	0.8 (1038) - 29 (7)	0.03 - 0.05					M, R	S	QC, N	M
Anatidae	Wood Duck	<i>Aix sponsa</i>	0 (1) - 2.5 (12)			0 (1)			M, R	S	QC, N, S	M
Anatidae	Mallard+	<i>Anas platyrhyn- chos</i>	8 (13) - 10.6 (66)	0.48 - 0.5		50 (4)			M, R	S	QC, N, S	M
Anatidae	Ring- necked Duck	<i>Aythya col- laris</i>	5.3 (19)						M, R	S	QC, N	M
Ardeidae	Green Heron	<i>Butorides virescens</i>	100 (1)				0.0049	0.0103	M	S	QC, N, S	A
Accipitridae	Northern Harrier+	<i>Circus cyaneus</i>				100 (1)			M	QC, N, S	QC, N	M

Table 3.	Table 3.	Table 3.	Table 3.	Table 3.	Table 3.	Table 3.	Table 3.	Table 3.	Table 3.	Table 3.	Table 3.
Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area	Char- ac- teris- tics of bird species af- fected by WNV in the Mon- treal area
Accipitridae	Sharp- shinned Hawk+	<i>Accipiter striatus</i>				86.4 (22)			M, R	QC, N, S	QC, N, S
Accipitridae	Cooper's Hawk+	<i>Accipiter cooperii</i>	100 (1)			65.4 (26)	0.0036	0.0103	M, R	QC, N, S	QC, N, S
Accipitridae	Northern Goshawk+	<i>Accipiter gentilis</i>				85.7 (7)			M, R	QC, N	QC, N
Accipitridae	Bald Eagle+	<i>Haliaeetus leucophalus</i>				25 (4)			M	N, S	QC, N, S
Accipitridae	Red- shouldered Hawk+	<i>Buteo lineatus</i>	100 (1)			100 (5)			M, R	QC, N, S	QC, N, S
Accipitridae	Broad- winged Hawk+	<i>Buteo platypterus</i>				70 (10)			M	S	QC, N, S
Accipitridae	Red- tailed Hawk+	<i>Buteo jamaicensis</i>				76.9 (13)	0.0013		M, R	QC, N, S	QC, N, S
Charadriidae	Killdeer	<i>Charadrius vociferus</i>		0.84 -					M	N, S	QC, N, S
Laridae	Ring- billed Gull	<i>Larus delawarensis</i>		0.87 -		0 (3)			M, R	QC, N, S	QC, N
Columbidae	Rock Pigeon	<i>Columba livia</i>	4.3 (23) - 55 (20)	0					R	QC, N, S	QC, N, S

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Columbidae	Mourning Dove	Zenaidura macroura	3.8 (26)	0 - 0.19		0 (2)	0.0137	0.0103	M, R	QC, N, S	QC, N, S
			- 57.69 (26)								
Cuculidae	Yellow-billed Cuckoo	Coccyzus americanus	5.9 (17)						M		QC, N, S
Strigidae	Great Horned Owl+	Bubo virginianus	44.4 (9)	0.68 - 0.9		50 (6)			R	QC, N, S	QC, N, S
Strigidae	Snowy Owl+	Bubo scandiacus				100 (2)			M	QC, N	
Strigidae	Barred Owl+	Strix varia				66.7 (3)			R	QC, N, S	QC, N, S
Strigidae	Northern Saw-whet Owl+	Aegolius acadicus				50 (8)			R	QC, N	QC, N
Picidae	Hairy Woodpecker	Picoides villosus	0 (14)						R	QC, N, S	QC, N, S
Picidae	Northern Flicker	Colaptes auratus		0.06 - 0.14			0.0178	0.0103	M, R	N, S	QC, N, S
Picidae	Pileated Woodpecker	Dryocopus pileatus	20 (5)						R	QC, N, S	QC, N, S

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Falconidae	American Kestrel	<i>Falco sparverius</i>	16 (152) - 100 (1)	0.79 - 0.93	0 (1)	0 (5)			M, R	QC, N, S	QC, N, S
Falconidae	Merlin+	<i>Falco columbarius</i>	100 (1)			65.5 (55)			M, R	QC, N, S	QC, N
Falconidae	Gyr Falcon	<i>Falco rusticolus</i>				100 (1)			M, R	QC	
Tyrannidae	Great Crested Flycatcher	<i>Myiarchus crinitus</i>	2 (50)						M		QC, N, S
Vireonidae	Warbling Vireo	<i>Vireo gilvus</i>					0.0189	0.0103	M		QC, N, S
Vireonidae	Red-eyed Vireo	<i>Vireo olivaceus</i>					0.0288	0.0206	M		QC, N, S
Corvidae	Blue Jay+	<i>Cyanocitta cristata</i>	0.8 (121) - 35.8 (134)	2.39 - 2.55	23.6 (886)	66.7 (6)	0.0093		R	QC, N, S	QC, N, S
Corvidae	American Crow+	<i>Corvus brachyrhynchos</i>	3.2 (157) - 68.3 (183)	1.04 - 1.62	40.1 (1418)	73.9 (46)	0.0549	0.0103	R	QC, N, S	QC, N, S
Hirundinidae	Tree Swallow	<i>Tachycineta bicolor</i>	2.6 (156)						M	S	QC, N, S

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Paridae	<i>Black-capped Chickadee</i>	<i>Poecile atricapillus</i>	0 (107)				0.0511	0.0309	R	QC, N	QC, N
Sittidae	White-breasted Nuthatch	<i>Sitta carolinensis</i>	0 (40) - 2.9 (35)						R	QC, N, S	QC, N, S
Troglodytidae	House Wren	<i>Troglodytes aedon</i>	5.9 (17)						M	S	QC, N, S
Turdidae	Eastern Bluebird	<i>Sialia sialis</i>	2.4 (126)						M. R	N. S	QC, N, S
Turdidae	Swainson's Thrush	<i>Catharus ustulatus</i>	2.13 (47) - 3.1 (32)						M		QC, N
Turdidae	Wood Thrush	<i>Hylocichla mustelina</i>	1 (101) - 15.6 (32)						M		QC, N, S
Turdidae	American Robin	<i>Turdus migratorius</i>	2.6 (76) - 10.11 (366)	1.04 - 1.1			0.0578	0.3093	M. R	QC, N, S	QC, N, S
Mimidae	Gray Catbird	<i>Dumetella carolinensis</i>	3.5 (2706) - 35 (17)	0.07 - 0.1			0.0158	0.0309	M. R	N. S	QC, N, S

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Mimidae	Brown Thrasher	<i>Toxostoma rufum</i>	3.7 (643)						M. R	N. S	QC, N, S
			10.5 (19)								
Sturnidae	<i>Common Star- ling</i>	<i>Sturnus vul- garis</i>	0.16 -	0.22		0.2045	0.1134	R		QC, N, S	QC, N, S
Bombycillidae	<i>Cedar Waxwing</i>	<i>Bombycilla cedro- rum</i>	20 (5)			0.0671	0.0825	M. R		QC, N, S	QC, N, S
Parulidae	<i>Ovenbird</i>	<i>Seiurus auro- capilla</i>	0.9 (115)	- 3.1 (32)		0.0024	0.0103	M		S	QC, N, S
Parulidae	<i>Common Yellowthroat</i>	<i>Geothlypis trichas</i>	0.7 (299)					M		S	QC, N, S
Parulidae	<i>American Redstart</i>	<i>Setophaga ruticilla</i>	0.4 (280)					M		S	QC, N, S
Parulidae	<i>Cape May Warbler</i>	<i>Setophaga tig- rina</i>			100 (1)			M			QC, N
Parulidae	<i>Northern Parula</i>	<i>Setophaga amer- i- cana</i>	4.7 (43)					M		S	QC, N, S
Parulidae	<i>Yellow War- bler</i>	<i>Setophaga pet- techia</i>		1		0.0368	0.0103	M			QC, N, S

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Passerellidae	Chipping Sparrow	Spizella passerina	3 (59)				0.0652	0.0103	M. R	S	QC, N, S
Passerellidae	Savannah Sparrow	Passerculus sandwichensis					0.0021	0.0412	M	S	QC, N
Passerellidae	Song Sparrow	Melospiza melodia	0 (13) - 3.4 (88)	1.2			0.0431	0.0928	M. R	QC, N, S	QC, N, S
Passerellidae	Eastern Towhee	Pipilo erythrophthalmus	0.7 (144) - 9.6 (197)						M	N. S	QC, N, S
Cardinalidae	Scarlet Tanager	Piranga olivacea	2.8 (71)						M		QC, N, S
Cardinalidae	Northern Cardinal	Cardinalis cardinalis	6.2 (503) - 52.2 (115)	0.38			0.0313	0.0309	R	QC, N, S	QC, N, S
Cardinalidae	Rose-breasted Grosbeak	Pheucticus ludovicianus	1 (98) - 5 (22)						M		QC. N
Cardinalidae	Indigo Bunting	Passerina cyanea	3.6 (28) - 2.2 (223)						M	S	QC, N, S
Icteridae	Eastern Meadowlark	Sturnella magna	100 (1)						M	N. S	QC, N, S

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Icteridae	Baltimore Oriole	<i>Icterus galbula</i>	8.3 (12)						M	S	QC, N, S
Icteridae	Red-winged Blackbird	<i>Agelaius phoeniceus</i>	0 (63)	0.9 - 0.99			0.1036	0.0206	M. R	QC, N, S	QC, N, S
Icteridae	Brown-headed Cowbird	<i>Molothrus ater</i>	1.8 (494)	0					M. R	QC, N, S	QC, N
Icteridae	Common Grackle	<i>Quiscalus quiscula</i>	0 (106)	1.39 - 2.04			0.0462	0.0206	M. R	QC, N, S	QC, N, S
Fringillidae	House Finch	<i>Haemorhous mexicanus</i>	2.5 (927)	1.29 - 1.8			0.0125	0.0309	M. R	QC, N, S	QC, N, S
Fringillidae	American Goldfinch	<i>Spinus tristis</i>	0.3 (337)				0.0666	0.0103	M. R	QC, N, S	QC, N, S
Passeridae	House Sparrow	<i>Passer domesticus</i>	1.6 (1042)	1.25 - 1.6		0 (1)	0.0508	0.0722	R	QC, N, S	QC, N, S

Species= bird species for which blood meal data are available in our study area (n=20); + = bird species

for which mortality data are available in available study area ($n=18$); ¹Minimum and maximum percentages of WNV sero-positive birds ($n = \text{sample size}$) (Komar et al. 2001, Ringia et al. 2004, Godsey et al. 2005, Komar et al. 2005, Gibbs et al. 2006, Dusek et al. 2009, Loss et al. 2009, Dubé et al. 2010, Chuang et al. 2011, Kilpatrick et al. 2013, Komar et al. 2013, Randall et al. 2013); ² Minimum and maximum index of host competence for WNV (Komar et al. 2003, Kilpatrick et al. 2007, Wheeler et al. 2009); ³ Percentage of dead birds positive to WNV ($n = \text{sample size}$) (CWHC); ⁴ a_s is the density of species s divided by the total density of the avian community, data come from the EPOQ database. The sum of all a_s is equal to one; ⁵ f_i is the fraction of total blood meals taken by *Cx. pipiens-restuans* from host s (INSPQ 2016). The sum of all f_i is equal to one; ⁶ = M: Migratory bird species; R: Resident bird species; ⁷ = S: South USA; N: North USA; QC: Quebec.

Figure captions

Figure 1. Yearly number of cases of West Nile virus (WNV) infection in humans and number of dead birds positive to WNV in Quebec 2002 - 2017. Insert: Correlation between the number of West Nile virus cases diagnosed in birds and the number of cases of WNV observed in humans in Quebec (log scales).

Figure 2. Study area with locations of collection of dead birds (CWHC), bird watching and counts (EPOQ), and collection of female *Cx. pipiens-restuans* on the island of Montreal, Quebec.

Figure 3. List L_1 : ranked list ($n = 18$) of dead birds found by WNV passive bird surveillance. RR stands for relative risk or ratio of dead birds positive to WNV. Quoted letters “M” and “R” stand for migratory and resident birds, respectively.

Figure 4. List L_2 : ranked list ($n = 23$) of bird species found from analysis of *Cx. pipiens-restuans* blood meals. Left panel: Relative abundance ($n \times a_s$) of bird species and relative feeding (fraction of *Cx. pipiens-restuans* blood meals) ($n \times f_s$). Dashed vertical lines at relative abundance and feeding “1” represent the ratio “1/n” where $n = 23$ is the bird species diversity. Right panel: relative host preference ($n \times p_s$) of *Cx. pipiens-restuans*. Quoted letters “M” and “R” stand for migratory and resident birds, respectively.

Figure 5. Proportion of *Culex pipiens-restuans* blood meals taken from birds of all species by week. Data points correspond to field data and the solid line through the data to the predicted proportions using the logistic regression model.

Figure 6. Selection process and result of the literature review.

Figure 7. List L_3 : ranked list ($n = 53$) of bird species found from the literature review. RR stands for relative risk or ratio of sero-positives. Quoted letters “M” and “R” stand for migratory and resident birds, respectively.







