

## ORIGINAL ARTICLE

# Wild Birds as Sentinels for Multiple Zoonotic Pathogens Along an Urban to Rural Gradient in Greater Chicago, Illinois

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## Impacts

- We assessed exposure and infection of wild birds with multiple zoonotic pathogens across a gradient of urbanization.
- Birds from more urban sites had higher exposure to West Nile virus than those from more forested sites, and overall seroprevalence was 3.5%. Blacklegged ticks, although rare on birds, harboured high infection prevalence (>50%) with the Lyme disease pathogen. Less than 1% of birds were shedding *Salmonella*.
- Elucidating the relationships among wild birds, zoonotic pathogens, vectors and the urban landscape will allow for assessment of disease risk in urban zones, where high-density human populations are at risk of exposure.

## Keywords:

Zoonoses; wild birds; urbanization; *Borrelia burgdorferi*; *Salmonella*; West Nile virus

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## Summary

Wild birds are important in the maintenance and transmission of many zoonotic pathogens. With increasing urbanization and the resulting emergence of zoonotic diseases, it is critical to understand the relationships among birds, vectors, zoonotic pathogens, and the urban landscape. Here, we use wild birds as sentinels across a gradient of urbanization to understand the relative risk of diseases caused by three types of zoonotic pathogens: *Salmonella* pathogens, mosquito-borne West Nile virus (WNV) and tick-borne pathogens, including the agents of Lyme disease and human anaplasmosis. Wild birds were captured using mist nets at five sites throughout greater Chicago, Illinois, and blood, faecal and ectoparasite samples were collected for diagnostic testing. A total of 289 birds were captured across all sites. A total of 2.8% of birds harboured *Ixodes scapularis* – the blacklegged tick – of which 54.5% were infected with the agent of Lyme disease, and none were infected with the agent of human anaplasmosis. All infested birds were from a single site that was relatively less urban. A single bird, captured at the only field site in which supplemental bird feeding was practised within the mist netting zone, was infected with *Salmonella enterica* subspecies *enterica*. While no birds harboured WNV in their blood, 3.5% of birds were seropositive, and birds from more urban sites had higher exposure to the virus than those from less urban sites. Our results demonstrate the presence of multiple bird-borne zoonotic pathogens across a gradient of urbanization and provide an assessment of potential public health risks to the high-density human populations within the area.

## Introduction

Wild birds play important roles in the maintenance and movement of zoonotic pathogens (Williams, 1957; Cooper, 1990; Reed et al., 2003; Benskin et al., 2009), and zoonotic pathogens comprise over 75% of all emerging human diseases (Taylor et al., 2001). Recently, the influence of the urban environment on zoonotic disease has been receiving more attention (i.e. Beran, 2008; Campos et al., 2008; Makita et al., 2008; Soriano et al., 2010). Key features of the urban environment that promote the transmission of pathogens include increased host contact rates and susceptibility to infection, high rates of pathogen introductions, pollution and stress that reduce host immune function, and warmer microclimates and reduced seasonality that promote vector populations and allow environmental persistence of some parasites (Bradley and Altizer, 2007). The role of birds in the maintenance and transmission of zoonotic pathogens in an urban environment has not been comprehensively addressed.

Some bird-borne zoonotic pathogens may have increased prevalence in urban areas owing to human-driven mechanisms. For example, supplemental wild bird feeding, which occurs commonly in the urban environment (Jones and James Reynolds, 2008), has been linked with increases in the prevalence of some bacterial species within wild birds (Brittingham et al., 1988). Supplemental feeding promotes disease emergence by creating high densities of birds, high concentration of faeces and stress owing to social interactions (Daoust and Prescott, 2007). One example of a class of zoonotic pathogens that is transmitted at bird feeders are bacteria within the genus *Salmonella*, which includes approximately 2500 serotypes that have been recovered from birds, mammals and reptiles. While most bird infections are subclinical, salmonellosis is a cause of sporadic mortality particularly among young birds in large breeding colonies and songbirds around feeders in winters. Among wild birds, the most commonly isolated serotype is *S. enterica* subspecies *enterica* serovar typhimurium, which appears to be adapting to songbird species that frequent bird feeders (Daoust and Prescott, 2007).

The increased incidence of some vector-borne zoonoses in the urban environment may reflect patterns of vector abundance. For example, urban environments harbour structural features that promote poor drainage and stagnant water, including catch basins, roadside ditches, sewage lagoons and manmade containers. These sites promote larval development of *Culex pipiens* mosquitoes (Geery and Holub, 1989), the main vector of West Nile virus (WNV) in eastern United States. A recent spatial analysis concluded that incidence of human WNV disease

was greatest in urban areas in the eastern United States owing to the urban distribution of the vector *Cx. pipiens* (Bowden et al., 2011). West Nile virus first arrived in North America in 1999 and rapidly spread from New York City to much of North and South America in one decade and has become one of the most widely distributed arboviruses in the world (Weaver and Reisen, 2010). In the last decade, approximately one million people in the United States are estimated to have been exposed to WNV, resulting in nearly 30 000 human clinical cases, and over 1000 fatalities (Gyure, 2009).

One ecological impact of urbanization is the loss of biodiversity in urban cores as compared to more rural or forested areas (McKinney, 2002). The dilution theory predicts that for some vector-borne generalist pathogens, the presence of hosts that support vector feeding but not pathogen amplification may dilute the transmission of the pathogen, and this phenomenon should be most apparent in natural areas with diverse host communities as compared to more fragmented or urban areas with reduced host diversity. Pathogen dilution occurs because vectors 'waste' their bites blood-feeding on hosts that are not efficient reservoirs for the pathogens (Dobson, 2004). The dilution theory has been supported in some, but not all, empiric and modelling studies across three zoonotic disease systems that all involve birds in their maintenance and transmission – Lyme disease, WNV and human anaplasmosis (Ostfeld and Keesing, 2000; Schmidt and Ostfeld, 2001; Allan et al., 2003; LoGiudice et al., 2003; Estrada-Pena et al., 2008; Swaddle and Calos, 2008; Loss et al., 2009; Ogden and Tsao, 2009). The relationships between avian host biodiversity and vector-borne zoonotic disease risk have been best studied in the WNV system. Because bird diversity decreases in urban areas, and because WNV incidence is greatest in urban areas, a correlation is present. Two independent studies, however, both concluded that it is not simply avian host biodiversity in a given area that sculpts WNV, but instead, it is the reservoir competence of hosts on which *Culex* mosquitoes feed that drive pathogen transmission and disease risk (Hamer et al., 2011a; Simpson et al., 2012).

Here, we hypothesize that wild birds across a continuum of urbanization will exhibit a gradient of infection for multiple zoonotic pathogens, in which birds captured in urban zones will be more highly infected than those captured in more rural or forested zones. The objective of this study was to assess the relationships among wild bird populations, zoonotic pathogens and the urban environment through sampling populations of birds collected at multiple sites that constitute a gradient of urbanization. Specifically, we aimed to determine (i) infestation prevalence with ticks and infection prevalence of ticks with *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, the

agents of Lyme disease and human anaplasmosis, respectively; (ii) prevalence of faecal shedding of *Salmonella* pathogens; and (iii) seroprevalence of WNV antibodies and infection prevalence WNV. Data on the occurrence of zoonotic pathogens within natural reservoirs in urban environments are critical for understanding potential health risks posed to the high density of humans that reside within urban and suburban environments.

## Materials and Methods

### Bird mist netting

In June and July 2010, birds were captured at five sites that constitute a gradient of urbanization in the greater Chicago-land area (Fig. 1). Sites 1–5 are urban green spaces, forest preserves and private open spaces in Des Plaines, Batavia, Wheaton, Maywood and Lemont, Illinois, respectively. One morning per week per site, we used eight to ten 12-m mist nets (Avinet, Dryden, NY, USA) to capture birds. The nets were run from sunrise for approximately 6 h on fair weather days and checked hourly. To determine mist netting success rate, 1 net hour is defined as the equivalent of one 12-m net run for 1 h. From each captured bird, vital data were obtained, including species, sex, age class (hatch-year or after hatch-year) and weight, and a federally issued leg band was attached before release. Recaptures of previously banded birds were noted. All birds were checked for ticks by inspecting the ears and region around the head. Ticks were removed and preserved in 70% ethanol. Blood was sampled by jugular venipuncture using a 29-gauge, half-inch insulin syringe (Becton Dickinson, Franklin Lakes,

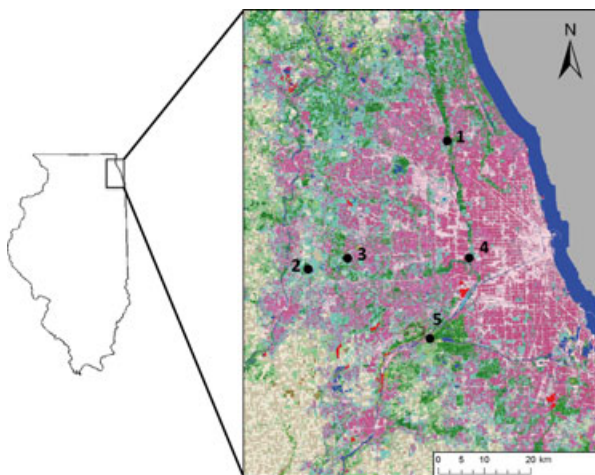
NJ, USA). The volume of blood collected varied by bird size but did not exceed 1% of the bird's body weight or 0.2 mL. Blood was added to 0.8 mL of BA-1 diluent (as described by Nasci et al. (2002) in a microcentrifuge tube. Blood was stored on ice packs in the field and centrifuged within 5 h. Serum and BA-1 was pipetted and placed in a 2.0-mL cryovial; clots and the serum were stored at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ . Faecal samples were taken with sterile cotton swabs from the cloaca of each captured bird. Swabs were incubated in 1 mL of buffered peptone water (Fluka, Buchs, Switzerland), a medium for the non-selective pre-enrichment of bacteria, in particular pathogenic members of the Enterobacteriaceae, at  $37^{\circ}\text{C}$  for 16–20 h, and then stored at  $-20^{\circ}\text{C}$ . All fieldwork was carried out under appropriate collecting permits with approvals from Michigan State University's Institutional Animal Use and Care Committee permit 02-07-13-000 and Lincoln Park Zoo's animal use committee.

### Detection of tick-borne pathogens

Ticks were identified to species and stage (Sonenshine, 1979; Durden and Keirans, 1996). Total DNA from ticks was extracted by using a DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's animal tissue protocol, but with modifications as described previously (Hamer et al., 2010). Nymphal ticks were extracted individually, whereas same-species larvae from the same individual animal were pooled for extraction. All ticks were tested for the presence of *B. burgdorferi* by using a quantitative PCR (qPCR) of a region of the 16S rRNA gene (Tsao et al., 2004) using a PCR enzyme kit (Universal PCR Master Mix; Applied Biosystems, Foster City, CA, USA). A six-point standard dilution series of DNA extracted from cultured *B. burgdorferi* ( $10^4$ – $10^{-1}$  organisms per  $3\ \mu\text{L}$  reaction volume) served as positive controls, and water served as a negative control. Reactions for qPCR were performed with an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). All ticks were tested for the presence of *A. phagocytophilum* by PCR of the *p44* gene using primers and protocol as described by Holden et al. (2003) in a  $25\text{-}\mu\text{L}$  reaction volume. Amplicons were visualized using agarose gel electrophoresis.

### Detection of *Salmonella*

The enriched cloacal samples were thawed and centrifuged at 7500 rpm for 10 min. The supernatant was removed, and the pelleted material was subjected to a DNA extraction as described earlier, but following the protocol for gram-negative bacteria. Two and a half microlitres of the eluted material was used as template for



**Fig. 1.** Map of five field sites in greater Chicago, Illinois. The background map is land satellite land cover imagery, in which urban areas are pink and purple, forested areas are green, and agricultural land is yellow.

PCR amplification with primers 139 and 141 to generate a 284-bp fragment of the *invA* gene that encodes a protein of a Type III secretion system that is essential for the invasion of epithelial cells by the bacterium (Rahn et al., 1992). This assay detects all *Salmonella enterica* subspecies as well as *Salmonella bongori*. The PCR was carried out in a total reaction volume of 25  $\mu$ L using a PCR enzyme kit (FailSafe PCR System; Epicentre, Madison, WI, USA) following the method described by Gaertner et al. (2008), followed by agarose gel electrophoresis. DNA from cultures of *Salmonella typhimurium* and *Salmonella* Enteritidis served as positive controls.

DNA from suspect-positive *Salmonella* samples was sequenced to confirm the species identity. Briefly, the PCR product was purified (Qiagen PCR purification kit; Qiagen, Valencia, CA, USA), and sequences were determined in both directions by using primers used for the original PCR on an ABI PRISM 3100 genetic analyzer (Applied Biosystems). Sequences were compared with published sequences using the basic local alignment search tool in GenBank (Altschul et al., 1990).

#### Detection of West Nile virus and antibodies to the virus

We used blocking enzyme-linked immunosorbent assay (ELISA) for detection of WNV antibodies in bird serum samples similar to the method of Blitvich et al. (2003) but with modifications as described by Hamer et al. (2008). Briefly, the inner 60 wells of a 96-well EIA/RIA medium-binding microtiter plate (Corning Incorporated 3591) were loaded with a 1 : 12 000 dilution of 4G2 capturing antibody and coating buffer and incubated overnight at 37°C. Plates were washed six times with PBS-Tween 20, and then, wells were blocked with a milk-PBS solution (BIO RAD non-fat dry milk) and incubated for 2 h at 37°C. Plates were washed and a 1 : 50 dilution of WNV antigen and PBS was loaded into wells and incubated for 2 h. Plates were washed and 100  $\mu$ L of field-collected serum (1 : 20 dilution with BA-1) was loaded along with positive and negative controls. The plate was incubated for 2 h, washed and wells were loaded with a 1 : 4000 dilution of 6B6C-1 monoclonal antibody (MAb) labelled with horseradish peroxidase and milk-PBS. After another 2-h incubation and washing, 100  $\mu$ L of tetramethylbenzidine (Sigma Aldrich Inc., St. Louis, MO, USA) was added and then incubated and stopped with 50  $\mu$ L of sulphuric acid. The reduction in optical density was determined with plate blanks subtracted at a wavelength of 450 nm on an automated plate reader (Molecular Devices, Sunnyvale, CA, USA). Per cent inhibition was calculated as  $(1 - (TS/CS) * 100)$ , where TS is the optical density of the test serum, and CS is the mean optical density of

the negative control serum. Two different positive controls and four negative controls were used on each plate. Samples testing positive on the first screen were serially diluted and tested to find the endpoint titre. Samples with a per cent inhibition of 60 or greater were considered positive for exposure to WNV.

We tested bird serum samples for the presence of WNV RNA as described previously (Hamer et al., 2008). RNA was extracted from serum using an ABI PRISM 6100 Nucleic Acid Prep Station following the Tissue RNA Isolation Protocol (Applied Biosystems; P/N 4330252). We extracted RNA from 100  $\mu$ L of bird serum in a 1 : 20 dilution with BA-1 using a protocol developed for the isolation of viral RNA from non-cellular samples on the ABI 6100 Nucleic Acid Prep Station. RNA was eluted in a final volume of 60  $\mu$ L. A region of the WNV RNA envelope gene was detected using real-time, reverse transcription polymerase chain reaction (RT-PCR) (Lanciotti et al., 2000). Thermocycling was performed on an ABI PRISM 9700HT sequence detector at the Research Technology Support Facility at Michigan State University, following the Taq-Man One-Step RT-PCR Master Mix Protocol (Applied Biosystems; P/N 04310299).

#### Urbanization metric

We quantified the land use around each site by computing an urbanization index (UI), similar to that used by Gomez et al. (2008), within a buffer of 2000 m radius as follows:

$$UI = (100\% - \% \text{green space} + \text{road density})/2$$

Green space was estimated by combining land classified as forested and as urban open space using remotely sensed data at 30-m resolution derived from Illinois Gap Analysis Project data within a geographic information system (ARCGIS 10.0; ESRI, Redlands, CA, USA). Land cover classes were derived from the National Land Cover Dataset as described by Anderson et al. (1976) in which forested lands are defined as having a tree-crown areal density of 10 per cent or more, are stocked with trees capable of producing timber or other wood products and exert an influence on the climate or water regime. Urban open space includes parks, botanical gardens, arboreta, cemeteries and golf courses; some areas that would otherwise meet the definition as forested are placed into this category because of their adjacency to urban features (Anderson et al., 1976). To better assess our hypotheses, we combined these landscape features into a 'green space' category, which discounts the urbanization score in our metric. Road density is the total metres of road within



the area encompassed by the 2000 m radius buffer and was obtained using the infrastructure data layers available in the Illinois Natural Resources Geospatial Data Clearinghouse (<http://www.isgs.uiuc.edu/nsdihome/webdocs/st-admin.html>). Road density is an ecologically relevant measure of the urban environment that captures elements of landscape fragmentation (Medley et al., 1995). To identify the extent to which our UI explained variation in pathogen prevalence, pathogen parameters (when data allowed) were plotted against the UI and regression coefficients ( $r^2$ ) were calculated.

## Results

### Bird captures

A total of 289 captures occurred across all study sites, representing 281 individual birds. The overall mist netting success rate was 48.4 birds per 100 net hours and differed among sites ( $df = 4$ ;  $F = 12.08$ ;  $P < 0.0001$ ), in which success was higher at Site 5 than at all other sites ( $P < 0.01$ ), with 81 birds per 100 net hours captures at this site. Totals of 67, 56, 51, 55 and 60 birds were captured at Sites 1–5, respectively. A total of 34 species of birds were captured, in which five species alone comprised over half (53.6%) of the data set: Gray Catbird (*Dumetella carolinensis*), American Robin (*Turdus migratorius*), American Goldfinch (*Carduelis tristis*), Brown-headed Cowbird (*Molothrus ater*) and Red-winged Blackbird (*Agelaius phoeniceus*). Across all captures, the sex ratio was fairly even (41.2% female, 36.0% male and 22.8% unknown sex), and a majority of birds were of the after-hatch-year age class (71.3% after hatch-year, 22.8% hatch-year and 5.2% unknown age class). A total of eight recaptures occurred during the study, including one

Wood Thrush (*Hylocichla mustelina*) that was captured three times, three Gray Catbirds and one each of Downy Woodpecker (*Picoides pubescens*), Indigo Bunting (*Passerina cyanea*) and American Goldfinch.

### Ticks on birds and their infection with *B. burgdorferi* and *A. phagocytophilum*

Ticks were found on eight birds representing 2.8% of all captures. All infested birds were from Site 5, where 13.3% of birds were infested. A total of 13 ticks were removed from eight birds. All ticks were identified as *Ixodes scapularis*, the blacklegged or deer tick, and both larvae and nymphs were present (Table 1). The host birds from which these ticks were removed include three hatch-year and five after-hatch-year birds. Eleven total tick samples were tested for pathogens (three larvae removed from the same bird were pooled for testing), of which 6 (54.5%) were positive for *B. burgdorferi*, and none were positive for *A. phagocytophilum* (Table 1). Among those ticks testing positive for *B. burgdorferi* was a single *I. scapularis* larva – owing to the negligible transovarial transmission in this system, this finding may implicate the Red-winged Blackbird from which it was removed as an infectious, competent reservoir host. However, an infected *I. scapularis* nymph was simultaneously removed from this bird, and therefore, co-feeding transmission cannot be ruled out.

### Faecal shedding of *Salmonella*

A random subset of 180 faecal swabs was tested for infection with *Salmonella*, representing 61.2%, 85.7%, 56.9%, 49.1% and 58.3% of birds collected from Sites 1–5,

**Table 1.** Infection of ticks removed from birds with *B. burgdorferi* and *A. phagocytophilum*. All birds with ticks were captured at Site 5 in suburban Chicago, Illinois, June–July 2010

Bird Species-ID	Bird Sex	Bird Age	Tick Species	Stage/Quantity	<i>B. burgdorferi</i>	<i>A. phagocytophilum</i>
COGR-1	M	HY	<i>I. scapularis</i>	LL/3	NEG	NEG
RWBL-1	F	HY	<i>I. scapularis</i>	NN/1	POS	NEG
BHCO-1	U	HY	<i>I. scapularis</i>	NN/1	NEG	NEG
RWBL-2	F	AHY	<i>I. scapularis</i>	NN/1	POS	NEG
			<i>I. scapularis</i>	LL/1	POS	NEG
HOWR-1	F	AHY	<i>I. scapularis</i>	NN/1	POS	NEG
			<i>I. scapularis</i>	NN/1	NEG	NEG
			<i>I. scapularis</i>	LL/1	NEG	NEG
RBGR-1	M	AHY	<i>I. scapularis</i>	NN/1	POS	NEG
RWBL-3	F	AHY	<i>I. scapularis</i>	NN/1	POS	NEG
RBGR-2	M	AHY	<i>I. scapularis</i>	NN/1	NEG	NEG

Common Grackle, COGR; Red-winged Blackbird, RWBL; Brown-headed Cowbird, BHCO; House Wren, HOWR; Rose-breasted Grosbeak, RBGR; M, male; F, female; U, unknown sex; HY, hatch-year; AHY, after hatch-year; LL, larvae; NN, nymph.

respectively. Only one sample tested positive (0.6% of all tested samples); this sample was from a female, after-hatch-year Red-winged Blackbird from Site 5. The final *invA* DNA sequence was 300 nucleotides in length and matched with 100% sequence homology to Genbank entries of *S. enterica* subspecies *enterica* including serovars Dublin, Enteritidis, Gallinarium, Typhi, and Paratyphi A.

#### Avian exposure to and infection with West Nile virus

Overall seroprevalence of antibodies to WNV among the birds of all sites was nine of 260 (3.5%), and all seropositive birds were after hatch-year. As the majority of bird captures took place prior to the seasonal occurrence of WNV amplification in this region, we also calculated the seroprevalence of after-hatch-year birds only, which was nine of 184 (4.9%). Seropositive birds included five

Northern Cardinals (*Cardinalis cardinalis*) and one each of Wood Thrush, Brown-headed Cowbird, Rose-breasted Grosbeak (*Pheucticus ludovicianus*) and Common Grackle (*Quiscalus quiscula*; Table 2). Seropositive birds came from four of the five field sites. The endpoint titres of these seropositive birds ranged from 1 : 20 to 1 : 80. One of the seropositive birds, an after-hatch-year male Wood Thrush, was captured on June 25, July 2 and July 6, and only the third blood sample taken tested seropositive. A total of 120 randomly selected blood samples were tested for the presence of WNV, of which none tested positive.

#### Urbanization metric

Urbanization indices for the five sites, from most to least urban, are 42.46 (Site 1), 37.98 (Site 4), 37.55 (Site 3), 35.07 (Site 5) and 1.15 (Site 2). Given the lack of patho-

**Table 2.** West Nile virus seroprevalence of wild birds captured from five sites in greater Chicagoland, Illinois, June–July 2010. For scientific names of birds, please refer to the American Ornithologists' Union Check-list of North American bird species (AOU 2011)

Sample size tested for WNV antibodies (percent seropositive)						
Avian species	Site 1	Site 2	Site 3	Site 4	Site 5	All sites
American Goldfinch		11	2	13		26
American Robin	11		10	13		34
Baltimore Oriole			1			1
Barn Swallow		3	5			8
Black-capped Chickadee	1	1	5			7
Brown-headed Cowbird	5 (20.0)	1	2	2	17	27 (3.7)
Blue Jay					2	2
Cedar Waxwing	1	2				3
Common Grackle			2 (50.0)	1	1	4 (25.0)
Common Yellowthroat				3		3
Dickcissel						
Downy Woodpecker	6	1	5		1	13
Eastern Phoebe		1	1			2
Eastern Towhee	1					1
Eastern Wood-peewee			1			1
European Starling			3	2		5
Field Sparrow	5					5
Gray-cheeked Thrush	1					1
Gray Catbird	17	14	1		6	38
House Wren						
Indigo Bunting	1			3	2	6
Mourning Dove					1	1
Northern Cardinal	4 (50.0)	5	3 (66.6)	2 (50.0)	1	15 (33.3)
Rose-breasted Grosbeak					9 (11.1)	9 (11.1)
Red-headed Woodpecker					1	1
Red-winged Blackbird	3		3		12	18
Savannah Sparrow	1	1		2		4
Song Sparrow	3	2	4	6		15
White-breasted Nuthatch					1	1
Wood Thrush	3 (33.3)					3 (33.3)
Yellow-sided Flycatcher	1					1
Yellow-throated Vireo			1			1
Yellow Warbler		4				2
	64 (6.3)	46	49 (6.1)	47 (2.1)	54 (1.9)	260 (3.5)

gen prevalence across more than one site for tick-borne pathogens and *Salmonella*, our urbanization analysis is restricted to an assessment of the nature and significance of urbanization on bird exposure to WNV. There was a positive trend between WNV seroprevalence and UI ( $r^2 = 0.53$ ;  $P = 0.08$ ), in which birds from sites with higher urbanization indices had higher exposure to WNV.

## Discussion

Our study demonstrates that multiple zoonotic pathogens, including WNV, the agent of Lyme disease, and *Salmonella*, as well as blacklegged ticks, are all associated with wild birds in greater Chicago, Illinois. These data are useful in understanding not only relative risk of diseases to wild bird populations, but also to the high-density human populations that characterize the area. Furthermore, we found that avian exposure to WNV marginally increases with level of urbanization. This relationship has been reported previously; for example, in Georgia the WNV antibody prevalence among songbirds at urban sites was approximately double that of birds from non-urban sites (Bradley et al., 2008). Gomez et al. (2008) found that the probability of mammals being WNV antibody-positive increased with the UI. The structural features of the urban environment that promote development of the mosquito vector (Bowden et al., 2011) as well as the patterns of avian host biodiversity and avian community reservoir competence for the virus (Hamer et al., 2011a; Simpson et al., 2012) likely contribute to this trend. Given the high density of humans in urban cores, the increased virus activity in these areas presents a heightened public health risk.

We found 3.5% of birds across all sites to be seropositive for WNV, with no virus-positive birds. This low exposure rate and lack of viremic birds is not unexpected considering the early summer time of sample collection in June and July, which is prior to the main WNV amplification event in the area that occurs in August (Ruiz et al., 2010). All seropositive birds in our study were after-hatch-year animals, and it is likely that the presence of circulating antibodies reflects exposure in a previous year. One exception, however, is a Wood Thrush that was captured and sampled on three separate occasions and seroconverted in early July – a finding that is likely indicative of recent exposure. One-third of the Northern Cardinals in our study were seropositive, with positive individuals collected at three different study sites. Northern Cardinals have been among the species with the highest seroprevalence in other studies; for example, 27%, 52% and 12.4% of Northern Cardinals were positive in studies in Georgia, Chicago and central Illinois, respectively (Ringia et al., 2004; Bradley et al., 2008; Loss et al., 2009).

Birds play many important roles in the ecology of tick-borne diseases, including pathogen reservoirs, blood meal hosts, dispersal agents for both the ticks and pathogens, as well as possibly serving as a mechanism for pathogen dilution (Giardina et al., 2000; LoGiudice et al., 2003) or pathogen acceleration (Hamer et al., 2011b). At Site 5, *I. scapularis* infested over 13% of all birds, and over 50% of these ticks were infected with *B. burgdorferi*. Hatch-year birds were likely to have been born on site, thus indicating local acquisition of *I. scapularis*. Our findings reflect the continued invasion of this tick and pathogen across the Midwest and constitute a public health risk. New populations of *I. scapularis* infected with *B. burgdorferi* were recently described in Chicago (Jobe et al., 2006, 2007). Human Lyme disease cases are increasing in Illinois – in 2000, there were 35 reported human cases of Lyme disease and in 2009 there were 136 cases (IDPH, 2011). *Ixodes scapularis* was found only at one field site, reflecting what is likely a patchy distribution and low population density and limiting the ability to understand the effects of the urban environment on tick and tick-borne pathogen prevalence. As our camera trap survey data show lower detections of deer with higher degrees of urbanization (Magle, unpublished data), we speculate that the lack of *I. scapularis* at some of our sites may relate to patterns of deer distribution, as deer are important hosts on which *I. scapularis* can complete its life cycle.

None of the bird-derived ticks we tested were positive for infection with *A. phagocytophilum*. Wild birds in North America and Europe can serve as reservoirs for this pathogen (Daniels et al., 2002; Skoracki et al., 2006). Future studies with larger sample sizes of larval ticks, as well as sero-surveillance of wild birds, will be useful in elucidating the influence of urbanization on the ecology of *A. phagocytophilum* and the role that birds play.

We found faecal shedding of *Salmonella* to be rare among the wild birds in our study, with <1% of faecal swabs found to be positive. This is likely because passive mist netting did not result in the capture of many birds that typically aggregate at bird feeders. Of interest is that the single bird that tested positive was from Site 5, which is the only site observed to have active bird feeders within the mist netting area. *Salmonella* pathogens are one of the main causes of human food-borne illness throughout the world, and wild bird infection usually reflects contamination of the environment by humans or livestock (Cizek et al., 1994).

Limitations of our study include a limited diversity of landcover from which birds were collected, and a season of collection that did not encompass the full mosquito season. Future longitudinal studies conducted at additional field sites to encompass more diversity in landcover, including rural sites and city centers, will be useful in

better elucidating the mechanisms of increased zoonotic pathogen prevalence in urban environments. Understanding of the various relationships between birds and emerging zoonotic diseases will be critical for managing disease risk.

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