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City sanitation and socioeconomics predict rat zoonotic infection across diverse neighbourhoods

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Abstract

Rat-associated zoonoses transmitted through faeces or urine are of particular concern for public health because environmental exposure in homes and businesses may be frequent and undetected. To identify times and locations with greater public health risks from rats, we investigated whether rat characteristics, environmental features, socioeconomic factors, or season could predict rat infection risk across diverse urban neighbourhoods. In partnership with a pest management company, we sampled rats in 13 community areas along an income gradient in Chicago, a large city where concern about rats has increased in recent years. We collected kidneys for *Leptospira* spp. testing and colon contents for aerobic bacteria such as *Salmonella* spp. and *Escherichia coli*. Of 202 sampled rats, 5% carried *Leptospira* spp. and 22% carried *E. coli*. Rats were significantly more likely to carry *Leptospira* spp. on blocks with more standing water complaints in higher-income neighbourhoods (OR = 6.74, 95% CI: 1.54–29.39). Rats were significantly more likely to carry *E. coli* on blocks with more food vendors (OR = 9.94, 2.27–43.50) particularly in low-income neighbourhoods (OR = 0.26, 0.09–0.82) and in the spring (OR = 15.96, 2.90–88.62). We detected a high diversity of *E. coli* serovars but none contained major virulence factors. These associations between environmental features related to sanitation and infection risk in rats support transmission through water for *Leptospira* spp. and faecal–oral transmission for *E. coli*. We also found opposing relationships between zoonotic infection risk and income for these two pathogens. Thus, our results highlight the importance of sanitation for predicting zoonotic disease risks and including diverse urban areas in pathogen surveillance to mitigate public health risks from rats.

KEYWORDS
disease reservoirs, sanitation, socioeconomic factors, urban rats, zoonoses

1 | INTRODUCTION

Living with rats creates challenges for public health in cities worldwide. Commensal rats (brown rats *Rattus norvegicus* and black rats *Rattus rattus*, hereafter 'rats') can carry several zoonotic pathogens that can be transmitted to humans via contact with rat urine (i.e., *Leptospira interrogans*) or faeces (e.g., *Escherichia coli*; Himsworth, Bidulka, et al., 2013; Himsworth, Parsons, Jardine, & Patrick, 2013). These rat-associated zoonoses are transmitted through environmental contamination and are of particular concern for public health because they do not require direct contact with rats. Exposure may thus be more frequent or undetected. For example, Leptospirosis is an emerging zoonotic disease with roughly 1 million cases and 58,000 deaths per year (Bharti et al., 2003; Costa, Hagan, et al., 2015). Although traditionally considered a rural disease, sporadic outbreaks of Leptospirosis have been reported in urban centres (Vinetz, Glass, Flexner, Mueller, & Kaslow, 1996) including tropical slums (Hagan et al., 2016) and more recently in European cities (Dupouey et al., 2014). These outbreaks have led to increasing concern about expanding urban rat populations because households infested with rats can have a higher risk of infection (Hagan et al., 2016). Rat faeces can also contain antimicrobial-resistant and pathogenic strains of bacterial pathogens known to cause diarrhoeal diseases in humans (i.e., *Salmonella enterica*, *Escherichia coli*, and *Staphylococcus aureus*) (Guenther et al., 2012; Himsworth et al., 2015). Thus, even living in proximity to rat infestations can increase public health risks for significant zoonotic infections.

To minimize public health risks via targeted rodent control, it is critical to understand the ecology of rats and rat-associated zoonoses to identify biological, spatial, and temporal predictors of rat infection. Although urban rat ecology is complex and a growing field of study, rats are often more abundant (Rael, Peterson, Ghersi, Childs, & Blum, 2016) and can be more likely to carry *L. interrogans* (Ayral et al., 2015) in neighbourhoods with lower incomes. This is likely because fewer resources are available for property maintenance and rodent control. Local habitat features such as sanitation and food waste may be strong determinants of spatial heterogeneity in rat infection because of their role in rat or pathogen ecology. For example, the presence of food waste in garbage can promote rat infestations (Murray et al., 2018) and potentially the transmission of faecal–oral pathogens such as *E. coli* and *Salmonella* due to faecal contamination and contact with food waste (Nelson, Jones, Edwards, & Ellis, 2008). Other sanitation concerns, such as standing water from poor drainage, can create microhabitat conditions suitable for transmission of *Leptospira* spp. via contact with contaminated surface water (Ganoza et al., 2006). Variation in rat infection risk is likely also due to individual rat characteristics because rats that are older (Himsworth et al., 2014) and have more injuries (Himsworth, Bidulka, et al., 2013; Himsworth, Parsons, Jardine, & Patrick, 2013) can be more likely to carry some pathogens. Seasonal changes in weather may also affect pathogen transmission via seasonal peaks in population density (Feng & Himsworth, 2014) and the persistence of bacteria in the environment (Van Elsas, Semenov, Costa, & Trevors, 2011). Because of these factors, rat-associated zoonotic disease risk varies across space and time. Understanding the spatiotemporal dynamics of rats and their zoonoses will help to mitigate public health risks.

Given that environmental and socioeconomic factors can promote rat abundance and that increased rat density may promote pathogen spread among rats, we investigated whether these factors could be used to predict areas of greater rat-associated zoonotic risk. We studied predictors of rat zoonotic infection risk in Chicago, Illinois, USA, a large city with increasing concerns about urban rats (Murray et al., 2018) and greater disparities in income than the national average (Asante-Muhammad, 2017). We sampled rats along an income gradient and tested them for the environmentally transmitted zoonotic pathogens *Leptospira* spp., *E. coli*, *Salmonella*, *Campylobacter* spp., and *Staphylococcus aureus*. We predicted that rats would be more likely to carry *Leptospira* spp. if they were sampled near areas with drainage issues, because this pathogen is transmitted through water, and more likely to carry *Salmonella* and *E. coli* if they were sampled in areas with more food waste because they are transmitted through faecal–oral contact. We also predicted that infection risk would be higher in areas with lower incomes and higher rat densities. Because rat characteristics (i.e., age, sex, injuries) and season have been found to influence whether rats carry certain pathogens, we also evaluated how these factors influenced pathogen status. Identifying the contexts in which urban residents are more at risk of rat-associated zoonoses will help mitigate public health concerns in cities struggling with rodent pests.

Impacts

- Rats were more likely to be infected with *Leptospira* spp. on blocks with standing water issues in higher-income neighbourhoods, which highlights that surveillance should not be limited to low-income communities.
- Rats were more likely to carry *Escherichia coli* on blocks with more food vendors, particularly in low-income neighbourhoods. Although we did not detect concerns for human disease, rat infection near restaurants provides opportunities for food contamination.
- Sanitation concerns and socioeconomic status in communities may be important in predicting some rat-associated zoonotic disease risks over large and diverse urban areas.

2 | METHODS

2.1 | Study area

We studied rat disease ecology in Chicago, the third largest city in the United States with a population of 2.7 million (United States Census Bureau, 2017), which borders Lake Michigan and has warm humid summers and cold winters (Wolfram Alpha, 2020). The city is composed of over 200 neighbourhoods, grouped into 77 community areas whose permanent boundaries were defined by the City of Chicago (CMAP, 2017). From 2013 to 2018, public complaints about rats increased by 34% and these complaints appear to reflect differences in rat abundance across community areas (Murray et al., 2018). Chicago neighbourhoods also differ in income, with affluent neighbourhoods earning median household incomes nearly seven times higher than the least affluent neighbourhoods (CMAP, 2017). To capture this variation in rat conflicts and socioeconomic status, we sampled rats in 13 community areas that varied in the number of rat

TABLE 1 Summary of community areas in Chicago selected for rat sampling

Community area	Rats sampled (*n*)	*Leptospira* spp. positive (prevalence)	*E. coli* positive (prevalence)	*E. coli* serovars detected	Median Household Income (USD)	Standing water complaints	Food vendors	Rat complaints
Armour Square (AS)	19	1 (0.05)	10 (0.53)	O10:H56, O129:H48, O153:H14, O17:H41, O32:H12, O7:H7, O71:H45, O8:H25	$24,336.46	1	213	158
Beverly (BE)	1	0 (0.00)	1 (1.00)	O45:H8	$90,765.77	11	56	99
Englewood (EN)	6	1 (0.17)	2 (0.33)	O109:H8, O4:H5	$19,853.78	5	62	631
Forest Glen (FG)	2	0 (0.00)	2 (1.00)	O8:H8	$101,558.60	8	63	324
Greater Grand Crossing (GG)	12	0 (0.00)	1 (0.08)	O150:H8	$26,515.05	12	140	490
Lake View (LV)	32	5 (0.16)	8 (0.25)	O13:H14, O153:H30, O170:H28, O4:H5, O62:H30, O7:H7, O71:H12	$76,854.22	49	1,094	1574
Logan Square (LS)	38	1 (0.03)	3 (0.08)	O109:H21, O132:H49	$59,216.40	54	424	1515
Near North Side (NN)	19	0 (0.00)	5 (0.26)	O132:H49, O163:H14, O39:H7, O71:H45	$84,975.80	25	1,043	355
New City (NC)	10	1 (0.10)	0 (0.00)	-	$30,420.92	10	145	586
North Lawndale (NL)	6	1 (0.17)	3 (0.50)	O132:H49, O18:H49	$22,383.49	12	98	827
South Lawndale (SL)	31	0 (0.00)	5 (0.16)	O132:H49, O153:H14, O174:H2	$30,700.56	17	273	1,142
Washington Park (WP)	1	0 (0.00)	0 (0.00)	-	$22,084.75	0	41	74
West Ridge (WR)	23	0 (0.00)	1 (0.04)	-	$46,091.23	33	417	1,042
All areas	202	10 (0.05)	43 (0.21)		$48,904.39	237	4,069	8,817

Note: Two-letter initials correspond with Figure 3.

complaints received per year by the City of Chicago via 311 reports and represented an income gradient (Table 1).

2.2 | Sample collection

In collaboration with a pest management company, Landmark Pest Management, we trapped rats in alleys using snap trap stations (pairs of Victor snap traps in JT Eaton aluminium stations). Traps were baited with cat food or anchovy paste and checked every 48 hr. To maximize trap success, we selected four alleys in each community area with the highest number of rat complaints. Within community areas, the distance between alleys was 1,678 ± 573 m (mean ± *SD*). We followed a standardized trapping design of 10 traps per alley to calculate trap success, which is the number of rats caught per trap and per trap night. Although we could not account for sprung traps (i.e., bycatch or false closure), we accounted for any lost traps due to theft or damage (Murray et al., 2018). To detect seasonal changes in infection risk and minimize sample degradation from warm weather, we trapped rats from March 1 to June 20, 2018 (average ± *SD* trap nights per alley = 54 ± 3; daily average ± *SD* temperature = 10.2°C ± 5.7) and from November 5 to December 5, 2018 (30 ± 0 trap nights per alley; 1.5°C ± 2.1). Trapped rats were immediately frozen at −20°C until processing. All rats were captured as part of ongoing pest management by a private company and so this study was exempt from IACUC Approval.

Rats were thawed to 4°C and underwent a full necropsy. We recorded rat sex and reproductive status as a proxy for age class (sexually mature vs. immature) using genital morphology, recorded the presence of cutaneous wounds, and collected morphometric measurements including mass, total length, tail length, hind foot length, and ear length (Table S1). We collected both kidneys for *Leptospira* spp. testing and colon contents for aerobic bacterial culture, such as *E. coli*, *Salmonella enterica*, *Campylobacter* spp., and *Staphylococcus aureus*. Biological samples were stored at −80°C in sterile Whirlpak bags until they were shipped to the Wyoming State Veterinary Laboratory (Laramie, WY) for diagnostic PCR for pathogenic *Leptospira* spp. and culture for aerobic bacteria. The Wyoming State Public Health laboratory serotyped all *Salmonella* isolates.

2.3 | Leptospira PCR

DNA was extracted from kidney tissue using the MagMAX-96 DNA Multi-Sample Kit (Applied Biosystems) following manufacturer's instructions. Four microliters of DNA extract was used in each *Leptospira* spp. PCR reaction targeting the *lig* gene found in pathogenic leptospires (Palaniappan et al., 2005). Primers Lig1 (5'- TCA ATC AAA ACA AGG GGC T-3') and Lig2 (5'- ACT TGC ATT GGA AAT TGA GAG-3') were used in a 50 µl reaction containing 24 µl GoTaq green Master Mix (Promega), nuclease-free water, 2 µl of 50 mM MgCl, and 650 nm of each primer. Cycling conditions of 95°C for 5 min, 35 cycles of 95°C for 30 s, 48°C for 45 s, 72°C for 30 s, and 72°C for 7 min (Palaniappan et al., 2005) . An amplicon size of 480 bp was evaluated on 1.5% agarose gel, and if present the case was considered positive. A positive control (DNA from *Leptospira interrogans* serovar Copenhageni ATCC BAA-1198D-5) and no template control (nuclease-free water) were used to ensure the PCR reaction was successful and there was no contamination.

2.4 | Aerobic culture

Intestinal contents were plated for isolation with a sterile swab onto multiple agar plates purchased and quality controlled from Hardy Diagnostics including Columbia blood agar with 5% sheep blood (CBA), MacConkey (MAC), and Campy blood free Karmali (CAMPY). Subsequently, the swab was placed in selenite broth (Selenite Cystine Broth, Hardy Diagnostics) for enrichment of *Salmonella* spp. The CBA, MAC plates, and selenite broth were incubated at 37°C in ambient O2. CAMPY plates were incubated at 41°C in microaerophilic conditions. At ~18–24 hr, the selenite broth was sub-cultured for isolation onto HardyCHROM^{tm} Salmonella, and Hektoen Enteric agar plates and incubated at 37°C ambient O2. Culture plates were read and documented once at 18–24 hr, and again at 36–48 hr. Colonies from each sample were analysed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Biotyper, Hamburg Germany) following the manufacturer's instructions for identification. *S. aureus*, *E. coli*, and *Salmonella* isolates were sub-cultured and then frozen at −80°C for further analysis.

2.5 | *E. coli* sequencing

Whole-genome sequencing (WGS) was performed on all *E. coli* isolates using the Illumina MiSeq platform with the Nextera XT library prep kit and V2 chemistry. Sequencing was done according to the manufacturer's protocols. Draft genome assemblies were created from the WGS data using the default settings in SKESA v.2.2 (Souvorov, Agarwala, & Lipman, 2018). The program ectyper v0.8.1 was used to predict the serotype from the draft assemblies for each isolate (National Microbiology Laboratory, 2019). To evaluate the virulence of each isolate, we ran AMRFinder v3.2.3 on each assembly using the default settings (Feldgarden et al., 2019). In addition to including known antimicrobial resistance genes, the database used with AMRFinder includes known virulence factors found in *E. coli* (e.g., Shiga toxin-producing genes). All sequence data are publicly accessible under the BioProject PRJNA230969 with the National Center for Biotechnology Information (Table S2).

2.6 | Sanitation data

We assessed whether poor sanitation promoted zoonotic infection risk in rats using several types of municipal sanitation data. We first tested whether rats were more likely to carry *Leptospira* spp. if they were trapped in alleys where public complaints had been made

TABLE 2 Comparison of candidate models containing predictors of *Leptospira* spp. or *Escherichia coli* infection in rats using AICc

Pathogen	Model	Model description	ΔAICc	Deviance	Random effect variance
Leptospira spp.	Sanitation and SES	Standing water complaints*Income	0.00	61.76	0.31
	Global	Injuries + Season +Sex + Reproductive +Water*Income + Water*Trap success	4.77	50.53	0.22
	Sanitation	Standing water complaints	7.22	72.98	0.33
	Sanitation and abundance	Trap success*Standing water complaints	7.34	69.10	0.27
	Individual biology	Injuries + Sex +Reproductive	10.52	68.28	1.72
	Null	Intercept	10.59	78.35	1.65
	Relative abundance	Trap success	11.97	77.73	1.54
	Socioeconomics	Median household income	12.03	77.79	1.63
	Season	Capture season	12.29	78.05	1.56
E. coli	Global	Injuries + Season +Sex + Reproductive +Food inspections*Income + Food inspections*Trap success	0.00	128.26	0.36
	Season	Capture season	9.95	160.21	1.11
	Individual biology	Injuries + Sex +Reproductive	24.83	165.09	1.83
	Sanitation and SES	Food inspections*Income + Proportion failed inspection*Income	25.39	171.65	1.63
	Sanitation	Food inspections + Proportion failed inspections	30.95	181.21	2.05
	Relative abundance	Trap success	30.76	181.02	2.22
	Sanitation and abundance	Trap success*Food inspections	28.07	174.33	1.61
	Null	Intercept	32.21	184.47	2.20
	Socioeconomics	Median household income	33.82	184.08	2.17

Note: Asterisk (*) indicates that both terms were in the model individually as well as their interaction.
SES, socioeconomic status.

about the presence of standing water. We accessed all public complaints regarding standing water sanitation violations made to City of Chicago Streets and Sanitation via 311 between 2015 and 2018 using the Chicago Data Portal (City of Chicago, 2020). We pooled complaints over multiple years to include areas with drainage issues that persisted for months.

We also evaluated whether rats were more likely to carry *E. coli* and *Salmonella* if they were trapped near areas with more food vendors. We predicted a positive association between *E. coli* prevalence and food vendors because food waste from restaurants may aggregate rats and promote the transmission of pathogens that spread via a faecal–oral route, such as *E. coli*. Food vending can be a significant source of food waste as vendors dispose of up to 20% of handled or prepared food annually and can therefore create areas with higher amounts of food waste relative to residential or commercial areas (Silvennoinen, Heikkilä, Katajajuuri, & Reinikainen, 2015). To include the locations of food vendors, we accessed all food inspections performed in 2018 by the Chicago Department of Public Health's Food Protection Program (Chicago Department of Public Health, 2020). From this dataset, we included all locations of businesses that sell food to the public. We also predicted that rat infection risk would be especially associated with food vendors with rodent-related sanitation complaints. To test this prediction, we calculated the proportion of vendors that failed their inspections due to rodent sightings or signs such as droppings, chew marks, or grease marks.

To relate rat infection status with sanitation data, we recorded the presence of standing water complaints or food inspections if they were within 150 m of the trapping alley using QGIS (QGIS Development Team, 2020). This buffer size approximates the size of rat home ranges in other cities (Byers, Lee, Patrick, & Himsworth, 2019; Combs, Puckett, Richardson, Mims, & Munshi-South, 2018) and corresponds to roughly one city block.

2.7 | Statistical analysis

We used binomial generalized linear mixed models to test whether rat zoonotic infection risk increased with sanitation concerns, lower socioeconomic status, season, rat relative abundance, and rat characteristics. Our response variable was the infection status (positive or negative) of each rat. We created nine candidate models for each pathogen, which reflect different hypotheses on the spatiotemporal predictors of rat zoonotic infection risk. These models included a (a) global model (all terms) and (b) null model (intercept only) as well as models related to (c) individual biology, (d) season, (e) sanitation, (f) socioeconomics, (g) rat relative abundance, (h) sanitation and socioeconomics, and (i) sanitation and rat relative abundance (Table 2). In all models, we censored trapping sites where fewer than four rats were captured to minimize bias on prevalence from low sample size (retained 82% of dataset). Capture site (i.e., alley) was treated as a random effect. We did not include highly correlated terms in the same model (R^2 > .6), continuous variables were standardized with a mean of 0 and standard deviation of 1 prior to analysis, and modelling was performed using the package lme4 in R (RStudio Team, 2016). The relative fit of each candidate model for each pathogen was compared using AICc, and all models within two ΔAICc of the best fit model were considered to have substantial support (Burnham & Anderson, 2002). We then used top-ranked models to predict the probability of infection in all community areas across the city using the predict.glm function in R.

3 | RESULTS

In total, 254 rats were trapped between March 1–June 20 and November 5–December 5, 2018. All trapped rats were presumed to be *Rattus norvegicus* based on the morphology of the ears and tail relative to body size (Aplin, Chesser, & Have, 2003) but this was not confirmed via genetic analyses. Of these, 202 were trapped between March 1–May 23 and November 8–December 1 and were in adequate condition to be necropsied with no visible signs of decomposition. These included 105 females (67 adults, 36 subadults) and 97 males (65 adults, 32 subadults; Table 1). Trap success ranged from 0.003 to 0.096 rats per trap per night across alleys. The overall prevalence of *Leptospira* spp. was 5.0% (10/202), 21.7% for *E. coli* (44/202), 1.0% for *Salmonella* (2/202, both serovar Enteriditis), and 1.9% for *Staphylococcus aureus* (4/202). We did not detect *Campylobacter* spp. or *Clostridium* spp. Based on these prevalence rates, we only analysed predictors of infection for *Leptospira* spp. and *E. coli* and the prevalence of both pathogens varied by neighbourhood (Figure 1).

For *Leptospira* spp., the best model contained sanitation, socioeconomic status, and their interaction (Table 2). Based on parameter 95% confidence intervals, rats were significantly more likely to carry *Leptospira* spp. if they were trapped on blocks with more standing water complaints, but this relationship was only significant as an interaction with income (odds ratio = 6.74, 95% C.I. = 1.54–29.39; Table 3). Alleys with standing water complaints in high-income neighbourhoods were more likely to have *Leptospira* spp.-positive rats than alleys with water complaints in lower-income neighbourhoods (Figure 2). Because the top model for *Leptospira* spp. infection

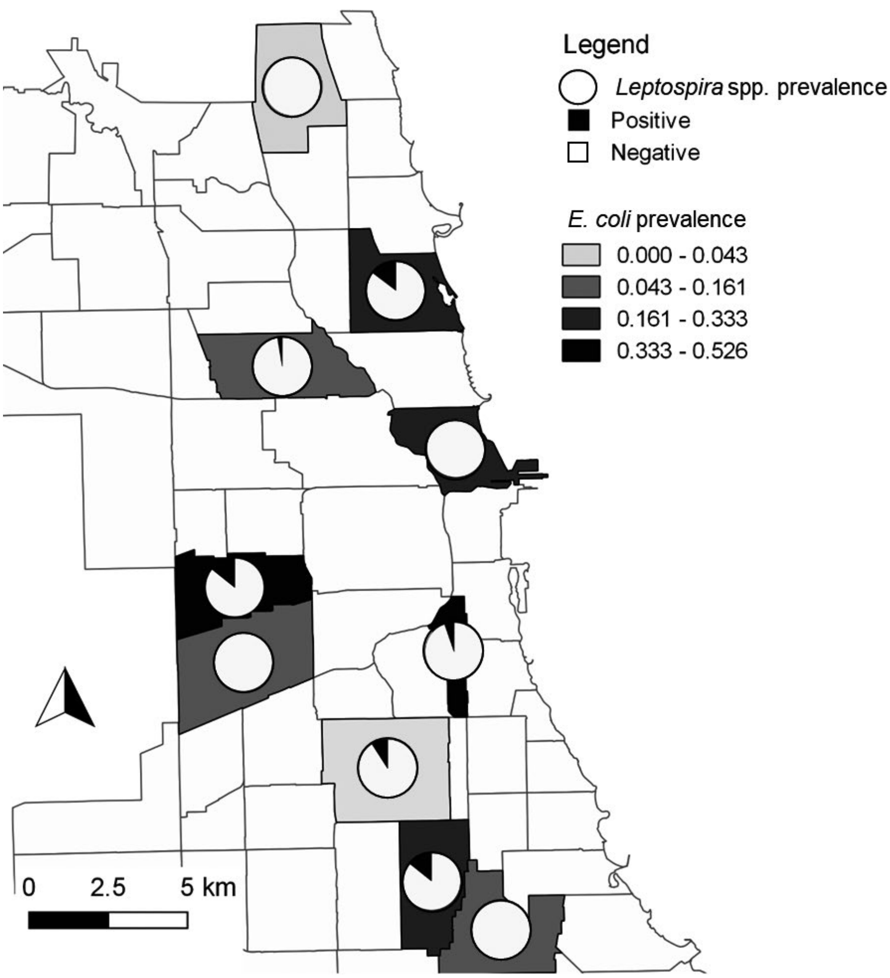

FIGURE 1 Map of study community areas showing pathogen prevalence in sampled rats. Community areas where ≥4 rats were sampled are shaded by the prevalence of *Escherichia coli* and pie charts show the prevalence of *Leptospira* spp. carriage in rats

TABLE 3 Parameter estimates in top-ranked candidate models associated with *Leptospira* spp. and *Escherichia coli* carriage in urban rats in Chicago

Pathogen	Model	Term	Estimate	*SE*	Pr (>|z|)	Odds Ratio (95% C.I.)
Leptospira spp.	Sanitation and SES	Intercept	−5.15	1.13	4.77×10^{-6}	5.81×10^{-3} (6.41×10^{-4}–5.28×10^{-2})
		Income	−1.13	0.88	0.20	0.32 (0.06–1.81)
		Water complaints	0.51	0.63	0.41	1.67 (0.49–5.67)
		Income*Water complaints	1.91	0.75	0.01	6.74 (1.54–29.39)
E. coli	Global	Intercept	−2.42	0.77	1.75×10^{-3}	0.09 (0.02–0.40)
		Sex (Male)	−1.62	0.58	5.14×10^{-3}	0.20 (0.06–0.62)
		Reproductive	0.85	0.55	0.12	2.34 (0.79–6.94)
		Season (Spring)	2.77	0.87	1.48×10^{-3}	15.96 (2.90–88.62)
		Trap success	−0.41	0.38	0.28	0.66 (0.31–1.40)
		Food inspections	2.30	0.75	2.30×10^{-3}	9.94 (2.27–43.50)
		Median income	−0.64	0.38	0.09	0.52 (0.25 – 1.09)
		Proportion failed inspections	−4.86	6.61	0.46	7.75×10^{-3} (1.83×10^{-8}–3.28×10^{3})
		Injuries	0.66	0.60	0.27	1.94 (0.60–6.33)
		Food Inspections*Income	−1.33	0.57	2.07×10^{-2}	0.26 (0.09–0.82)
		Trap success*Inspections	−0.16	0.36	0.68	0.85 (0.39–1.85)

contained only spatial variables, we predicted the probability of *Leptospira* spp. infection for an individual rat trapped in any Chicago community area based on the number of standing water complaints, income, and their interaction. Probability of infection ranged from <.001 to >.25 between communities (Figure 3).

Of the 41 *E. coli* isolates that were successfully sequenced, we detected 25 unique serovars (Table 1). None of the *E. coli* serovars contained major virulence factors (e.g., Shiga toxin 1 (*stx1*) or 2 (*stx2*) genes) that are associated with pathogenic *E. coli* strains. The global model best explained variation in rat *E. coli* carriage (Table 2). Rats were significantly more likely to carry *E. coli* if they were trapped on blocks with more food inspections (OR = 9.94, 2.27–43.50; Table 3). The interaction between food inspections and income was also significant, such that the probability of *E. coli* carriage was higher with food vendors in lower-income community areas (OR = 0.26, 0.09–0.82; Figure 4). Rats were also significantly more likely to carry *E. coli* in the spring (OR spring = 15.96, 2.90–88.62; sample prevalence in spring =39%, fall =8%) or if they were female (OR males = 0.20, 0.06–0.62; female =27%, male =15%).

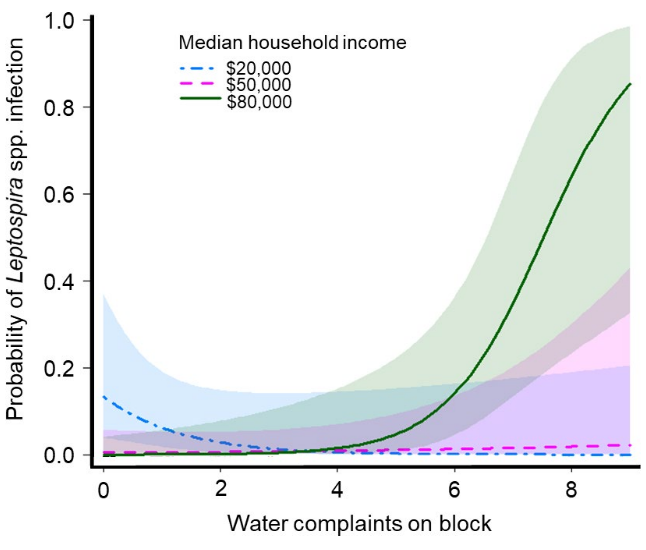

FIGURE 2 Interaction between the probability of *Leptospira* spp. carriage in Chicago rats, standing water complaints in the alley of capture, and median household income. Dashed and solid lines represent mean estimates of the probability of *Leptospira* spp. carriage at varying income levels. Shaded bands show 95% confidence intervals

4 | DISCUSSION

In this study, we quantified the prevalence of several environmentally transmitted rat-associated zoonotic pathogens across diverse neighbourhoods in Chicago, a large city where concern about rats is growing. We confirmed that rats in Chicago carry pathogenic *Leptospira* spp. and diverse serotypes of *E. coli*. Both pathogens were associated with poor sanitation and had opposing relationships with socioeconomic status, such that the likelihood of exposure to zoonotic bacteria varies by community based on income. We were more likely to detect *Leptospira* spp. in higher-income areas with standing water issues. Conversely, we were more likely to detect *E. coli* in lower-income areas with higher densities of food vendors. Our results demonstrate the importance of public sanitation data and incorporating diverse urban areas in pathogen surveillance to predict zoonotic disease risks across cities.

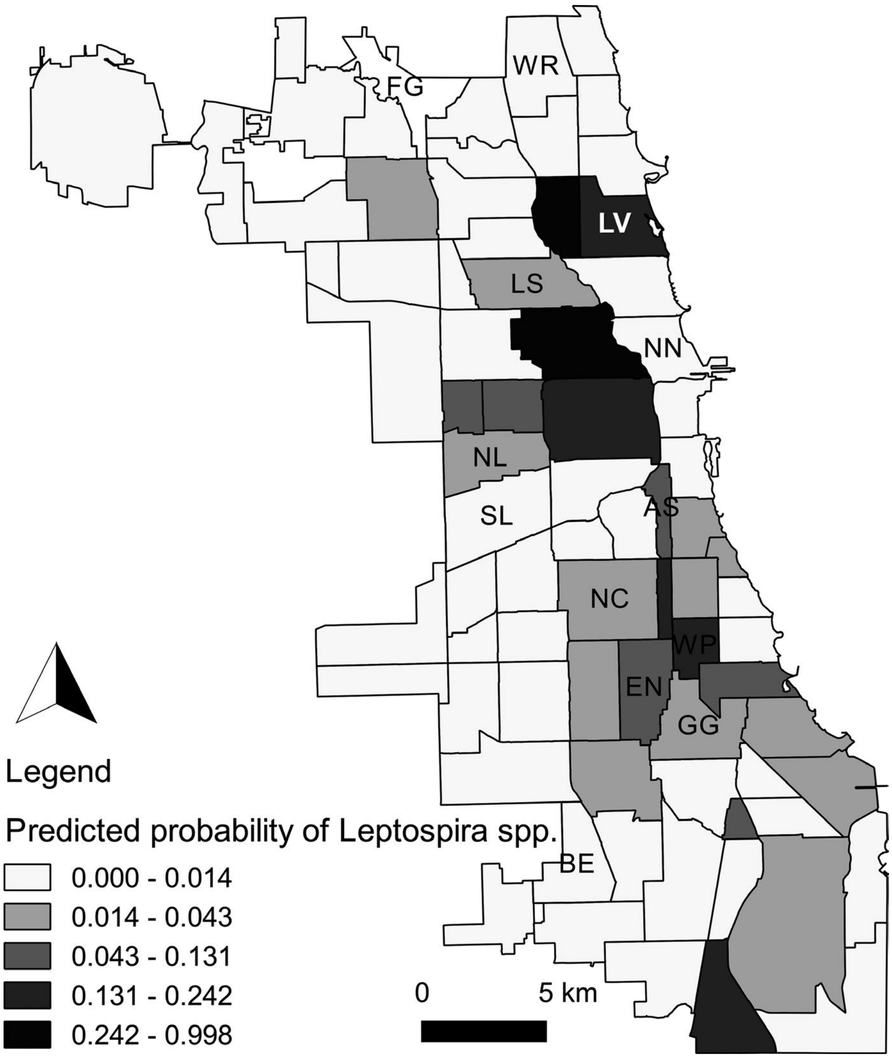

FIGURE 3 Map of Chicago community areas shaded by predicted probability of *Leptospira* spp. in urban rats based on standing water complaints, median household income, and their interaction in the top performing model. The 13 community areas where rats were sampled are labelled with initials corresponding to Table 1

Contact with water sources contaminated with *Leptospira* spp. from rat urine, either through recreational or occupational exposure, is a common route of transmission for Leptospirosis (Mwachui, Crump, Hartskeerl, Zinsstag, & Hattendorf, 2015). In Chicago, the positive relationship between rat *Leptospira* spp. infection and standing water complaints in higher-income areas highlights public health concerns in urban alleys with poor drainage. Potential for exposure to *Leptospira* spp. in alleys is not unique to Chicago. For instance, Vinetz et al. (1996) similarly suspected Leptospirosis transmission to involve contact with rat urine in urban alleys in Baltimore, MD. In addition to humans, domestic dogs can contract Leptospirosis from contact with contaminated water (White et al., 2017), and so alleys with enough standing water to warrant complaints may be hotspots for transmission between people, domestic animals, and wildlife. In fact, the prevalence of *Leptospira* spp. infection has been increasing for Chicago dogs (White et al., 2017), suggesting a potential concomitant increase in exposure for urban residents. In these instances, mitigation efforts might aim to identify and resolve standing water issues to minimize *Leptospira* spp. risk.

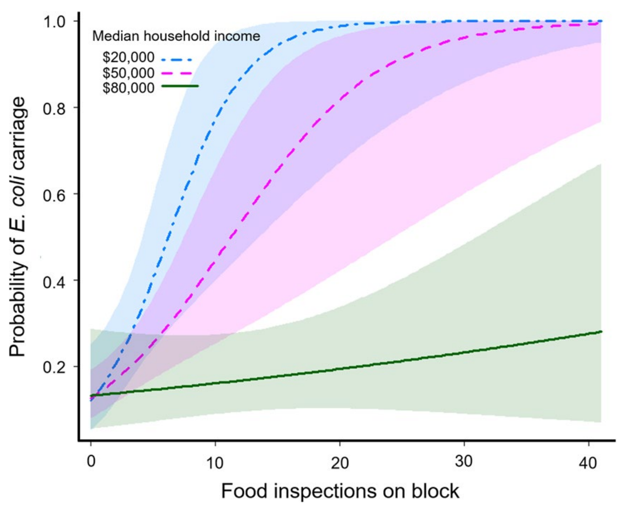

FIGURE 4 Relationships between the probability of *Escherichia coli* carriage in rats in Chicago and the interaction between food inspections on the block of capture and median household income. Dashed and solid lines represent mean estimates of the probability of *E. coli* carriage at varying income levels. Shaded bands show 95% confidence intervals

The mechanisms supporting the unanticipated interaction between standing water and higher incomes for *Leptospira* spp. infection is less clear. Leptospirosis is typically associated with urban slums (Hagan et al., 2016) and so we predicted, as in other studies (Ayral et al., 2015), that economically disadvantaged communities are more likely to be at risk of rat-associated zoonoses. This hypothesized link between socioeconomic status and public health risks from rats is complex but likely related to higher environmental exposure or fewer resources to mitigate rat infestations. Contrary to our predictions, alleys with standing water in high-income neighbourhoods had an order of magnitude higher likelihood of *Leptospira* spp. in rats relative to lower-income neighbourhoods. The importance of spatial predictors for *Leptospira* spp. infection informed our predictions of rat infection risk across the city, identifying seven community areas where predicted infection risk exceeds 10% (Figure 4). Our results highlight that surveillance for rat-associated zoonotic pathogens should not be limited to low-income communities.

Municipal sanitation data provided new insights to predict public health risks from *E. coli* carriage in rats. Rats were more likely to carry *E. coli* on blocks with higher numbers of food vendors, which may represent areas with greater rat attractants from food waste (Silvennoinen et al., 2015), potentially aggregating rats. None of the *E. coli* serovars we detected contained major virulence factors and thus are not cause for concern regarding human disease. However, the relationships we found between food inspections and *E. coli* infection in rats is of interest for public health because food vendors represent locations where opportunities for food contamination and spatial overlap between rats and humans are high. Although the *E. coli* serovars we detected pose low risk for human disease, and we found very low prevalence of other food-borne pathogens *Salmonella enterica* and *Campylobacter* spp., management strategies could emphasize proper containment of food waste to minimize rat attractants and risk of food contamination (Murray et al., 2018). Contrary to our predictions, we found no relationship for food vendors with rodent-associated sanitation complaints, potentially because all food vendors produce food waste or because rodent concerns included mice.

We found the highest prevalence of *E. coli* near food vendors in low-income neighbourhoods. Restaurants in lower-income neighbourhoods may pose additional risks for rat-associated pathogens if there are fewer resources available for decontamination, rodent control, or waste management. Although disparities in infectious disease with socioeconomic status are complex (Gibney & Leder, 2019), previous work has detected increased risk of food-borne illness with lower incomes (Jalava, Ollgren, Eklund, Siitonen, & Kuusi, 2011). This risk can be partially caused by access to food vendors; previous studies have found increased bacterial contamination (Koro, Anandan, & Quinlan, 2010) on produce from vendors in low-income areas. Food vendors in low-income areas may have fewer resources to mitigate rodent infestations because they are often challenged with lower revenue and higher costs associated with security and employee retention (Mauer et al., 2006). The patterns we observed in *E. coli* risk with income may be a symptom of the growing disparities in income and investment between Chicago neighbourhoods (Theodos, Hangen, Meixell, & Rajasekaran, 2019). Addressing these inequities and the challenges faced by lower-income food vendors may mitigate these health risks exacerbated by rat activity.

Unlike the aforementioned factors, we did not find an association between rat abundance and *E. coli* or *Leptospira* spp. prevalence, suggesting environmental exposure may be more important than density-dependent transmission. Previous work has also suggested that rats are exposed to *E. coli* from the local environment and act as "pathogen sponges" (Himsworth et al., 2015). Exposure to a wide variety of pathogen sources in the urban environment, such as garbage, food waste, sewage, and pet waste in alleys, could explain the high diversity of *E. coli* serotypes in Chicago rats (Table 1).

Our results suggest several avenues for future research to predict public health risks from rat infestations. Detection of *Leptospira* spp. in this study was based on PCR analysis of kidney tissue and was unable to identify the pathogen to species, a common limitation of rat studies (Boey, Shiokawa, & Rajeev, 2019). Additional testing to identify the species and serovar would help advance pathogen ecology in urban environments. Our analysis also does not determine active shedding of leptospires in urine. However, the load of leptospires in rat kidneys and urine are highly correlated (Costa, Wunder, et al., 2015). To more accurately infer risk of transmission, future studies could quantify leptospires in water samples from urban alleys (Ganoza et al., 2006) and quantify the amount of *Leptospira* spp. present in rat tissues, for example using qPCR. It is also worth noting that standing water complaints may not accurately reflect the extent of standing water throughout the study area and the number of complaints may overestimate the number of standing water bodies in high-income neighbourhoods. Future studies should consider accounting for this by performing field visits to compare reported environmental features to observations. Future work could also elucidate the mechanisms driving the higher prevalence of *E. coli* we detected in the spring trapping season. This pattern may be due to higher ambient temperatures facilitating *E. coli* persistence in the environment (Van Elsas et al., 2011) or based on other aspects of rat life history because of the significant differences in carriage between males and females.

In this study, we highlighted municipal sanitation data as an important predictor to identify areas where rats are more likely to carry environmentally transmitted zoonotic pathogens. The increase in *E. coli* carriage with increased food inspections is especially concerning because these areas promote spatial overlap between rats and humans. Socioeconomic status was also important in predicting rat infection risk and yielded opposing relationships between income, sanitation, and likelihood of infection for *Leptospira* spp. and *E. coli*. Both types of data represent an accessible method to predict risks from rats over large and diverse urban areas. Advances such as these in identifying the contexts where urban residents are exposed to pathogens in rat excreta will help municipalities devise and deploy effective public health mitigation strategies around the world.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

ETHICAL APPROVAL

All rats were captured as part of ongoing pest management by a private company and so this study was exempt from IACUC Approval.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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