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Authors

Taff, Conor C
Weis, Allison M
Wheeler, Sarah
et al.

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Influence of Host Ecology and Behavior on *Campylobacter jejuni* Prevalence and Environmental Contamination Risk in a Synanthropic Wild Bird Species

Conor C. Taff,[a,b] Allison M. Weis,[c] Sarah Wheeler,[d] Mitchell G. Hinton,[a] Bart C. Weimer,[c] Christopher M. Barker,[e] Melissa Jones,[a] Ryane Logsdon,[f] Woutrina A. Smith,[g] Walter M. Boyce,[e] Andrea K. Townsend[h]

Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, Davis, California, USA[a]; Lab of Ornithology and Department of Ecology & Evolutionary Biology, Cornell University, Ithaca, New York, USA[b]; Department of Population Health and Reproduction, School of Veterinary Medicine, 100K Pathogen Genome Project, University of California, Davis, Davis, California, USA[c]; Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, California, USA[d]; Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, Davis, California, USA[e]; Department of Evolution & Ecology, University of California, Davis, Davis, California, USA[f]; Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Davis, California, USA[g]; Department of Biology, Hamilton College, Clinton, New York, USA[h]

ABSTRACT

Campylobacter jejuni is a foodborne pathogen that often leads to human infections through the consumption of contaminated poultry. Wild birds may play a role in the transmission of _C. jejuni_ by acting as reservoir hosts. Despite ample evidence that wild birds harbor _C. jejuni_, few studies have addressed the role of host ecology in transmission to domestic animals or humans. We tested the hypothesis that host social behavior and habitat play a major role in driving transmission risk. _C. jejuni_ infection and host ecology were studied simultaneously in wild American crows (_Corvus brachyrhynchos_) in Davis, CA, over 3 years. We found that 178 of 337 samples tested were culture positive (53%), with infection varying by season and host age. Among adult crows, infection rates were highest during the winter, when migrants return and crows form large communal roosts. Nestlings had the highest risk of infection, and whole-genome sequencing supports the observation of direct transmission between nestlings. We deployed global positioning system (GPS) receivers to quantify habitat use by crows; space use was nonrandom, with crows preferentially occupying some habitats while avoiding others. This behavior drastically amplified the risk of environmental contamination from feces in specific locations. This study demonstrates that social behavior contributes to infection within species and that habitat use leads to a heterogeneous risk of cross-species transmission.

IMPORTANCE

Campylobacter jejuni is the most common cause of gastroenteritis in industrialized countries. Despite efforts to reduce the colonization of poultry flocks and eventual infection of humans, the incidence of human _C. jejuni_ infection has remained high. Because wild birds can harbor strains of _C. jejuni_ that eventually infect humans, there has long been speculation that wild birds might act as an important reservoir in the _C. jejuni_ infection cycle. We simultaneously studied infection prevalence, social behavior, and movement ecology in wild American crows (_Corvus brachyrhynchos_). We found that social behavior contributed to patterns of infection and that movement behavior resulted in some areas having a high risk of transmission while others had a low risk. The incorporation of ecological data into studies of _C. jejuni_ in wild birds has the potential to resolve when and how wild birds contribute to domestic animal and human _C. jejuni_ infection, leading to better control of initial poultry contamination.

Campylobacter jejuni is the leading cause of gastroenteritis in industrialized countries (1, 2), with most infections in humans resulting from the consumption of contaminated and improperly cooked poultry (3). Generally, infected individuals recover in a few days, but in some cases, infection can lead to hospitalization (~15% of culture-positive cases in the United States [1]), chronic autoimmune disorders (~2% of cases [4, 5]), and even death (~0.06% of cases [1]). These health concerns demand that food producers manage flock infection and disinfect suspect meat at a considerable cost, yet some surveys suggest that even with the precautions taken, up to 70% of poultry sold in U.S. and United Kingdom grocery stores is contaminated with *C. jejuni* (6, 7). Given the human health and financial costs caused by *C. jejuni*, it is unsurprising that major research efforts have been devoted to reducing flock infection (8–11), developing genetically resistant lines of poultry (12), and limiting transmission from poultry to humans (13, 14). Despite these efforts, human infection rates in the United States have failed to match CDC targets and have actually increased 14% over the past 10 years (1). Although most research is carried out in the laboratory or on poultry farms,

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Address correspondence to Conor C. Taff, cct63@cornell.edu.

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scientists have long known that *C. jejuni* can also be carried by wild birds (e.g., see reference 15), and there has been extensive speculation that wild bird populations might act as reservoirs of *C. jejuni*, thereby contributing to domestic animal infection or even to direct human infection via environmental contamination from feces (reviewed in reference 16).

Studies documenting *C. jejuni* prevalence in wild animals have increased rapidly in the past 15 years (17–20). Many studies now show that diverse families of wild birds harbor *C. jejuni* (reviewed in reference 16) and that, at least in some cases, these strains are similar to those that infect poultry, livestock, and humans (9, 21). The majority of this work has been conducted in Europe, but a smaller number of studies in the United States demonstrate similar patterns (22, 23). Relatively few studies, however, go beyond a simple culture test to detect the presence of *C. jejuni*. In rare cases, direct transmission from wild birds to humans has been demonstrated beyond any doubt (24, 25), but direct transmission is difficult to verify, and the actual transmission rates are probably underestimated. One recent study concluded that 2.1 to 3.5% of annual human *C. jejuni* cases in Oxfordshire, United Kingdom, are directly attributable to wild birds (26). Despite ample evidence that *C. jejuni* infection in birds is common, the importance of wild birds in the human *C. jejuni* infection cycle is still unclear.

Studies that survey wild birds for *C. jejuni* often implicitly assume that high prevalence is a good proxy for high risk of transmission to humans or domestic animals (22). Carriage of human-pathogenic strains by wild birds is a necessary antecedent for undesirable cross-species transmission, but host ecology and behavior are also likely to be major determinants of the actual risk of transmission posed by specific wild birds. In a recent review, Waldenström and Griekspoor (16) argue that the lack of information on the ecology of wild hosts infected by *C. jejuni* has limited our ability to make informed risk assessments. Even basic host ecology studies of wild bird species that carry *C. jejuni* might greatly increase our understanding of factors that contribute to transmission. For example, Ramos et al. (27) demonstrated that *C. jejuni* infection rates vary among populations of yellow-legged gulls (*Larus michahellis*), and that these variations are attributable to the amount of human refuse in the diet at both the individual and population level. How pervasive such patterns are and how strongly they influence the risk of cross-species *C. jejuni* transmission are open questions.

American crows (*Corvus brachyrhynchos*), members of the corvid family, are excellent candidates for studying links between host ecology and *C. jejuni* association. Corvids are a globally distributed clade of birds known for their close association with domestic animals, agricultural fields, and urban areas. Corvids have the highest rates of *C. jejuni* infection of any sampled clade (16, 28, 29) and, among those families with relatively high infection rates, corvids arguably have the most direct contact with humans and domestic animals. We established previously that *C. jejuni* infection is common in American crows in the suburban town of Davis, CA, and that at least some of the isolates found have characteristics of strains that are pathogenic to humans (28). In addition to the high prevalence of *C. jejuni* infection, American crows exhibit social behavior and movement patterns that may influence the risk of transmission from crows to domestic animals or directly to humans (30). Each winter, crows form large communal roosts that may facilitate both within-species transmission and amplify cross-species transmission risks in areas surrounding the roost (31). Crows are also strong flyers and social foragers; thus, the risk of contamination may be spread far from the roost but may also be highly concentrated in areas that attract crows (e.g., feedlots or particular crops) rather than be equally distributed across the landscape.

Here, we build on previous work on the molecular characteristics of *C. jejuni* isolates from American crows (28) by integrating those results with host ecology. Specifically, we conducted extensive year-round sampling of crow feces to look for evidence of seasonal prevalence patterns associated with social behavior and differences in *C. jejuni* prevalence in feces between adults and nestlings. We hypothesized that *C. jejuni* prevalence would be higher in nestlings than adults, due to a less-developed immune system and high exposure to social sources of infection from parents and nest mates (32). We also predicted that overall *C. jejuni* prevalence in feces would be highest at the peak of the communal roosting season, when thousands of crows spend each night in close proximity and up to 58% of crows have visible fecal staining on their feathers from roost mates perched on higher branches (31). For nestlings, we predicted that the shared nest environment would drive transmission, with nest mates tending to be either all uncolonized or all colonized with a similar strain of *C. jejuni*. Finally, we combined our information on prevalence with movement data from global positioning system (GPS) receivers deployed on adult crows to describe winter foraging behavior and patterns of landscape use during the roosting season.

MATERIALS AND METHODS

Study population and general field methods. We studied *C. jejuni* infection in wild American crows in Davis, CA, from May 2012 until June 2015. During that time, year-round fecal samples were collected for *C. jejuni* testing from adults and nestlings. Crow density varied seasonally, because migratory crows overwintered in Davis from October to March each year. During this period, migrants and year-round residents formed a large communal roost each night. Each morning before dawn, crows dispersed from the roost to daytime feeding locations. In the winters of 2014 and 2015, periodic roost counts were conducted before dawn to estimate the onset and duration of roosting along with the peak winter roost attendance (as described in reference 31). Resident crows also formed much smaller roosts in the summer between May and August. These roosts were unpredictable in size and location and generally only included 100 to 400 individuals. Samples were collected from summer roosts when possible, but data from summer roosts were sparse, and no samples were collected in August or September when the birds did not roost in large numbers.

After migrants left the winter roost, local crows began their breeding season, which typically lasted from early April until late June. During the 2012, 2013, and 2014 breeding seasons, breeding activity was monitored closely at crow territories on and around the University of California, Davis campus (described in reference 33). Once a nest was located, a regular census was conducted until hatching, and then each nest was visited to collect samples for *C. jejuni* testing. In 2012, fecal samples were collected from most nestlings that were visited, but in 2013 and 2014, samples were collected only opportunistically when a nestling defecated during handling.

Similar samples were collected from adult birds captured during the winter roosting season in 2014 and 2015. Adults were captured using a drop-in trap that was baited and set before dawn so that crows would encounter it when leaving the roost. After capture, crows were held in plastic carriers until processing; a fresh fecal sample was collected from each carrier as crows were removed. Some of the captured adults were fitted with GPS receivers to quantify winter habitat use (see below). All trapping, banding, and sampling procedures were conducted with approval from the United States Geological Survey (USGS) Bird Banding

Laboratory permit no. 23777, the California Department of Fish and Wildlife permit no. 12065, and University of California Davis IACUC protocol no. 16897.

***Campylobacter* testing.** All fecal samples were collected by swabbing fresh feces and storing the swab in Amies clear gel transport medium (Remel BactiSwab; Fisher Scientific) in a cooler until submission for testing (within 6 h of collection) (28). In addition to the captured nestling and adult samples described above, feces were collected periodically from directly under the large winter roost or the much smaller summer roost. In order to collect the freshest samples, fecal samples were always collected before sunrise. To prevent ground contamination, only the top portion of the feces was collected. In total, 119 samples were tested from the winter roost between 31 October and 3 April, 71 samples from the small and less-predictable summer roost between 23 April and 31 July, 102 samples from nestlings between 6 May and 29 June, and 45 samples from captured adults between 8 January and 14 March. All samples were submitted for testing to the U.C. Davis Veterinary Medicine Teaching Hospital Clinical Laboratory Services Facility. Samples were cultured and, if candidate colonies were identified, subjected to biochemical testing to confirm the presence of *C. jejuni* (28). For a subset of samples ($n = 16$), duplicate swabs were collected and submitted for independent testing to evaluate the repeatability of the lab results. We calculated 95% binomial confidence intervals of infection prevalence for each type of sample using the 'binconf' function of the 'Hmisc' package in R; we report the Wilson score interval, as recommended by Agresti and Coull for binomial proportions (34).

GPS deployment. In January 2015, eight GPS receivers were deployed on adult crows captured in the drop-in trap. The receivers were 15-g FLR-II units produced by Telemetry Solutions (Concord, CA, USA), which recorded locations that were accurate to within about five meters. Crows fitted with receivers weighed 353 to 398 g, so that the weight of the receiver was always <4.5% of the bird's body weight. Receivers were secured by a backpack harness that looped around each wing to attach at the breast; harnesses were made from 1.5-mm nylon cord and fastened with copper crimps, superglue, and stitches applied by needle and thread at each connection point. After the harnesses were attached, crows were released into a holding aviary for at least 1 h to ensure that the GPS unit was functioning properly and that the harness was safely attached. Crows were released following this holding period. Receivers were programmed to attempt to collect nine location points each day at the following fixed times: 1:00, 3:00, 7:00, 9:00, 11:00, 13:00, 15:00, 17:00, 19:00, and 23:00. To preserve battery life, the units were programmed to time out if a location fix could not be established within 65 s, so the complete data set for each bird did not always contain all scheduled locations.

The receivers were equipped for remote transfer so that locations could be downloaded to a base station held within ~30 m of the receiver without needing to recapture the bird. Surveys of the roost occurred well before dawn 2 to 3 times per week after the receivers were deployed to download locations. At these visits, observers systematically walked under each tree and held the base station aloft to establish connections. An unmanned base station was also deployed in the field at areas of known crow activity to maximize chances of recovering location data. Location recovery efforts were continued until the roost had completely dispersed during the first week of April 2015.

Spatial analysis. The Quantum Geographic Information Systems (QGIS) software (Quantum GIS Development Team, 2015, http://qgis.osgeo.org) was used to match our GPS locations with landscape characteristics using the USDA CropScape cropland 2014 data layer (USDA 2014; http://nassgeodata.gmu.edu/CropScape/). The cropland data layer draws basic land use data from the National Land Cover Database 2011 layer (35) but further subdivides agricultural areas into specific crop types. Nearly all nocturnal (19:00 to 7:00) locations were at or near the roost in developed areas, while diurnal (7:00 to 19:00) locations were more variable. Thus, diurnal and nocturnal time budgets were analyzed separately. Locations were pooled across individual birds before calculating the amount of time spent in each land use or crop type as a simple average of the number of locations in each area divided by the total number of locations collected. To provide context for interpreting habitat use, the total percentage of land in the study area for each habitat or crop type was also calculated. The study area was defined as a circle with a 12.5-km radius from the roost, which contained ~95% of the observed daytime crow locations. Although the individual tracked birds did not utilize the entire area of this circle, this boundary was chosen because it best represented the choice of habitat that crows could have exploited given the distance that they traveled from the roost each day. Calculating habitat use with a 95% minimum convex polygon rather than a circle yielded qualitatively similar conclusions, and only the results using a 12.5-km buffer are presented for simplicity. We calculated the approximate home range of each individual crow using minimum convex polygons from all observed locations.

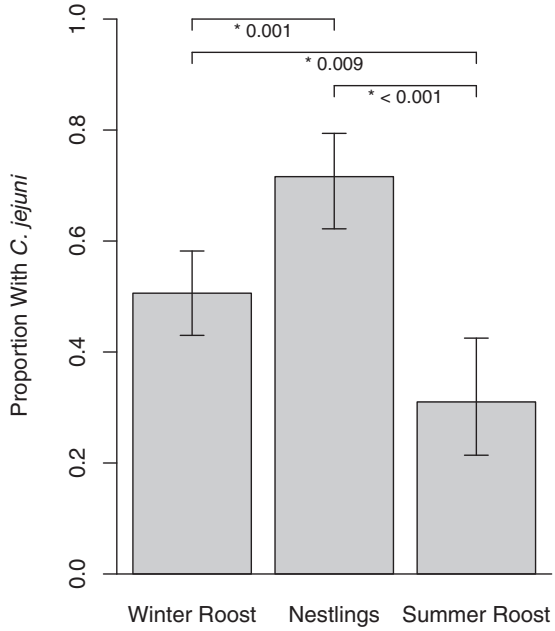

FIG 1 Overall prevalence of *Campylobacter jejuni* in fecal samples collected from the winter roost (including adults captured during the roosting season, $n = 164$), from nestlings ($n = 102$), or from the summer roost ($n = 71$). The infection prevalence for each pair of sample types was significantly different by Fisher's exact test. Error bars illustrate the 95% binomial confidence interval for prevalence estimates. Asterisks (*) indicate significant differences with a P value of <0.05.

The predicted amount of crow feces was calculated per square kilometer per day in each habitat type at the peak of the roosting season by multiplying the percentage of time spent in each habit by the maximum number of crows at the roost (~6,000) and by the estimated number of fecal samples per day per crow (Table 1). We recorded the number of defecations by nine adult crows held in aviaries for 6.3 ± 2.8 h (mean ± standard deviation). These individuals defecated 7.9 ± 3.5 times per hour (mean ± standard deviation), but this rate was almost certainly elevated by the stress of capture, especially immediately after placement in the aviary. For the purposes of illustration, a very conservative daytime defecation rate of 18 fecal samples per day per crow was adopted (1.5 feces per hour). This rate should not be considered definitive but provides a minimum estimate of crow defecation across the landscape.

A randomization test was used to determine if daytime habitat use was biased toward or against certain habitat types. First, the habitat type was identified at 50,000 regularly spaced locations within the 12.5-km buffer surrounding the roost. Using this set of locations as a starting point, 1,430 points were randomly sampled (the number of daytime crow observa-

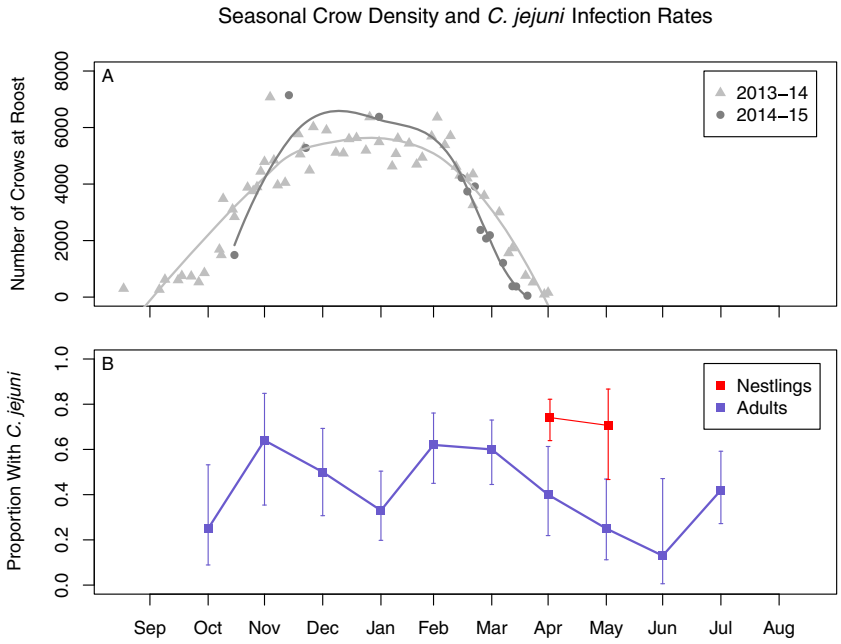

FIG 2 Crow roost size (A) and year-round prevalence of *C. jejuni* in crow feces for adults and nestlings (B). Nestlings were sampled only in April and May, because crows do not breed at other times of the year. No fecal samples were collected in August and September, because crows did not roost communally in those months, making sample collection difficult. Roost point counts are plotted with a smoothed localized regression line to illustrate seasonal patterns. Prevalence estimates show the percentage of isolates that tested positive for *C. jejuni* in each month, pooling all samples across years. Error bars illustrate the 95% binomial confidence interval for each prevalence estimate. The total number of samples tested varied by month.

tions) 10,000 times, and for each set of points, the percentage of locations in each habitat type was calculated. Repeated sampling of the same point was allowed to account for the fact that crows could be observed in the same location multiple times. The observed crow usage of each habitat was then compared to the distribution generated by random sampling. Crows were considered to significantly favor a habitat if the observed value was greater than the 97.5th percentile of the random distribution or significantly disfavor a habitat if the observed value was less than the 2.5th percentile of the random distribution. No similar test was performed for nighttime locations, because these locations were focused solely on the roost.

Genomic analysis. To assess the similarity of *C. jejuni* isolates obtained from nest mates, we analyzed whole-genome sequences (WGS) of isolates collected from 23 individually marked nestlings from 9 nests (see collection methods above). For this analysis, isolates were included when at least two positive samples were available from separate nestlings in the same nest. High-molecular-weight genomic DNA (gDNA) was obtained from colonies isolated from blood agar plates after growth at 37°C under microaerophilic gas conditions, as previously described by the 100K Pathogen Genome Project (36–38). Briefly, the cells were lysed with an enzyme cocktail, mixed by vortexing, and purified gDNA was isolated using whole-genome kits, according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). gDNA purity and integrity were confirmed on a 2200 TapeStation with the genomic DNA ScreenTape (Agilent Technologies, Santa Clara, CA, USA) (39–41). gDNA with $A_{260/280}$ and $A_{260/230}$ of >1.8 was used for library construction.

Following isolation, gDNA was sheared using the Covaris E220 with the 96 microTUBE plate (Covaris, Inc., Woburn, MA, USA) (42). Libraries were made using Kapa high-throughput (HTP) library preparation kit (KR0426 version 3.13; Kapa Biosystems, Wilmington, MA, USA) with dual-SPRI size selection (43). Libraries were constructed using the Agilent Bravo (Agilent Technologies, Santa Clara, CA). Library quantitation was done using Kapa SYBR Fast qPCR kits (Kapa Biosystems) to ensure a starting concentration of 400 ng and a fragment insert size between 350 and 450 bp (43). Libraries were indexed using Bioo Scientific NEXTflex-96 DNA barcodes version 13.05 (Bioo Scientific Corp., Austin, TX) and Integrated DNA Technologies Weimer 384 TS-LT DNA barcodes. Sequencing was done by BGI@UCDavis (Sacramento, CA, USA) on an Illumina HiSeq 2000 platform using paired-end 100 bp (PE100) reads (Illumina, Inc., San Diego, CA, USA) (44, 45).

Sequences were aligned using progressiveMauve (46, 47), and contigs were reordered using the reorder contigs option in Mauve under standard parameters using *C. jejuni* subsp. *jejuni* NCTC 11168 as the reference genome (see Fig. S1 in the supplemental material). Genomic distances were determined using the Genome-to-Genome Distance Calculator (GGDC) (http://ggdc.dsmz.de/distcalc2.php) (48, 49). Distances were calculated using formula 2, as recommended for draft genomes. Distance matrices were translated into Newick tree format in the T-Rex Web server software using the neighbor-joining method (50, 51). Trees were edited using Dendroscope 3.0 (52) and Geneious version 6.1.8 (53).

To assess whether nest mate isolates were more similar than expected by chance, we conducted a randomization test using our strain GGDCs. For this test, the GGDC matrix was held constant while randomly reassigning nestlings to different *C. jejuni* strains and then calculating the average nest mate similarity based on the matrix of GGDC values. This procedure was iterated 100,000 times to generate an expected nest mate similarity distribution assuming random infection; the empirical isolate similarity that was observed was then compared to the random distribution. Given the *a priori* prediction that nest mates would have more similar strains than expected by chance, a *P* value was calculated as the number of random permutations with more similar nestling isolates divided by the total number of permutations (100,000). Randomization tests and all other statistical procedures were conducted in R version 3.2.2 (R Core Development Team, Vienna, Austria).

Accession number(s). Data from isolates that were sequenced as part of this study are accessible through NCBI (SRA accession numbers SRR1815854 to SRR1815856, SRR1815858, SRR1815860, SRR1816035, SRR1816037 to SRR1816040, SRR1816044, SRR1816046 to SRR1816049,

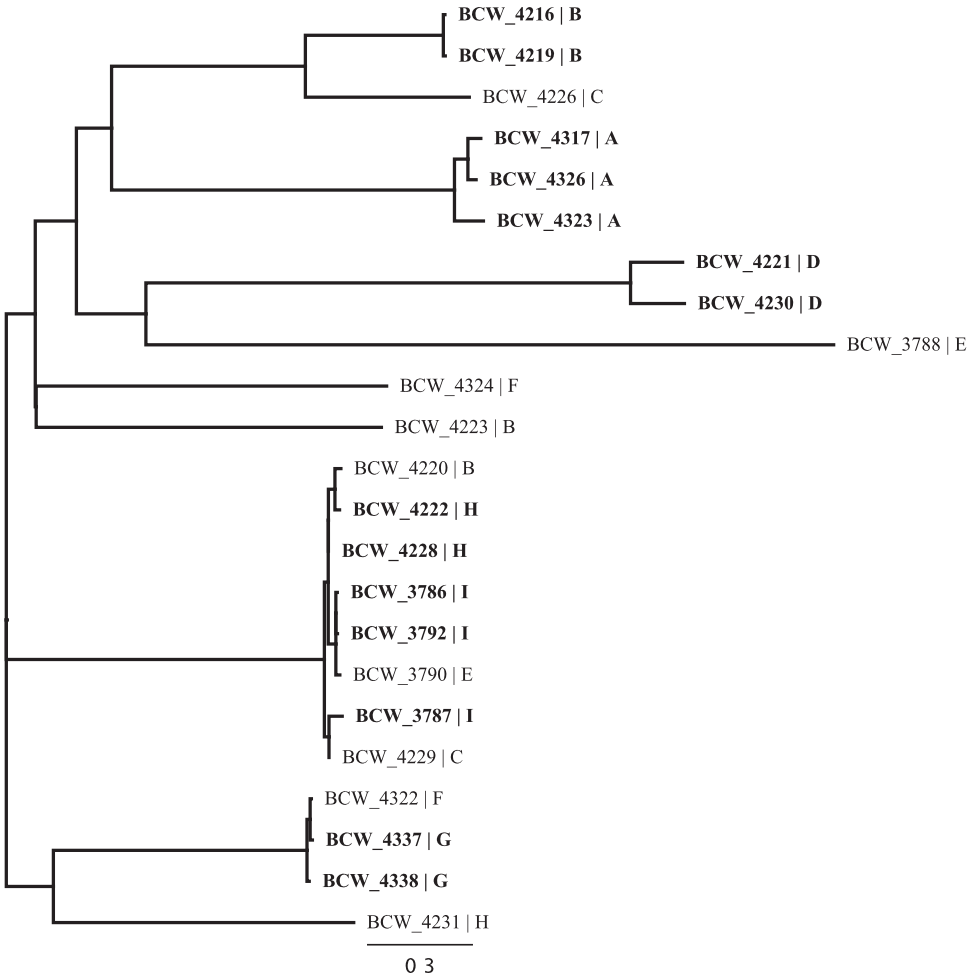

FIG 3 Genomic distance relationship of *Campylobacter jejuni* strains collected from nests where at least two nestlings sampled were positive ($n = 9$ nests) and where full-genome sequences were available. Tips with the same letter indicate isolates that came from different nestlings in the same nest. Nest mates with GGDC values of <0.003 are shown in bold. Isolates from the same nest had smaller GGDC values than expected by chance ($P = 0.006$). The isolate identification numbers can be used to find NCBI accession numbers from Table S2 in the supplemental material.

SRR1816118, SRR1816122, SRR1816123, SRR1816125, SRR1816131, and SRR1816132; see Table S2 in the supplemental material).

RESULTS

***Campylobacter* prevalence by date, subject age, and social behavior.** Overall, 337 individual fecal samples were examined for *C. jejuni*. Of those, 178 (53%) tested positive for *C. jejuni*. Prevalence varied by crow age and season. Nestlings had the highest prevalence, with 73 out of 102 (72%) samples testing positive. Samples collected in the winter from captured adults or swabs under the roost had intermediate prevalence, with 51% of samples testing positive (22 of 45 captured adults and 61 of 119 roost swabs). The summer roost had the lowest prevalence, with 22 of 71 (31%) fecal samples testing positive. Differences in prevalence among samples collected during winter roosting, during summer roosting, or from nestlings all were significant (Fig. 1; Fisher's exact test, $P < 0.01$). Sixteen of these samples were submitted in duplicate, and all produced the same test result (9 positive, 7 negative). The difference in prevalence for adult samples collected during winter or summer roosting was consistent with the hypothesis that roost size drives transmission (Fig. 2A and B), but our data did not allow us to test for a causal link between roost size and transmission *per se*.

We also found that social proximity in the nesting environment was a strong predictor of *C. jejuni* infection (likelihood ratio test comparing a full and reduced logistic mixed model with nestling infection status as the binary response, presence of a colonized nest mate as a predictor, and nest identity as a random effect: $n = 69$ nestlings from 26 nests, $\chi^2 = 21.4$, $P < 0.0001$). In general, nest mates were either all colonized with *C. jejuni* or all uncolonized, with only a few nests (4 out of 26) containing a mix of colonized and uncolonized individuals.

GGDC calculations for each pair of sequenced isolates from these nestlings indicated that many nest mates were colonized by very similar strains of *C. jejuni* (GGDC for nest mates, 0.012 ± 0.003; GGDC for non-nest mates, 0.021 ± 0.001; Fig. 3). The randomization test demonstrated that the GGDC for strains for *C. jejuni* isolates from the same nest was significantly ($P = 0.006$) smaller than expected by chance. In a few cases, strains from the same nest were genomically divergent, suggesting that multiple strains may be infecting individuals in a

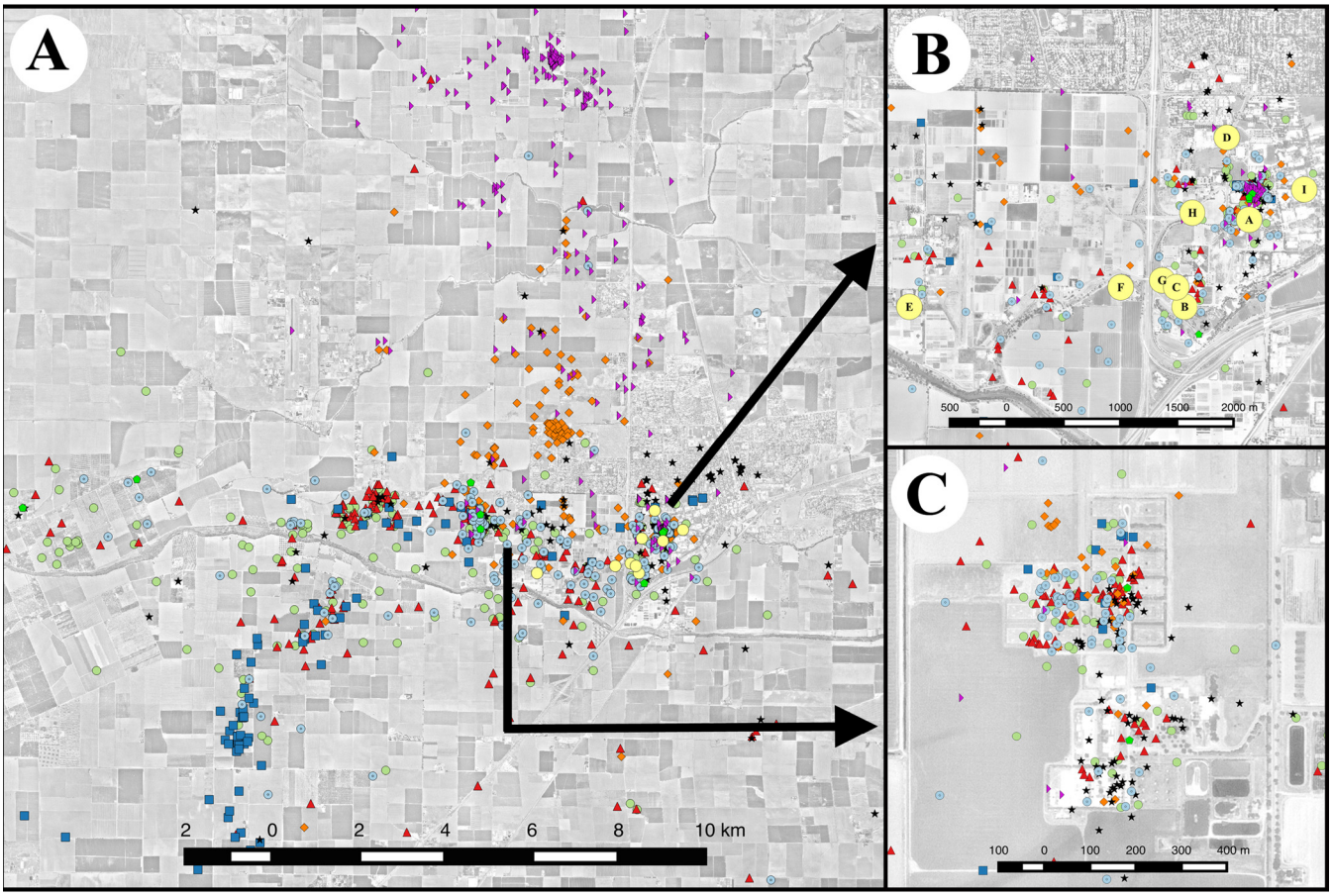

FIG 4 (A) Map of the study site displaying 2,147 GPS locations collected from American crows. The large yellow circles indicate the nests included in Fig. 3. The remaining symbols indicate the 7 individual birds from which locations were obtained. Insets show zoomed-in views of the nests included in Fig. 3, with the letters corresponding to those included on the phylogeny (B) and the primate research center (C). This map uses satellite imagery available from the U.S. Geological Survey; the map was constructed using the QGIS software.

single nest (i.e., acquired from both parents and/or from adult helpers-at-the-nest [54]).

Movement data and habitat use. Location data were collected from seven of the eight GPS receivers deployed. The bird carrying the eighth unit was never seen after initial deployment, and we assume that this bird did not return to the communal roost that we were monitoring, although the lack of data could also have been caused by a hardware malfunction. From the seven birds we reencountered, we collected a total of 2,147 locations between 9 January and 27 March 2015 (Fig. 4). Of these locations, 717 were collected at night and 1,430 during the day. The average home range size (and standard deviation) for these seven birds based on minimum convex polygons was 235 ± 207 km^2 (range, 78 to 684 km^2).

On average, crows forayed 7.7 ± 4.1 km (mean ± standard deviation) from the roost each day, with a maximum observed distance of 29 km. During the night, 96.7% of the collected locations were in developed areas, with the vast majority occurring at the communal roost. Daytime locations were recorded from both developed (25.9%) and agricultural (71.5%) areas, with the majority of daytime foraging occurring in the agricultural areas surrounding the town. Overall, daytime habitat usage differed from random expectations in every habitat category that we assessed except for areas of low-intensity development (Table 1). After accounting for the amount of each habitat type that was available, crows preferentially spent time in fallow fields, grasslands, hay fields, almond trees, walnut trees, and areas of medium- or high-intensity development (Table 1; randomization test, $P < 0.04$) and avoided tomatoes, winter wheat, sunflower, alfalfa, rice, and other crops (Table 1; randomization test, $P < 0.001$). We also noted heavy daytime use of two particular locations outside the roost. First, 3.2% of the daytime locations were at a local dairy barn, which encompassed <0.00001% of the available land area in the study site. Second, 19.4% of the daytime locations were at a local primate research center, which encompassed <0.001% of the available land area (Table 2).

DISCUSSION

Although many studies have suggested that wild birds play a role in *C. jejuni* transmission, the contributions of host ecology, movement, and social behavior to the risk of cross-species transmission have been largely unexplored. We documented high year-round prevalence of *C. jejuni* in American crows inhabiting an urban-to-rural landscape in Davis, CA. Further, we demonstrated that patterns of infection and environmental contamination are dependent on social behavior and space use; thus, studies focused solely

TABLE 1 Winter habitat use by American crows based on locations from GPS receivers compared to baseline percentage of each habitat

Habitat type	Night use (19:00 to 7:00) (%)	Day use (7:00 to 19:00) (%)	Baseline use %	Day vs baseline *P* value[a]	Crows/km^2/day	Estimated feces/km^2/day
Developed	96.7	25.9	11.3		27.3	491
Open or low intensity	10.5	7.7	6.5	0.08	14.0	252
Medium intensity	80.0	12.6	4.1	+<0.0001	32.1	578
High intensity	6.2	2.3	0.7	+<0.0001	35.1	632
Agricultural	3.3	71.5	87.7		10.0	180
Alfalfa	0.7	7.3	14.7	−<0.0001	6.2	112
Almonds	0.1	8.9	5.1	+<0.0001	21.8	392
Fallow/idle cropland	0.4	19.5	16.2	+0.002	14.3	257
Grassland/pasture	0.1	4.8	1.8	+0.0001	34.5	621
Other hay/nonalfalfa	0.0	4.9	3.8	+0.034	16.6	299
Sunflowers	0.4	2.2	7.8	−<0.0001	3.3	59
Tomatoes	0.8	4.9	15.3	−<0.0001	3.9	70
Walnuts	0.1	9.0	4.5	+<0.0001	24.4	439
Winter wheat	0.0	5.7	8.3	−<0.0001	8.6	155
Rice	0.0	0.0	3.2	−<0.0001	0.0	0
Other crops	0.7	4.3	7.0	−<0.0001	8.0	144
Other (e.g., wetlands, barren)	0.0	2.6	1.0	+<0.0001	31.8	572

[a] From randomization test.

on infection prevalence may serve as a poor proxy for actual transmission risk at a given location and time. Overall *C. jejuni* prevalence in feces varied between seasons with high and low social aggregation, while patterns of movement and habitat use served to amplify fecal contamination at certain times of the year and in certain locations. As a result, the risk of cross-species transmission of *C. jejuni* appeared to be concentrated in a predictable pattern across the landscape. Our study is among the first to combine extensive sampling and whole-genome sequencing of *C. jejuni* with relevant information on host ecology, movement, and social behavior.

Even with very high rates of infection, it is still unclear how important crows are in the overall *C. jejuni* transmission cycle that includes domestic animals and humans. At the peak of the winter roosting season, approximately 6,000 crows congregated at our study site. We estimated that 51% of those birds were infected with *C. jejuni*; however, many of the collected isolates may be crow specific (28) and thus might not be a threat for human infection. Furthermore, even with high rates of infection, most infected feces will not result in transmission because they may not contact another host, or the bacteria may die before another host is contacted. Still, with more than 6,000 birds occupying a small area at one time of the year, the cumulative risk of transmission might be high, especially considering the way that nonrandom space use can amplify the risk of transmission in specific areas. For example, the strong habitat associations with a local dairy barn and primate research center likely resulted in extensive exposure to crow feces at those locations during the peak of the roosting season. Even if each fecal deposition event represents a very low risk of cross-species transmission, the collective effect might be large. Although a primate facility is clearly not a typical landscape feature that crows encounter, we expect that similar patterns would be found at any animal facility where crows can access easy and abundant food resources.

TABLE 2 Sample collection data by specific location

Specific location	Collected at night (%)	Collected during day (%)	Total area (km^2)	No. of crows/day	No. of fecal samples/day
Primate center	0.0	19.4	0.25	1,164	20,952
Local dairy barn	<0.1	3.2	0.001	192	3,456

Given the high prevalence that we observed in this study, it is likely that crows are continually reexposed to *C. jejuni* and that these exposures result in repeated infection, or that infection is long-lasting. Unfortunately, the methods used in this study did not allow collection of longitudinal data from the same individuals, so it is not clear how long crows shed *C. jejuni* in feces after the initial infection. In other wild birds, very little is known about the length of infection or the possibility of cross-strain immunity (16). In one aviary-based study, Waldenström et al. (55) found that experimental infection of European robins (*Erithacus rubecula*) with *C. jejuni* isolated from a song thrush (*Turdus philomelos*) resulted in detectable bacteria shedding for 6.8 days, but infection of the same species with a human-pathogenic strain only resulted in 0 to 1 day of detectable shedding. In contrast, broiler chickens typically remain colonized throughout their lifetime (up to 7 weeks [56]), although experimental administration of different *C. jejuni* strains can produce variation in the persistence of infection (57). In one case, 3-week-old herring gulls (*Larus argentatus*) that were naturally infected with *C. jejuni* were taken into an aviary, and all had cleared their infection by 4 weeks after capture, indicating that the total infection length was less than 7 weeks (58). Although there is some indication that infection may result in acquired immunity, relatively little is known about the effectiveness or strain specificity of immunity, and reinfection by identical or novel strains is common (32, 56). Assuming that infection persists for 1 to 7 weeks in crows, the average individual would need to be exposed to *C. jejuni* frequently to maintain the 51% prevalence that we observed in the winter. At present, it is uncertain how this high rate of exposure is maintained, but there are a few potentially contributing factors.

Foraging ecology and habitat use may contribute to high rates of crow infection. Foraging guild has been implicated as a driver of *C. jejuni* infection, and opportunistic ground feeders, such as crows, are among the most colonized groups (20). Unsurprisingly, then, a high prevalence of *C. jejuni* infection has been found in corvids around the world (15, 25, 28, 29, 59). Our study suggests that social behavior may also be an important consideration in understanding the high rates of corvid infection. For adults, we found that infection was highest in the winter months and lowest in the summer. Although we could not test directly for a causal relationship between roost size and infection prevalence, the seasonal change in prevalence broadly parallels population-level changes in social aggregation. Whether this seasonal pattern is driven by roosting behavior or some other factor is unknown at present. For example, during the winter roosting season, migrants intermingle with local birds, and it is possible that an infusion of novel *C. jejuni* strains, rather than roosting density *per se*, drives seasonal differences. We could not evaluate this possibility in our study because samples collected under the winter roost came from birds of unknown migratory origin. Regardless of the mechanism driving seasonal changes in prevalence, the highest infection rates occurred when crows roosted communally in one densely packed area each night and foraged together in large flocks during the day. During these months, up to 58% of individuals had fecal staining on their feathers (31); because birds preen feathers regularly with their beaks, the potential for fecal-oral bacterial transmission is high. In addition to preening, crows often forage socially on highly concentrated food resources, which may result in frequent fecal-oral transmission during foraging. In general, *C. jejuni* does not survive well in the environment outside the wet and warm conditions of its host (60), but the social behaviors of crows could permit transmission even if *C. jejuni* survival in feces is brief. For nestlings, we found strong evidence that the infection status of nest mates influenced the risk of infection and that infected nest mates had more similar isolates than expected by chance.

Whether crows represent a major source of domestic animal and, ultimately, human *C. jejuni* infection remains uncertain, but our study indicates that data on infection prevalence and molecular characteristics of isolates alone will be insufficient for understanding *C. jejuni* transmission dynamics. Rather, more studies are needed that combine laboratory techniques and sampling of wildlife for *C. jejuni* with rigorous field work characterizing the ecology, movement, and behavior of potential wild bird vectors. Interestingly, some data suggest that the size of crow roosts has increased and that their locations have shifted from rural to increasingly urban areas over the last 50 years, with unknown impacts on disease dynamics (61). As human-altered landscapes continue to bring wildlife, humans, and domestic animals into increasingly close contact, understanding the shifting ecological interactions and patterns of cross-species transmission will only become more important.

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