AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

FOOD MICROBIOLOGY

Strain-Specific Differences in Survival of *Campylobacter* spp. in Naturally Contaminated Turkey Feces and Water

Lesley Good,[a] William G. Miller,[d] Jeffrey Niedermeyer,[a] Jason Osborne,[b] Robin M. Siletzky,[a] Donna Carver,[c] Sophia Kathariou[a]

[a]Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, North Carolina, USA
[b]Department of Statistics, North Carolina State University, Raleigh, North Carolina, USA
[c]Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, USA
[d]Produce Safety and Microbiology Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Albany, California, USA

ABSTRACT *Campylobacter jejuni* and *Campylobacter coli* are leading causes of human foodborne illness, with poultry as a major vehicle. Turkeys are frequently colonized with *Campylobacter*, but little is known about *Campylobacter* survival in turkey feces, even though fecal droppings are major vehicles for *Campylobacter* within-flock transmission as well as for environmental dissemination. Our objective was to examine survival of *Campylobacter*, including different strains, in freshly excreted feces from naturally colonized commercial turkey flocks and in suspensions of turkey feces in water from the turkey house. Fecal and water suspensions were stored at 4°C, and *Campylobacter* populations were enumerated on selective media at 48-h intervals. *C. jejuni* and *C. coli* isolates were characterized for resistance to a panel of antibiotics, and a subset was subtyped using multilocus sequence typing. *Campylobacter* was recovered from feces and water for up to 16 days. Analysis of 548 isolates (218 *C. jejuni* and 330 *C. coli*) revealed that *C. jejuni* survived longer than *C. coli* in feces ($P = 0.0005$), while the reverse was observed in water ($P < 0.0001$). Strain-specific differences in survival were noted. Multidrug-resistant *C. jejuni* isolates of sequence type 1839 (ST-1839) and the related ST-2935 were among the longest-surviving isolates in feces, being recovered for up to 10 to 16 days, while multidrug-resistant *C. coli* isolates of ST-1101 were recovered from feces for only up to 4 days. Data on *Campylobacter* survival upon excretion from the birds can contribute to further understanding of the transmission dynamics of this pathogen in the poultry production ecosystem.

IMPORTANCE *Campylobacter jejuni* and *Campylobacter coli* are leading foodborne pathogens, with poultry as a major reservoir. Due to their growth requirements, these *Campylobacter* spp. may be unable to replicate once excreted by their avian hosts, but their survival in feces and the environment is critical for transmission in the farm ecosystem. Reducing the prevalence of *Campylobacter*-positive flocks can have major impacts in controlling both contamination of poultry products and environmental dissemination of the pathogens. However, understanding the capacity of these pathogens to survive in transmission-relevant vehicles such as feces and farmhouse water remains poorly understood, and little information is available on species- and strain-associated differences in survival. Here, we employed model conditions to investigate the survival of *C. jejuni* and *C. coli* from naturally colonized turkey flocks, and with diverse genotypes and antimicrobial resistance profiles, in turkey feces and in farmhouse water.

KEYWORDS *Campylobacter*, *Campylobacter coli*, *Campylobacter jejuni*, turkey, antimicrobial resistance, feces, survival, water

Campylobacter spp. are zoonotic bacterial pathogens that are leading agents for human foodborne illness worldwide (1–4), annually resulting in an estimated 0.8 million cases of foodborne illnesses in the United States alone (1). In addition to acute

Citation Good L, Miller WG, Niedermeyer J, Osborne J, Siletzky RM, Carver D, Kathariou S. 2019. Strain-specific differences in survival of *Campylobacter* spp. in naturally contaminated turkey feces and water. Appl Environ Microbiol 85:e01579-19. https://doi.org/10.1128/AEM.01579-19.

Editor Eric V. Stabb, University of Illinois at Chicago

Address correspondence to Sophia Kathariou, skathar@ncsu.edu.

Received 15 July 2019
Accepted 8 September 2019
Accepted manuscript posted online 13 September 2019
Published 30 October 2019

gastroenteritis, human campylobacteriosis can be followed by severe autoimmune sequelae and constitutes the leading antecedent to Guillain-Barré syndrome (5). In the United States and other industrialized nations, *Campylobacter jejuni* is responsible for the majority (approximately 85%) of human campylobacteriosis cases, with *Campylobacter coli* being responsible for most of the remainder (4, 6), and contaminated poultry is considered to be a leading vehicle for human campylobacteriosis (7–9). Poultry, including chickens and turkeys, are frequently colonized by *C. jejuni* and *C. coli*, which can then be shed in large numbers in the feces (10–15).

Knowledge of *Campylobacter* survival outside its avian hosts remains poorly characterized. *C. jejuni* and *C. coli* are unable to grow below 30°C but can survive for variable lengths of time, with survival markedly better at low temperatures, such as 4°C (16–20). Survival in water can be enhanced by association with other microbes, including other bacteria and waterborne protozoa, such as *Acanthamoeba castellanii* and *Tetrahymena pyriformis* (21–24). *C. jejuni* that had been internalized by protozoa in water from a broiler farm survived longer than *C. jejuni* that remained extracellular and also exhibited increased tolerance to disinfection (22).

Campylobacter cells are shed, often in high numbers, in the feces of asymptomatic birds (7). Thus, poultry feces constitute a major vehicle for transmission of *Campylobacter* to the birds within a flock and for subsequent environmental contamination. In addition to coprophagy-mediated transmission within the flock as birds peck on feces-contaminated litter, birds can become infected through water contaminated with the fecal droppings (10, 11, 25). *Campylobacter*'s capacity to colonize chickens is enhanced upon passage through the birds' gastrointestinal (GI) tract and shedding in the fecal droppings (26–30). *Campylobacter* in the poultry feces can be then transmitted to other flocks and farms via insects, such as flies, and other vectors, including farm equipment and human traffic, with potential for downstream dispersal and contamination of the natural environment, e.g., surface water and soil (10, 25, 31–33).

In spite of its clear food safety and public health relevance, *Campylobacter* survival in poultry feces remains poorly understood. The limited available information is focused on survival in chicken droppings. *C. jejuni* was found to survive for up to 5 to 6 days in naturally or artificially contaminated laying hen feces at ambient temperature (20°C), with survival significantly higher in naturally contaminated feces (34–36). *C. jejuni* inoculated into feces and litter from *Campylobacter*-negative flocks survived significantly longer in feces than in litter, with survival found to be higher at lower temperatures (20°C versus 25 or 30°C) and independent of relative humidity (36). However, major gaps remain in our knowledge of the potential impact of species- (i.e., *C. jejuni* or *C. coli*) and strain-specific attributes, including genotype and antimicrobial resistance, on survival. Reports about *Campylobacter* survival in turkey feces have been lacking, even though turkeys are frequently colonized with *C. jejuni* and *C. coli*, including multidrug-resistant strains (12, 15, 37, 38). The objective of the current study was to employ model conditions in order to characterize survival of *Campylobacter* spp. in turkey feces and in water from turkey farms. To enhance the relevance of the findings to commercial turkey farm systems, we investigated the survival of *C. jejuni* and *C. coli* strains of diverse antimicrobial resistance profiles and genotypes in feces excreted by flocks that were already naturally colonized by these species and strains, as well as in water from the turkey farmhouse.

RESULTS

***Campylobacter* spp. in feces and water could be recovered for up to 16 days at 4°C, with a progressive decline during this period.** At time 0, *Campylobacter* populations in the fecal composite samples ranged from 1.4×10^6 to 3.2×10^6 CFU/g. Due to its growth requirements for high temperature and microaerobic atmosphere, *Campylobacter* was not expected to grow in these samples, and indeed *Campylobacter* levels progressively declined with time in all samples. As shown with two representative flocks, population levels in the samples declined slowly (1- to 3-log reduction) over the first 8 days of storage in the feces or in the water suspension (Fig. 1 and data not

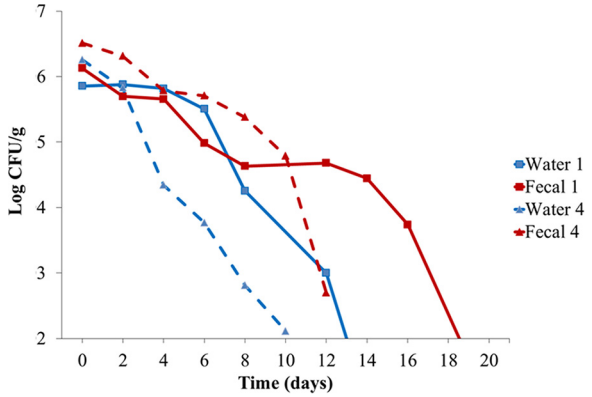

FIG 1 *Campylobacter* spp. survival in turkey feces and water suspensions. Fecal composite samples (samples 1 and 4) and water suspensions were prepared from two representative *Campylobacter*-positive turkey flocks as described in Materials and Methods. Total *Campylobacter* populations were enumerated on selective media (mCCDA) immediately prior to incubation at 4°C (time 0) and at 48-h intervals thereafter, as described in Materials and Methods.

shown). By day 16, *Campylobacter* spp. levels were approaching or had already reached the limit of detection, and by day 20, no isolates could be recovered from any of the samples (Fig. 1). The rate of decline of total *Campylobacter* spp. was not significantly different ($P > 0.05$) between the fecal composite sample and the suspension of the same composite sample in water; the average number of days at which *Campylobacter* spp. fell below the limit of detection was 10 and 10.7 for fecal composite samples and water samples, respectively.

Relative prevalence of *C. jejuni* increased with time in fecal composite samples, while *C. coli* predominated at later time points in water suspensions. *Campylobacter* species designations (*C. jejuni* or *C. coli*) were determined for 548 isolates obtained between time 0 and 16 days from the feces or the water suspensions (Table S1 in the Supplemental Material). Of the 548 isolates, 218 were *C. jejuni*, with the remaining 330 being *C. coli*; other *Campylobacter* species were not detected. The overall relative prevalence of *C. jejuni* in the feces was approximately 40% at time 0 but increased in the later time points to approximately 90% by day 14 (Fig. 2). The reverse was observed in the water suspensions, where the relative frequency of *C. coli* increased with time ($P \leq 0.0005$). Of the 55 *Campylobacter* isolates obtained from the water suspensions after 8 days, all but one were *C. coli* (Fig. 2).

***C. jejuni* and *C. coli* with specific antimicrobial resistance profiles differ in their survival in feces and in water suspensions.** Antimicrobial resistance profiles were determined for tetracycline (T), streptomycin (S), kanamycin (K), erythromycin (E), and the (fluoro)quinolones nalidixic acid and ciprofloxacin (Q) and are indicated with acronyms that reflect the observed resistance traits. Several antimicrobial resistance (AMR) profiles were identified among the isolates, including five among *C. jejuni* and nine among *C. coli*. Of the five AMR profiles in *C. jejuni*, two clearly predominated both in the feces and in the water suspensions. The majority of the *C. jejuni* isolates ($n = 148$, approximately 68%) had AMR profile TSKQ, i.e., resistant to all tested antimicrobials except for erythromycin, while 57 isolates (26% of all *C. jejuni*) had profile TKQ, i.e., resistant to tetracycline, kanamycin, nalidixic acid, and ciprofloxacin but susceptible to streptomycin and erythromycin (Fig. 3). The remaining three AMR profiles were infrequent; TSQ was detected in seven isolates, while TK and T were detected in three isolates each (Table S1). In *C. coli*, the leading AMR profiles in both feces and water suspensions included resistance to tetracycline and kanamycin (profile TK), followed by AMR profiles TKQ and TQ (Fig. 4). The multidrug resistance *C. coli* profile TSEKQ, i.e., resistance to all six tested antimicrobials, was noteworthy in constituting a noticeable (approximately 20%) fraction of the *C. coli* isolates in the early time points (days 0 to 2) but not thereafter (Fig. 4).

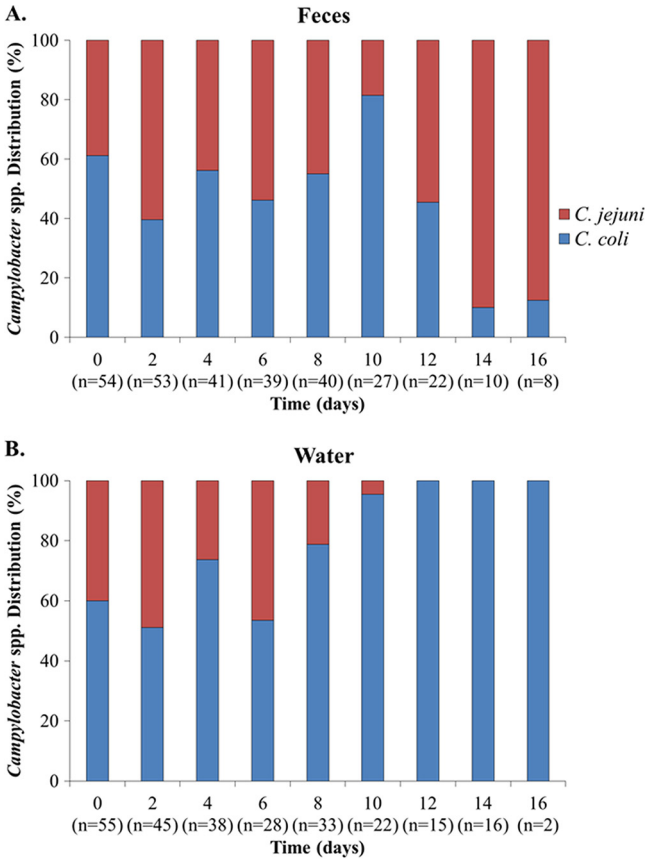

FIG 2 Relative survival of *Campylobacter jejuni* and *C. coli* in turkey feces and water suspensions. *Campylobacter* colonies from the mCCDA plates used for enumerations of total *Campylobacter* populations from the feces and the water suspensions at different time points (see legend to Fig. 1) were purified and speciated as described in Materials and Methods. *C. jejuni* and *C. coli* are shown in red and blue, respectively.

Survival in feces differed significantly across strains of *C. jejuni* with different AMR profiles ($P < 0.01$) (Fig. 3A). However, significant differences were not noted regarding survival in water ($P > 0.05$), where *C. jejuni* with profiles TSKQ and TKQ predominated in water suspensions through day 8 (Fig. 3B). As discussed above, total *C. jejuni* populations declined markedly in the suspensions thereafter (Fig. 2B), and only one *C. jejuni* isolate was obtained from the water suspensions after 8 days (Fig. 3B). Significant survival differences in feces were also noted across *C. coli* isolates with different AMR profiles, with the differences remaining significant in water suspensions as well ($P \leq 0.0001$). As indicated above, the relative prevalence of *C. coli* with the multidrug resistance profile TSEKQ diminished markedly after the early time points, both in feces and in the water suspension (Fig. 4). This TSEKQ profile was exclusively encountered among *C. coli*, in fact, and similar to results from previous studies of *Campylobacter* in turkeys in this region (14), no erythromycin-resistant *C. jejuni* isolates were identified in the study.

MLST genotyping of a panel of *C. jejuni* TSKQ isolates from different time points from the feces and the water suspensions (0, 2, 8, and 10 days) revealed that all shared the same sequence type, ST-1839, which was also detected in all but one of the seven tested *C. jejuni* isolates with AMR profile TKQ (Fig. 5, Table S2). The closely related ST-2935 (one allele difference from ST-1839) was detected in all three tested isolates of AMR profile TSQ, which were relatively uncommon and identified primarily in the feces and at later time points (days 12, 14, and 16) (Tables S1 and S2 and Fig. 5). One may speculate that *C. jejuni* ST-1839, of AMR profiles TSKQ or TKQ, and the related ST-2935 (AMR profile TSQ) may have high fitness for survival of *Campylobacter* after excretion.

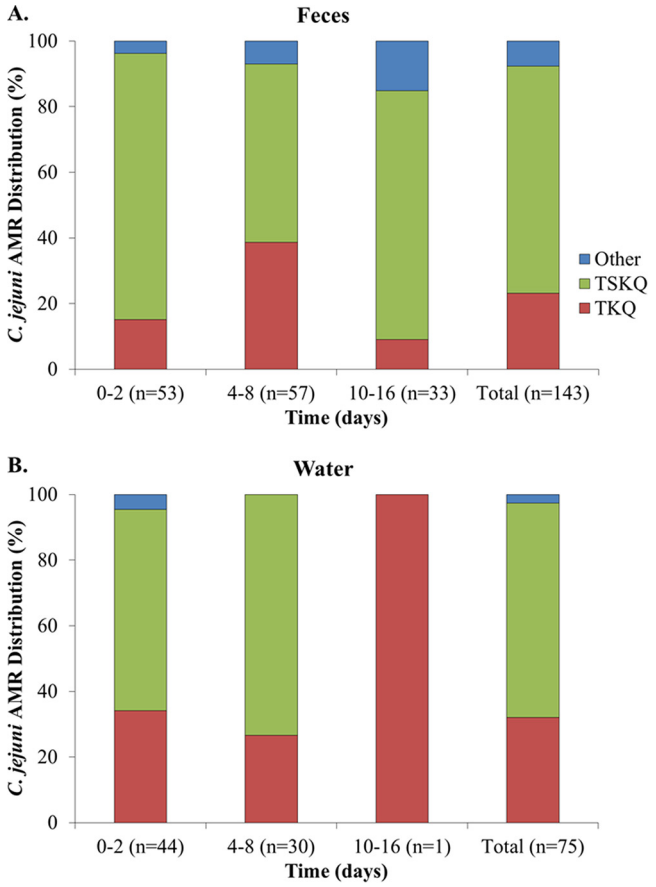

FIG 3 Relative prevalence of *Campylobacter jejuni* strains with different antimicrobial resistance (AMR) profiles at different time intervals in (A) feces and (B) water suspensions. AMR profiles were determined as described in Materials and Methods. AMR profile acronyms specify resistance to the following antimicrobials: tetracycline (T), streptomycin (S), kanamycin (K), and the (fluoro)quinolones nalidixic acid and ciprofloxacin (Q). The combination of letters indicates the specific AMR profile, e.g., TSEKQ indicates resistance to all tested antimicrobials, while TKQ indicates resistance to only tetracycline, kanamycin, and the quinolones nalidixic acid and ciprofloxacin.

It is also of interest that ST-2935 isolates were primarily detected at later time points, and only from feces, raising the possibility that they might represent variants of ST-1839 with enhanced capacity to survive in feces. Even though ST-1839 and ST-2935 are genetically similar based on the concatenated allele sequences (Fig. 5), further sequence analysis will be needed to more adequately assess the genetic differences that may account for differences in AMR profiles and potentially in fitness.

A number of STs were detected among *C. coli*. ST-1833 and the closely related (one-allele difference) ST-1192 were detected among *C. coli* isolates of the two leading AMR profiles, TK and TKQ, isolated from feces and water at various time points (0, 2, 10, and 16 days) (Tables S1 and S2 and Fig. 5). *C. coli* with the third-ranking AMR profile, TQ, had ST-1161, related to both ST-1833 (two-allele differences) and ST-1192 (one-allele difference). Also related (two-allele differences) to these STs was *C. coli* with ST-7731 and AMR profile TEKQ (Tables S1 and S2 and Fig. 5). Noteworthy was ST-1101, which was encountered among the multidrug-resistant *C. coli* TSEKQ and was phylogenetically remote, sharing no alleles with the other *C. coli* STs (Table S2 and Fig. 5). As discussed above, *C. coli* TSEKQ isolates were primarily obtained from early time points, and their relative prevalence diminished markedly with time.

DISCUSSION

The turkey flocks employed for materials in the current study were from a region (eastern North Carolina) dense in turkey production, and turkey flocks from this region

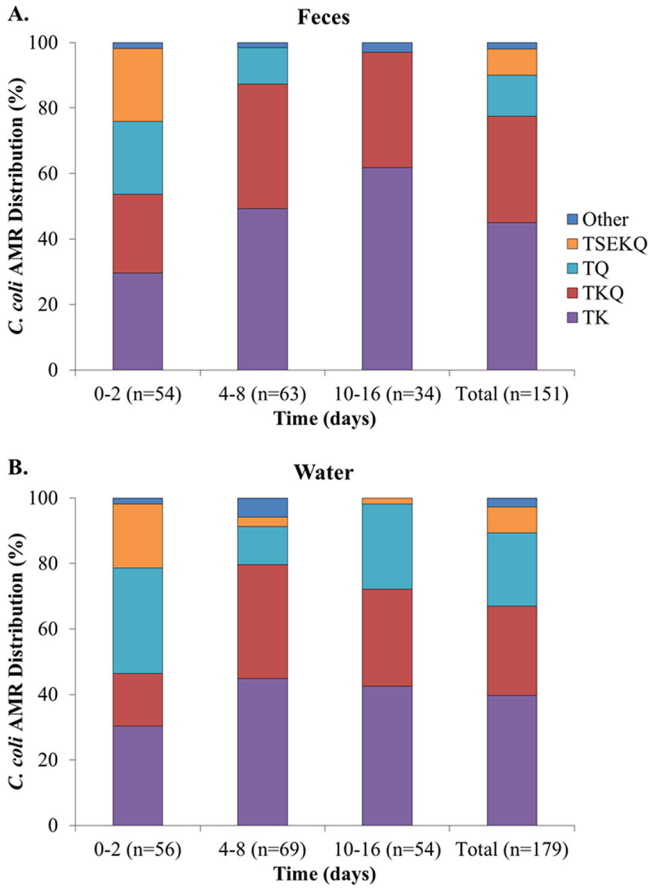

FIG 4 Relative prevalence of *Campylobacter coli* strains with different antimicrobial resistance (AMR) profiles at different time intervals in (A) feces and (B) water suspensions. AMR profiles were determined as described in Materials and Methods. AMR profile acronyms specify resistance to the following antimicrobials: tetracycline (T), streptomycin (S), erythromycin (E), kanamycin (K), and the (fluoro)quinolones nalidixic acid and ciprofloxacin (Q).

were previously found to be frequently colonized with both *C. jejuni* and *C. coli* (14, 38). Similarly, both species were encountered in the current study. Interestingly, *C. jejuni* survived significantly longer than *C. coli* in the feces, while the opposite was noted when feces were suspended in water from the turkey house. Such species-specific differences in survival in poultry feces versus water suspensions have not been reported before, and the underlying mechanisms remain to be elucidated. One possibility is that *C. coli* might have higher fitness than *C. jejuni* in protozoa present in the farm water. Protozoa were commonly found in the water systems of broiler farms, and the survival of *C. jejuni* and *C. coli* was shown to be significantly enhanced when cocultured with protozoa from the water systems (22). The presence of protozoa was not assessed in the turkey-house water in the current study, and the extent to which *C. coli* may have a fitness advantage in waterborne protozoa remains to be demonstrated, e.g., via cocultures with protozoa. Furthermore, studies using feces from flocks colonized with *C. jejuni* and *C. coli* strains different from those prevailing here will be valuable to determine whether the findings that we observed here pertain generally to *C. coli* versus *C. jejuni*.

Strain-specific differences were noted in survival, supporting previous data which were obtained with broiler feces naturally colonized with *C. jejuni* and stored at 20°C. In that study, genotyping employed *fla* typing, and of the five detected *fla* types, some tended to predominate in later time points after shedding (34). Our findings also strongly suggested that multidrug-resistant *C. coli* isolates with resistance profile TSEKQ had impaired capacity to persist in feces or water compared to other *C. coli* strains.

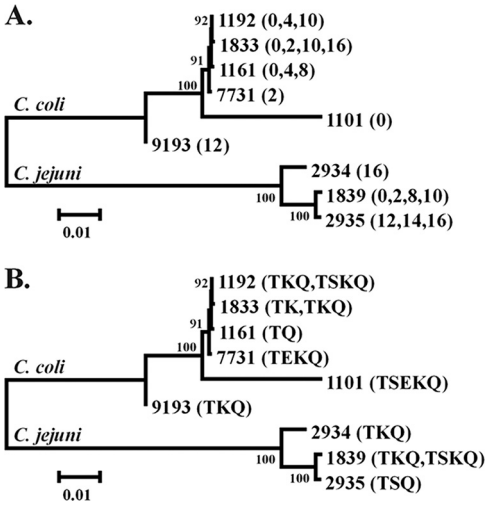

FIG 5 Phylogenetic tree of *Campylobacter jejuni* and *C. coli* strains with different sequence types (STs). The tree includes information on (A) time point at which the strains with the specific STs were obtained and (B) the AMR profiles of the same strains. The phylogenetic tree was constructed based on the concatenated sequence of the seven alleles as described in Materials and Methods.

Traits that may compromise the fitness of these strains have yet to be elucidated. In another study with commercial turkeys in the same region (eastern North Carolina), we discovered that *C. coli* TSEKQ isolates of ST-1161, ST-1149, and ST-906, related (one- to three-allele differences) to ST-1101 of *C. coli* TSEKQ in the current study, were frequently isolated from the cecal contents of young turkeys in the first 5 weeks of life, but their frequency declined significantly as birds aged (39). Such data had led to the hypothesis that these strains may have relatively low fitness in regard to their capacity to compete with other *Campylobacter* strains and other microbes in the gut as birds age (39). The current data suggest that *C. coli* TSEKQ with ST-1101 may also have relatively impaired fitness after excretion. It would be of interest to identify potential linkages between the low fitness of these strains in the feces from the relatively old birds in the current study (flocks were, on average, 12.8 weeks old when feces were collected) and their previously observed significant decrease in cecal prevalence with increasing age of the flocks (39).

In the current study, *Campylobacter* could be recovered from most fecal samples for up to 12 to 16 days at 4°C. This was noticeably longer than the observed survival of *C. jejuni* either naturally present or spiked in chicken feces which were then incubated at higher temperatures than we employed (20 to 30°C); in those studies, *C. jejuni* could only be recovered from the chicken feces for up to 5 to 6 days (34–36). Storage temperature may be a key reason for the observed differences, since *Campylobacter* survival is enhanced at low temperature (17, 19, 40). The low temperature (4°C) employed in the current study was intentionally chosen to enhance the potential to detect differences in survival among different strains of *C. jejuni* and *C. coli* shed by the birds in the feces. *Campylobacter* strains can differ in their cold tolerance (41–44), and such differences may contribute to the current findings. In addition to temperature, other attributes, including the avian source (chicken versus turkey) and the *Campylobacter* species and strains involved, may contribute to the observed differences in survival between our study and others.

Previous studies of survival of *Campylobacter* shed by colonized poultry involved only *C. jejuni* (34–36), whereas both *C. jejuni* and *C. coli* were shed in the feces and monitored in the current study. Previous studies also did not compare survival in feces and farm water and did not provide information on AMR profiles or STs of the *C. jejuni* strains that were investigated (34–36). Inclusion of ST or other readily cross-comparable genotypic designations, as well as antimicrobial resistance data, will be increasingly

useful for meaningful comparisons of the findings from different studies. It will, in addition, facilitate placement of findings from diverse regions and laboratories in the wider context of *Campylobacter* strain diversity and antimicrobial resistance.

In the current study, we characterized AMR profiles and genotypes of *C. jejuni* and *C. coli* strains isolated following different lengths of time in the feces or the water suspensions. Even though strains with certain AMR profiles and MLST-based genotypes differed in their capacity to survive in these materials, the underlying factors remain to be identified. Current data permitted the identification of associations between strain-specific traits such as resistance and genotype and relative survival capacity but do not constitute evidence of causality. The potential contribution of AMR profiles to persistence cannot be assessed with the current design of the study, which specifically investigated strains that had naturally colonized the flocks and were excreted in the feces in the farm environment. Such strains are genotypically diverse, and multiple attributes can contribute to their performance in feces and water. Genetic characterization of these strains via whole-genome sequence analysis is expected to be valuable in identifying potential genome content associations with strain-specific differences in survival in transmission-relevant vehicles that we observed in the current study.

The finding that *C. jejuni* survived significantly longer in feces than *C. coli* is important from a public health perspective. *C. jejuni* accounts for over 85% of human campylobacteriosis. Thus, higher relative fitness of *C. jejuni* in the feces may suggest greater potential for preharvest colonization of turkey flocks with this species. As mentioned earlier, *Campylobacter* can spread within a turkey house directly via coprophagy or among different turkey houses and farms via vectors such as flies, equipment, and transport crates as well as via human traffic, e.g., through footwear, clothing, and vehicles (10, 25, 31–33). During slaughter and processing, birds that became colonized with *Campylobacter* at the farm are more likely to lead to contamination of the carcasses with *Campylobacter* from the birds' intestinal contents (11, 45).

The current study monitored levels of *Campylobacter* populations naturally shed in the feces of *Campylobacter*-positive turkeys. This alleviates potential pitfalls associated with inoculation of feces or water with laboratory-grown strains of *C. jejuni* or *C. coli* and enhances the real-life relevance of the survival assessments. Further characterization of *Campylobacter* survival outside the poultry host will enhance our understanding of *Campylobacter* transmission within the farm ecosystem, as well as potential dissemination of the pathogen to the natural environment. Findings from such studies can inform assessments of the food safety and public health risk posed by different *Campylobacter* species and strains in the poultry production ecosystem, contributing to science-based interventions to reduce the disease burden of campylobacteriosis.

MATERIALS AND METHODS

Sample collection. Fecal droppings and water samples were obtained from seven turkey flocks grown conventionally at six different commercial turkey farms in eastern North Carolina between 29 July 2012 and 11 April 2013. Flock surveillance for a different project at our laboratory had indicated that these turkey flocks were *Campylobacter* positive and colonized with different *Campylobacter* species (*C. jejuni* and *C. coli*) and strains. All turkeys were males (toms) with an average age of 90 days (approximately 12.8 weeks) at the time that fecal droppings were collected for the present investigation.

Collection and processing of fecal droppings and water. During the visit to each turkey flock, eight fresh individual turkey droppings (two from each quarter of the turkey house) were collected. Droppings were considered to be fresh upon visual observation of voiding. During the same visit, 50 ml of water was collected from the same turkey house from drinking water available for the birds. Fecal and water samples were brought to the laboratory on ice within 3 hours of collection and processed on the same day.

In the laboratory, a fecal composite sample was prepared in a 50-ml sterile plastic Falcon tube (Becton, Dickinson and Co., San Jose, CA) by combining all eight individual turkey droppings and mixing them using a sterile metal spatula. Water suspensions were made in 15-ml sterile plastic Falcon tubes (Becton, Dickinson and Co.) by adding 1 g of the fecal composite sample into 9 ml of the water from the turkey house and vortexing. The fecal composite and the water suspension were stored in the dark at 4°C.

Isolation and enumeration of *Campylobacter* spp. Beginning on day 0 (immediately prior to placement of the samples at 4°C), dilutions of both the fecal composite sample and the water suspension were plated on the selective medium modified charcoal-cefoperazone-deoxycholate agar (mCCDA)

(Oxoid, Ogdensburg, NY) for isolation and enumeration of *Campylobacter* spp. Plates were incubated microaerobically using Campy GasPaks (Becton, Dickinson and Co.) at 42°C, and colonies were enumerated after 48 h of incubation. Enumerations were done at 48-h intervals until the number of *Campylobacter* colonies declined to near or below the detection limit (100 CFU/g feces or 100/ml of the water suspension). At every time point, 10 to 16 presumptive *Campylobacter* colonies were isolated and purified on Mueller-Hinton agar (MHA) (Becton, Dickinson and Co.) following microaerobic incubation at 42°C for 48 h as described above. Purified cultures were preserved at −80°C as described (14).

Species and antimicrobial resistance profile determinations. *Campylobacter* species designations (*C. jejuni* or *C. coli*) of a panel of 545 isolates from four flocks, each grown on a different farm, are summarized in Table S1. Designations were determined by multiplex PCR using primers specific for *hip* and *ceu*, as described (46). Isolates were tested as described (37) for susceptibility to a panel of six antibiotics, tetracycline (T), streptomycin (S), erythromycin (E), kanamycin (K), and the (fluoro)quinolones nalidixic acid and ciprofloxacin (Q). The pan-sensitive strain *C. jejuni* ATCC 33560 (American Type Culture Collection) was used as a quality control strain. All isolates were simultaneously grown on MHA plates to ensure viability. Antimicrobial resistance profiles are presented with acronyms that reflect the encountered resistance traits from among the tested antimicrobials. For instance, T represents an isolate with resistance only to tetracycline, while TKQ indicates an isolate with resistance to tetracycline, kanamycin, nalidixic acid, and ciprofloxacin but susceptible to the remaining two tested antimicrobials, and isolates with profile TSEKQ were resistant to all six tested antimicrobials.

Multilocus sequence typing and phylogenetic tree construction. A subset of isolates was characterized by multilocus sequencing typing (MLST) as described (47). The isolates were chosen to represent the two *Campylobacter* species that were encountered, i.e., *C. jejuni* and *C. coli*, the different time points, as well as the different antimicrobial resistance profiles. Thus, efforts were made to include isolates representing each specific species/antimicrobial resistance profile from each time point. All sequence data were deposited in PubMLST (https://pubmlst.org/campylobacter/) (48), and the MLST profiles identified in this study were downloaded from PubMLST as in-frame concatenated allele sequences. The concatenated sequences were aligned using ClustalX, and a dendrogram was constructed within MEGA v. 6 (49) using the neighbor-joining method with the Kimura 2-parameter distance estimation method.

Statistical analysis. Statistical analyses employed Chi-square and Fisher's exact tests and were conducted using SAS v. 9.3 (SAS Institute, Cary, NC). Linear models for mean log CFU were developed treating time points either as a factorial effect or as a continuously valued regressor with effect quantified by a slope. Sample type (feces or water) was included as a factorial effect, along with a possible interaction term with time point to allow for the possibility that the change over time depended on sample type. Fisher's exact test was used to determine whether there were differences among strains with different antimicrobial resistance (AMR) profiles in terms of how long they survived in feces or water. Two-way contingency tables were constructed between time and strain (AMR profile), separately for each species and sample type (feces and water). Time was dichotomized according to $t < 4$ days or $t \geq 4$ days.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.01579-19.

SUPPLEMENTAL FILE 1, PDF file, 0.6 MB.

ACKNOWLEDGMENTS

This study was supported by USDA-NIFA grant 2011-51110-31050.

We are grateful to turkey industry collaborators for their support throughout the project. We are grateful to Michael Mann and Rusty Romine for assistance in sample collection and to all turkey industry staff for access to farm samples. We thank all members of our laboratory for their support in this study.

REFERENCES

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, Jones JL, Griffin PM. 2011. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 17:7–15. https://doi.org/10.3201/eid1701.p11101.
2. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. 2015. Global epidemiology of *Campylobacter* infection. Clin Microbiol Rev 28:687–720. https://doi.org/10.1128/CMR.00006-15.
3. Kirk MD, Pires SM, Black RE, Caipo M, Crump JA, Devleesschauwer B, Döpfer D, Fazil A, Fischer-Walker CL, Hald T, Hall AJ, Keddy KH, Lake RJ, Lanata CF, Torgerson PR, Havelaar AH, Angulo FJ. 2015. World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. PLoS Med 12:e1001921. https://doi.org/10.1371/journal.pmed.1001921.
4. Friedman CR, Neimann J, Wegener HC, Tauxe RV. 2000. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations, p 121–138. *In* Nachamkin I, Blaser MJ (ed), *Campylobacter*, 2nd ed. ASM Press, Washington, DC.
5. Nyati KK, Nyati R. 2013. Role of *Campylobacter jejuni* infection in the pathogenesis of Guillain-Barré syndrome: an update. Biomed Res Int 2013:852195. https://doi.org/10.1155/2013/852195.
6. Fitzgerald C. 2015. *Campylobacter*. Clin Lab Med 35:289–298. https://doi.org/10.1016/j.cll.2015.03.001.
7. Andreoletti O, Budka H, Buncic S, Collins JD, Griffin J, Hald T, Hendrik Havelaar A, Hope J, Klein G, McLauchlin J, Messens W, Müller-Graf C, Nguyen-The C, Noerrung B, Peixe L, Prieto Maradona M, Ricci A, Sofos J, Threlfall J, Vågsholm I, Vanopdenbosch E, Hofshagen M, Kruse H, McCarthy N, Monteiro Pires S, Newell D, Strachan N. 2010. Scientific opinion on quantification of the risk posed by broiler meat to human campylobacteriosis in the EU. EFSA J 8:1437.
8. Domingues AR, Pires SM, Halasa T, Hald T. 2012. Source attribution of

human campylobacteriosis using a meta-analysis of case-control studies of sporadic infections. Epidemiol Infect 140:970–981. https://doi.org/10.1017/S0950268811002676.
9. Lindmark H, Boqvist S, Ljungström M, Agren P, Björkholm B, Engstrand L. 2009. Risk factors for campylobacteriosis: an epidemiological surveillance study of patients and retail poultry. J Clin Microbiol 47:2616–2619. https://doi.org/10.1128/JCM.00826-09.
10. Jacobs-Reitsma WF. 1997. Aspects of epidemiology of *Campylobacter* in poultry. Vet Q 19:113–117. https://doi.org/10.1080/01652176.1997.9694753.
11. Herman L, Heyndrickx M, Grijspeerdt K, Vandekerchove D, Rollier I, De Zutter L. 2003. Routes for *Campylobacter* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. Epidemiol Infect 131:1169–1180. https://doi.org/10.1017/s0950268803001183.
12. Wallace J, Stanley K, Jones K. 1998. The colonization of turkeys by thermophilic campylobacters. J Appl Microbiol 85:224–230. https://doi.org/10.1046/j.1365-2672.1998.00470.x.
13. Miller WG, Mandrell R. 2005. Prevalence of *Campylobacter* in the food and water supply: incidence, outbreaks, isolation and detection, p 101–163. *In* Ketley J, Konkel ME (ed), *Campylobacter jejuni*: new perspectives in molecular and cellular biology. Horizon Bioscience, Wymondham, Norfolk, United Kingdom.
14. Wright SL, Carver DK, Siletzky RM, Romine S, Morrow WEM, Kathariou S. 2008. Longitudinal study of prevalence of *Campylobacter jejuni* and *Campylobacter coli* from turkeys and swine grown in close proximity. J Food Prot 71:1791–1796. https://doi.org/10.4315/0362-028X-71.9.1791.
15. Luangtongkum T, Jeon B, Han J, Plummer P, Logue CM, Zhang Q. 2009. Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiol 4:189–200. https://doi.org/10.2217/17460913.4.2.189.
16. Park SF. 2002. The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. Int J Food Microbiol 74:177–188. https://doi.org/10.1016/s0168-1605(01)00678-x.
17. Hazeleger WC, Janse JD, Koenraad PM, Beumer RR, Rombouts FM, Abee T. 1995. Temperature-dependent membrane fatty acid and cell physiology changes in coccoid forms of *Campylobacter jejuni*. Appl Environ Microbiol 61:2713–2719.
18. Rodgers JD, Clifton-Hadley FA, Marin C, Vidal AB. 2010. An evaluation of survival and detection of *Campylobacter jejuni* and *C. coli* in broiler caecal contents using culture-based methods. J Appl Microbiol 109:1244–1252. https://doi.org/10.1111/j.1365-2672.2010.04748.x.
19. Bronowski C, James CE, Winstanley C. 2014. Role of environmental survival in transmission of *Campylobacter jejuni*. FEMS Microbiol Lett 356:8–19. https://doi.org/10.1111/1574-6968.12488.
20. Hilbert F, Scherwitzel M, Paulsen P, Szostak MP. 2010. Survival of *Campylobacter jejuni* under conditions of atmospheric oxygen tension with the support of *Pseudomonas* spp. Appl Environ Microbiol 76:5911–5917. https://doi.org/10.1128/AEM.01532-10.
21. Young KT, Davis LM, DiRita VJ. 2007. *Campylobacter jejuni*: molecular biology and pathogenesis. Nat Rev Microbiol 5:665–679. https://doi.org/10.1038/nrmicro1718.
22. Snelling WJ, McKenna JP, Lecky DM, Dooley J. 2005. Survival of *Campylobacter jejuni* in waterborne protozoa. Appl Environ Microbiol 71:5560–5571. https://doi.org/10.1128/AEM.71.9.5560-5571.2005.
23. Buswell CM, Herlihy YM, Lawrence LM, McGuiggan JT, Marsh PD, Keevil CW, Leach SA. 1998. Extended survival and persistence of *Campylobacter* spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining. Appl Environ Microbiol 64:733–741.
24. Vieira A, Karlyshev AV, Seddon AM. 2015. *Campylobacter-Acanthamoeba* interactions. Microbiology 161:933–947. https://doi.org/10.1099/mic.0.000075.
25. Ellis-Iversen J, Ridley A, Morris V, Sowa A, Harris J, Atterbury R, Sparks N, Allen V. 2012. Persistent environmental reservoirs on farms as risk factors for *Campylobacter* in commercial poultry. Epidemiol Infect 140:916–924. https://doi.org/10.1017/S095026881100118X.
26. Cawthraw SA, Wassenaar TM, Ayling R, Newell DG. 1996. Increased colonization potential of *Campylobacter jejuni* strain 81116 after passage through chickens and its implication on the rate of transmission within flocks. Epidemiol Infect 117:213–215. https://doi.org/10.1017/s0950268800001333.
27. Ringoir DD, Korolik V. 2003. Colonisation phenotype and colonisation potential differences in *Campylobacter jejuni* strains in chickens before and after passage in vivo. Vet Microbiol 92:225–235. https://doi.org/10.1016/s0378-1135(02)00378-4.
28. Jones MA, Marston KL, Woodall CA, Maskell DJ, Linton D, Karlyshev AV, Dorrell N, Wren BW, Barrow PA. 2004. Adaptation of *Campylobacter jejuni* NCTC11168 to high-level colonization of the avian gastrointestinal tract. Infect Immun 72:3769–3776. https://doi.org/10.1128/IAI.72.7.3769-3776.2004.
29. Chen L, Geys H, Cawthraw S, Havelaar A, Teunis P. 2006. Dose response for infectivity of several strains of *Campylobacter jejuni* in chickens. Risk Anal 26:1613–1621. https://doi.org/10.1111/j.1539-6924.2006.00850.x.
30. Conlan AJK, Line JE, Hiett K, Coward C, Van Diemen PM, Stevens MP, Jones MA, Gog JR, Maskell DJ. 2011. Transmission and dose-response experiments for social animals: a reappraisal of the colonization biology of *Campylobacter jejuni* in chickens. J R Soc Interface 8:1720–1735. https://doi.org/10.1098/rsif.2011.0125.
31. Hald B, Skovgard H, Pedersen K, Bunkenborg H. 2008. Influxed insects as vectors for *Campylobacter jejuni* and *Campylobacter coli* in Danish broiler houses. Poult Sci 87:1428–1434. https://doi.org/10.3382/ps.2007-00301.
32. Hald B, Skovgård H, Bang DD, Pedersen K, Dybdahl J, Jespersen JB, Madsen M. 2004. Flies and *Campylobacter* infection of broiler flocks. Emerg Infect Dis 10:1490–1492. https://doi.org/10.3201/eid1008.040129.
33. Jones NR, Millman C, van der Es M, Hukelova M, Forbes KJ, Glover C, Haldenby S, Hunter PR, Jackson K, O'Brien SJ, Rigby D, Strachan NJC, Williams N, Lake IR, ENIGMA Consortium. 2017. Novel sampling method for assessing human-pathogen interactions in the natural environment using boot socks and citizen scientists, with application to *Campylobacter* seasonality. Appl Environ Microbiol 83:e00162-17. https://doi.org/10.1128/AEM.00162-17.
34. Ahmed MFM, Schulz J, Hartung J. 2013. Survival of *Campylobacter jejuni* in naturally and artificially contaminated laying hen feces. Poult Sci 92:364–369. https://doi.org/10.3382/ps.2012-02496.
35. Bui XT, Wolff A, Madsen M, Bang DD. 2012. Reverse transcriptase real-time PCR for detection and quantification of viable *Campylobacter jejuni* directly from poultry faecal samples. Res Microbiol 163:64–72. https://doi.org/10.1016/j.resmic.2011.10.007.
36. Smith S, Meade J, Gibbons J, McGill K, Bolton D, Whyte P. 2016. The impact of environmental conditions on *Campylobacter jejuni* survival in broiler faeces and litter. Infect Ecol Epidemiol 6:31685. https://doi.org/10.3402/iee.v6.31685.
37. Gu W, Siletzky RM, Wright S, Islam M, Kathariou S. 2009. Antimicrobial susceptibility profiles and strain type diversity of *Campylobacter jejuni* isolates from turkeys in eastern North Carolina. Appl Environ Microbiol 75:474–482. https://doi.org/10.1128/AEM.02012-08.
38. Lee BC, Reimers N, Barnes HJ, D'Lima C, Carver D, Kathariou S. 2005. Strain persistence and fluctuation of multiple-antibiotic resistant *Campylobacter coli* colonizing turkeys over successive production cycles. Foodborne Pathog Dis 2:103–110. https://doi.org/10.1089/fpd.2005.2.103.
39. Niedermeyer JA, Ring L, Miller WG, Genger S, Parr Lindsey C, Osborne J, Kathariou S. 2018. Proximity to other commercial turkey farms affects colonization onset, genotypes and antimicrobial resistance profiles of *Campylobacter* in turkeys: suggestive evidence from a paired-farm model. Appl Environ Microbiol 84:e01212-18. https://doi.org/10.1128/AEM.01212-18.
40. González M, Hänninen M-L. 2012. Effect of temperature and antimicrobial resistance on survival of *Campylobacter jejuni* in well water: application of the Weibull model. J Appl Microbiol 113:284–293. https://doi.org/10.1111/j.1365-2672.2012.05342.x.
41. Chan KF, Le Tran H, Kanenaka RY, Kathariou S. 2001. Survival of clinical and poultry-derived isolates of *Campylobacter jejuni* at a low temperature (4°C). Appl Environ Microbiol 67:4186–4191. https://doi.org/10.1128/AEM.67.9.4186-4191.2001.
42. Cools I, Uyttendaele M, Caro C, D'Haese E, Nelis HJ, Debevere J. 2003. Survival of *Campylobacter jejuni* strains of different origin in drinking water. J Appl Microbiol 94:886–892. https://doi.org/10.1046/j.1365-2672.2003.01916.x.
43. Bronowski C, Mustafa K, Goodhead I, James CE, Nelson C, Lucaci A, Wigley P, Humphrey TJ, Williams NJ, Winstanley C, ENIGMA Consortium. 2017. *Campylobacter jejuni* transcriptome changes during loss of culturability in water. PLoS One 12:e0188936. https://doi.org/10.1371/journal.pone.0188936.
44. Oh E, Chui L, Bae J, Li V, Ma A, Mutschall SK, Taboada EN, McMullen LM, Jeon B. 2018. Frequent implication of multistress-tolerant *Campylobacter jejuni* in human infections. Emerg Infect Dis 24:1037–1044. https://doi.org/10.3201/eid2406.171587.

45. Berndtson E, Danielsson-Tham M-L, Engvall A. 1996. *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. Int J Food Microbiol 32:35–47. https://doi.org/10.1016/0168-1605(96)01102-6.
46. Smith K, Reimers N, Barnes HJ, Lee BC, Siletzky R, Kathariou S. 2004. *Campylobacter* colonization of sibling turkey flocks reared under different management conditions. J Food Prot 67:1463–1468. https://doi.org/10.4315/0362-028x-67.7.1463.
47. Miller WG, Englen MD, Kathariou S, Wesley IV, Wang G, Pittenger-Alley L, Siletz RM, Muraoka W, Fedorka-Cray PJ, Mandrell RE. 2006. Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. Microbiology 152:245–255. https://doi.org/10.1099/mic.0.28348-0.
48. Jolley KA, Bray JE, Maiden MCJ. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res 3:124. https://doi.org/10.12688/wellcomeopenres.14826.1.
49. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197.