ABSTRACT

GOOD, LESLEY DOREEN. Differential Survival and Fitness of Turkey-Derived *Campylobacter* spp. in Vehicles (Feces, Water) of Special Relevance for Pre-Harvest Transmission. (Under the direction of Dr. Sophia Kathariou).

Campylobacter jejuni and *C. coli* are leading bacterial agents of human foodborne illness in the United States and other industrialized nations. There is a high prevalence of both species in conventionally grown turkeys pre-harvest, and many strains exhibit resistance to multiple antibiotics. Antibiotic resistance, particularly to macrolides and fluoroquinolones, could impact fitness for survival. However, little is known about relative survival of different *Campylobacter* strains outside their animal host, in vehicles such as feces and water that are of high relevance for pre-harvest transmission.

The purpose of this study was to examine the differential survival of turkey-derived *Campylobacter* in vehicles of special relevance for pre-harvest transmission. To assess relative survival of different strains naturally present in turkey feces, fresh feces were collected and pooled to create an 8-dropping fecal composite and a fecal drinker water suspension. Fecal composite and drinker water suspension samples were stored in capped sterile plastic tubes at 4°C and total *Campylobacter* populations were enumerated on a selective medium (mCCDA). Enumeration occurred at 48-hour intervals until *Campylobacter* was either no longer detectable in the sample or counts approached the detection limit for reliable counts (2.0×10^3). Isolates from each time point (total, n = 548) were purified on Mueller Hinton Agar. For each isolate, species designations were determined by multiplex PCR and antibiotic susceptibility profiles were determined for five antibiotics: tetracycline (T), streptomycin (S), erythromycin (E), kanamycin (K), and nalidixic acid and ciprofloxacin (both nalidixic acid and ciprofloxacin are indicated as Q).

Additionally, three *Campylobacter* strains were selected for fitness trials, each representing a different commonly-encountered resistance profile: TSKQ *C. jejuni* (2MT8-3); TKQ *C. coli* (2MT6-4); and TSEKQ *C. coli* (2WT0-4). Sterile water suspensions were created by transferring cells from a broth culture into water both individually and in mixed-strain pairs. Strains were tested in pairs (*C. jejuni* 2MT8-3 TSKQ vs. *C. coli* 2WT0-4 TSEKQ and *C. jejuni* 2MT8-3 TSKQ vs. *C. coli* 2MT6-4 TKQ) for each of seven independent trials.

Overall, *Campylobacter* was isolated from feces and water for up to 12 to 16 days. There was no significant difference in survival of total *Campylobacter* spp. between feces and water. However, *C. jejuni* survived longer than *C. coli* in feces ($p = 0.0005$), whereas *C. coli* survived longer than *C. jejuni* in water ($p < 0.0001$). When time was dichotomized as $t < 4$ days and $t \geq 4$ days, the long-surviving strains included *C. jejuni* with resistance profiles TSKQ and TKQ ($p = 0.0077$, feces) and *C. coli* with profiles TK, TQ and TKQ ($p < 0.01$, feces, $p < 0.01$, water). *C. coli* resistant to all five antibiotics (TSEKQ) appeared unable to survive longer than 4 days in feces. In the fitness trials, each strain (*C. jejuni* 2MT8-3 TSKQ, *C. coli* 2WT0-4 TSEKQ, and *C. coli* 2MT6-4 TKQ) was able to survive equally up to 8 days in individual suspensions. In mixed-strain culture, TSKQ *C. jejuni* outcompeted TSEKQ *C. coli* on and after Day 2.

This study provides data that can be used to monitor relative survival of different species and strains of *Campylobacter* outside their avian host, in vehicles of high relevance for pre-harvest transmission. Further research is needed in order to attribute differential survival of *Campylobacter* to specific antibiotic resistance genes or other strain attributes. Results will inform efforts to reduce pre-harvest transmission of this pathogen in turkeys.

Differential Survival and Fitness of Turkey-Derived *Campylobacter* spp. in Vehicles (Feces, Water) of Special Relevance for Pre-Harvest Transmission

by
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DEDICATION

For Mom and Dad.

BIOGRAPHY

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: Review of Relevant Literature

1.1 *Campylobacter* as a Human Pathogen

Campylobacter spp., Gram-negative, motile, spiral-shaped bacteria, are a leading cause of acute gastroenteritis in humans *(13, 59)*. Each year in the United States, *Campylobacter* spp. are responsible for an estimated 0.8 million cases of foodborne illnesses, accounting for approximately 9% of all foodborne illness from major pathogens and 15% of foodborne hospitalizations *(73)*.

Species responsible for gastroenteritis in humans are typically referred to as "thermophilic" campylobacters, as they grow optimally at 42°C *(32)*. Such campylobacters are able to grow at 37°C – 42°C and will not grow at all at temperatures under 30°C *(46, 64)*. The two species of *Campylobacter* implicated in human disease are *C. jejuni* and *C. coli*. *C. jejuni* is more commonly implicated (>85% of cases) compared to *C. coli* (up to 15% of cases) *(32)*. Both species are obligate microaerophiles, requiring reduced concentrations of oxygen for growth (5% O_2, 10% CO_2, 85% N) *(3, 25)*. Virulence factors specific to *Campylobacter* spp. include chemotaxis, motility, and flagella, which are required for attachment and colonization of the gut epithelium *(3, 64)*

Although all age groups are at risk of infection, incidence of campylobacteriosis is highest in children less than 5 years of age *(14)*. It is unclear why there is an age discrepancy in campylobacteriosis. It is possible that parents of young children are more likely than other segments of the population to seek medical care when their children show symptoms of gastroenteritis. Similarly, it is possible that doctors are more likely to order diagnostic medical tests when treating young children. There may also be a yet undefined trait among young children that makes them more susceptible to campylobacteriosis *(63)*.

Ingesting as few as 400-800 bacteria has been shown to be sufficient for gastroenteritis in humans *(7, 46, 63)*. For a consumer, this means that as little as one drop of contaminated raw chicken juice can easily constitute an infectious dose *(46)*. The incubation period for campylobacteriosis can range from 18 hours to 8 days, with a mean incubation period of 3.2 days *(63)*. In otherwise healthy adults, campylobacteriosis is self-limiting and typically resolves itself within one week after the onset of symptoms *(46, 96)*. In rare cases that require medical treatment, antibiotics such as erythromycin and fluoroquinolones are usually prescribed *(46)*.

Although protective immunity is documented after chronic exposure to high risk foods such as raw milk, control and prevention of campylobacteriosis is critical to public health *(63)*. Infection can lead to long-term sequelae such as the development of Guillain-Barré Syndrome (GBS) or, in rare cases, death *(13, 59, 63)*. Infection with *C. jejuni* is the most common predisposing factor to developing GBS; a person's risk of developing GBS during the 2 months following a *C. jejuni* infection is approximately 100 times higher than that of the general population *(42, 55)*

1.2 *Campylobacter* species

1.2.1 *Campylobacter jejuni*

C. jejuni has been implicated in a higher percent of human disease compared to *C. coli*, therefore it has been studied more in-depth *(32)*. The reasons that *C. jejuni* has an advantage in causing clinical illness in humans compared to *C. coli* are not fully understood.

1.2.2 *Campylobacter coli*

The prevalence of human disease due to *C. coli* may be underestimated. One study in Germany surveyed and genotyped *C. coli* strains from human cases over the course of one calendar year *(36)*. Of 861 total isolates, 18.6% were *C. coli*. This number is higher than official reports for Germany and other countries *(36)*. The discrepancy could be due to different identification methods or overestimation of *C. jejuni*.

1.2.3 Other species of *Campylobacter*

More than 95% of *Campylobacter* strains isolated from cases of human disease are either *C. jejuni* or *C. coli (49)*. Three other species of *Campylobacter* known to cause gastroenteritis in humans are *C. fetus, C. lari and C. upsaliensis*. *C. fetus* is a known cause of spontaneous abortion in sheep and cattle, and has occasionally been isolated from these animals while healthy. *C. upsaliensis* is commonly isolated from the intestinal contents of dogs. *C. lari* is more often associated with birds and the environment, having been isolated from wild birds, oysters, mussels, pigs, and poultry. None of these species of *Campylobacter* contribute extensively to human disease *(49)*.

While *Campylobacter* does not grow outside the host, it can survive for long periods of time in cool moist places out of direct sunlight, such as retail refrigeration conditions *(25)*. *C. jejuni* cells survive best at 4°C, as noted by several studies *(16, 39, 70)*.

1.3 *Campylobacter* as a Zoonotic Pathogen

Campylobacter is a commensal organism commonly found in the intestines of cattle, sheep, swine, and avian species *(46, 89)*. The high body temperature of avian species makes them a particularly good host for *Campylobacter (46)*. Handling and consumption of raw or undercooked poultry constitute leading risk factors for contracting campylobacteriosis *(20, 25, 51)*. *Campylobacter* reservoirs and routes of transmission on a poultry farm remain poorly understood, but possible environmental sources include drinkers, shed entrances, anterooms, and, perhaps most importantly, other animals on the farm (both domestic and wildlife) *(11, 23, 40, 64)*. Infected poultry can then spread *Campylobacter* to other birds through horizontal transmission *(44, 87)*. It has been shown that in an otherwise *Campylobacter*-free flock, one colonized bird can spread *Campylobacter* to all other birds within the flock within approximately 3 days *(57)*. In cases where poultry flocks are not colonized with *Campylobacter*, those flocks can become contaminated during transport, likely as the result of contaminated crates *(38, 57, 75)*.

Infected poultry transmit *Campylobacter* to humans through two primary routes: direct or indirect *(20, 25)*. Direct contact involves contact with an animal that is shedding *Campylobacter*. By nature poultry processing employees such as slaughterhouse workers are at increased risk for contracting campylobacteriosis *(20)*. Indirect contact is achieved through the handling or consumption of contaminated meat products *(20)*. Between 50-80% of human cases of *C. jejuni* infection can be both directly and indirectly attributed to contaminated poultry *(25)*.

1.4 *Campylobacter* Colonization and Prevalence in Turkeys

C. jejuni and *C. coli* are two species of *Campylobacter* commonly found in poultry, including turkeys *(53, 76)*. Prevalence of *C. jejuni* relative to *C. coli* in turkey flocks varies among flocks and farms for reasons that are yet to be determined, however, it appears that *C. coli* is more prevalent in young turkeys (brooders) whereas *C. jejuni* is more prevalent in older birds (grow-outs) *(95)*. The fastidious nature of *Campylobacter* spp. can be used to facilitate isolation and culture from other gut microflora *(25)*.

Most of the *Campylobacter*-related poultry research that has been done to date focuses on broiler chickens *(25)*. Broiler chickens are colonized with *Campylobacter* spp. primarily in the small intestine and in mucus overlying the epithelial cells in the ceca *(60)*. As few as 40 viable *Campylobacter* cells have been shown to colonize chicks or chickens *(60)*. Isolation of *Campylobacter* among broiler chickens typically occurs between three and four weeks of age, but typically not before two weeks of age *(44)*. Once isolated from a broiler chicken, it only takes 3-7 days for *Campylobacter* to have spread and colonized 100% of the broilers on the same farm *(43, 57)*. Carriage rates show seasonal variation, with the highest rates occurring during warm summer months *(10, 21, 22)*.

Farm-level factors can impact the *Campylobacter* status of a broiler flock. In a study of 495 broiler flocks conducted in The Netherlands, Bouwknegt et al. showed that risk of *Campylobacter* colonization is correlated with age (≥ 29 days), season (summer or fall), presence of other animals on the farm or within 1 km, and number of broiler houses on the premises (≥5) *(10)*. Of these variables, presence of other farms within 1 km had the highest impact on *Campylobacter* prevalence *(10)*.

Turkey flocks have been shown to be colonized with *Campylobacter* at higher rates than chicken flocks (46% vs. 35%, respectively); odds of colonization increase with factors like the presence of a manure heap or unchlorinated water *(4)*. Turkeys should be studied more in-depth, as they may have unique colonization attributes as compared to other poultry such as broiler chickens.

The relatively small amount of research that has been done on turkeys to this point indicates that *Campylobacter* spreads quickly and easily throughout turkey flocks. Typically, turkeys are not colonized with *Campylobacter* spp. under two weeks of age, although some studies have detected *Campylobacter* colonization at earlier time points *(50, 66, 76, 84, 91)*. At two to three weeks of age, it is common for 100% of turkeys within a flock to be colonized *(50, 76, 91)*. Lack of colonization in turkeys less than three weeks old is likely due to the presence of protective maternal antibodies, as has been shown in broilers *(71)*.

Wallace et al. discovered that turkey flocks are colonized with several different biotypes of *Campylobacter* simultaneously *(91)*. Within the gastrointestinal tract of mature turkeys at slaughter, numbers of *Campylobacter* increase with distance from the beak. Within the turkey intestine, *Campylobacter* numbers are highest in turkey ceca *(91)*.

Horizontal, not vertical, transmission is considered to be an important factor in *Campylobacter* colonization within turkey flocks *(76, 91)*. *Campylobacter* strains have been shown to persist on farms and colonize successive turkey flocks *(50)*. Several environmental factors including cattle or other farm animals on or adjacent to poultry farms, excessively moist litter, farm workers' boots, and transport crates can contribute to such persistence and horizontal spread of *Campylobacter* within both turkey and broiler farming operations *(21, 22, 43, 52, 68, 69, 76, 99)*. However, a study of turkey flocks reared in close proximity to swine farms showed no effect of swine colonization on turkey *Campylobacter* colonization patterns *(95)*. Specifically, swine herds were colonized with mostly *C. coli*, whereas turkeys flocks were colonized with varying combinations of *C. jejuni* and *C. coli*, indicating that spp. prevalence is likely host associated *(95)*. Well-managed farm hygiene practices, such as proper litter maintenance and controlling traffic between farms, can reduce or prevent *Campylobacter* colonization *(76)*.

Campylobacter contamination can occur at any stage of turkey production *(66)*. *Campylobacter* is a common component of the gut flora of many types of livestock, domestic pets, and wildlife, allowing for ample opportunities to contaminate the processing environment *(25)*. The avian gut temperature is 42°C, allowing optimal growth conditions for *Campylobacter* spp. *(25)*. These bacteria are often shed in the feces of asymptomatic animals, providing additional sources for environmental contamination and routes for horizontal transmission to birds *(25, 57)*.

Both conventionally-raised and organically-raised turkeys are colonized with *Campylobacter*, although conventionally-raised turkeys are colonized at higher rates *(54)*. *C. coli* was the predominant species in conventionally-raised turkeys, whereas organically-raised turkeys were colonized predominantly with *C. jejuni (54)*. Relative colonization of turkey flocks by *C. jejuni* vs. *C. coli* varies among farms and is also dependent on bird age. Young turkeys (brooders) are more likely than grow-out turkeys (7-14 weeks of age) to be colonized with *C. coli*, whereas grow-out birds are more likely to be colonized with *C. jejuni* *(95)*.

A *Campylobacter*-negative turkey flock can become contaminated during processing, as shown in Fig. 1. When a *Campylobacter*-positive flock is processed, working surfaces and equipment within the slaughter facility can become contaminated such that even after cleaning and disinfection, the surfaces can spread *Campylobacter* to otherwise negative flocks *(66)*. *Campylobacter* has been found in deep poultry muscle tissue, although at lower rates than on the surface *(25)*. This is not surprising, as the nature of slaughter and evisceration of poultry enables significant contamination with intestinal contents *(25, 66)*. It is unclear whether the presence of *Campylobacter* in deep muscle tissue is a reflection of gut contamination or a true systemic infection of the bird. Handling and processing contribute greatly to the spread of contamination on carcasses *(53)*. As Fig. 1 indicates, production (i.e. grow-out) is the most frequently-studied contamination point during turkey production. More attention should be paid in the future to other parts of the production cycle.

Research conducted with broilers shows that transport-induced stress can increase the shedding of *Campylobacter* that contributes to carcass contamination *(94)*. Similarly, after feed withdrawal, transport, and holding time at the abattoir, *Campylobacter* colonization rates in turkeys could be higher than at the farm *(93)*. This is possibly due to a decline in lactic acid within the emptied crop of turkeys.

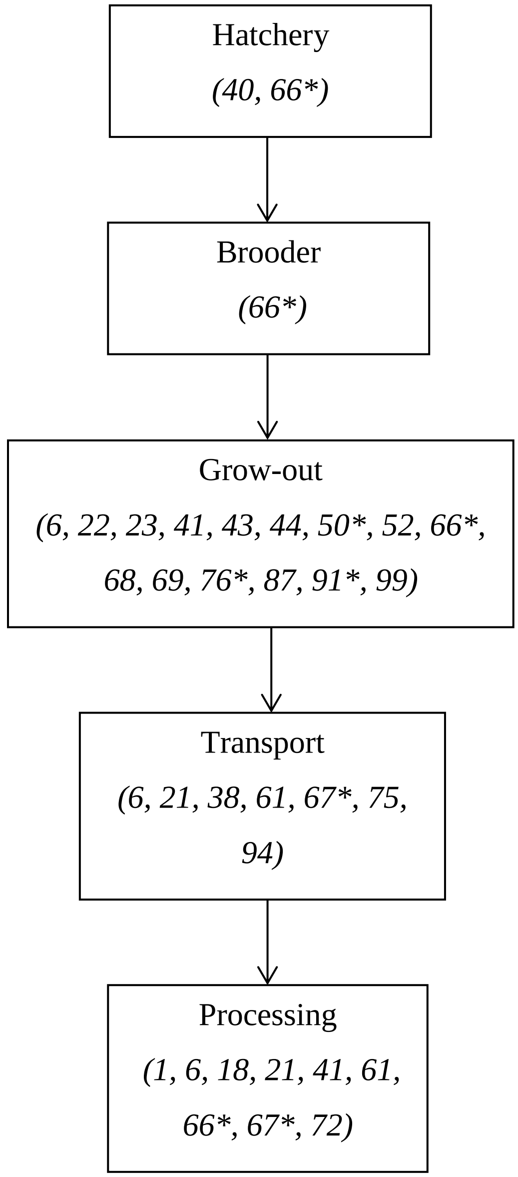

Fig. 1: Different stages of poultry production and processing**.** At each stage, potential for transmission of *Campylobacter* has been documented. Relevant articles for transmission at each stage are indicated.

* Indicates that the study was performed on turkeys. All others were performed on chickens.

1.5 Regional Differences in *Campylobacter* Colonization

Prevalence and species of *Campylobacter* that colonize turkeys vary by geographic region. A study of turkey ceca at processing plants in Ohio revealed high prevalence of *Campylobacter* colonization in both conventionally-raised (83% colonization) and organically-raised (87% colonization) turkeys *(54)*. Predominant species varied by production type, as *C. jejuni* was most commonly isolated from organically-raised turkeys, and *C. coli* most commonly isolated from conventionally-raised turkeys *(54)*. The results from the conventional turkey ceca are similar to those seen in turkey feces from farms in eastern North Carolina, where a study of 15 flocks showed a *Campylobacter* colonization rate of 87% *(95)*.

By comparison, turkey carcasses at processing plants in North Dakota only showed a 34.9% prevalence of *Campylobacter (53)*. While the distribution varied among the two plants studied, *C. jejuni* was more commonly isolated from each plant (51.6% and 76.8% of total *Campylobacter*, respectively) than *C. coli* (40.5% and 14.6% of total *Campylobacter*, respectively) *(53)*.

In northern Germany, a study of turkey meat at processing plants showed 29.2% of samples to be *Campylobacter*-positive *(5)*. Unlike studies conducted in the United States, where the species *C. jejuni* and *C. coli* comprise nearly all *Campylobacter* species, the samples from Germany included presumptive *C. fetus.* Specifically, *Campylobacter* isolated from the turkey meat in that study were 61.9% *C. jejuni*, 14.3% *C. coli*, and 23.8% presumptive *C. fetus,* based on biochemical validation using catalase and oxidase reactions *(5)*.

1.6 Genetic Diversity of *Campylobacter* in Turkeys

Disease burden of different types of *Campylobacter* can be determined using source attribution through microbial typing, which compares subtypes of isolates causing human disease to the distribution of subtypes isolated from different sources *(25)*. Common subtyping approaches are the use of pulsed-field gel electrophoresis (PFGE) or multi-locus sequence typing (MLST) *(25)*.

PFGE is a phenotypic subtyping method that relies on separation of large DNA fragments using electric current *(59)*. This method has been instrumental in linking *Campylobacter* isolates from humans to isolates from broiler chickens (as opposed to other animals or wild birds) *(25)*. Although PFGE is widely used in source attribution of foodborne illness such as campylobacteriosis, a major drawback is interlaboratory variability in methodology and interpretation *(59)*.

MLST is a genotypic subtyping method relying on DNA sequences rather than DNA fragment size, and therefore it yields unambiguous data *(59)*. MLST has been successfully used to trace the epidemiology of *Campylobacter (62, 81)*.

Much of the current knowledge of *Campylobacter* strain types comes from MLST and PFGE *(25)*. MLST data show that both *C. jejuni* and *C. coli* are highly diverse. According to information in the pubMLST database (accessed October 4, 2013), *C. jejuni* and *C. coli* combined have 6,869 distinct sequence types (ST) *(45)*.

PFGE is the preferred method of "DNA fingerprinting" used by PulseNet, the national molecular subtyping network for foodborne disease surveillance, in all 50 states *(27)*. PFGE assists epidemiologists in distinguishing between outbreaks and sporadic cases of campylobacteriosis by determining which cases are of the same origin (i.e., the strains colonizing an animal source, or isolated from a vehicle such as milk or water, are the same strains causing human disease) *(27, 28)*. For both *C. jejuni* and *C. coli,* PFGE and MLST results show a high level of agreement in the detection of strain types and clonal complexes *(92)*.

Within restricted geographic areas, multidrug resistant *C. coli* represents a clonal population. MLST shows that of 59 *C. coli* isolates taken from turkeys in eastern North Carolina, 69% represent one of three STs *(19)*. Likewise, a different study of turkey-derived *C. coli* isolates from eastern North Carolina used PFGE to reveal two distinct *fla* types over three successive flocks on the same farm *(50)*. In this study, prevalence of the types varied significantly between flocks, suggesting that *Campylobacter* persists in environmental reservoirs between flocks *(50)*. MDR *C. jejuni* is similarly clonal within eastern North Carolina *(34)*. Of 41 turkey-derived isolates, PFGE revealed that most were one of three STs *(34)*. This suggests dissemination of certain clonal groups within turkey production *(34)*.

When assessed across the entire U.S., *Campylobacter* clonal groups are much more diverse. Miller et al. used MLST to study host-associated *C. coli* across the U.S. *(56)*. Their results revealed much diversity in *C. coli* associated with swine, turkeys, and chickens. However, cattle proved to host a clonal population of *C. coli*, as 83% of *C. coli* cattle isolates belonged to one common ST *(56)*.

In a recent Danish study of turkeys both before and after slaughter, PFGE was used to investigate strain types that colonize flocks throughout production *(9)*. PFGE analysis of positive flocks revealed only one strain type of *Campylobacter*. This could indicate that flocks are colonized by only one type, or it could be a result of selectivity bias of different enrichment media. Alternately, it could indicate different survival abilities among different strains when subjected to stress conditions such as rapid temperature change, oxygen tension, and nutrient deprivation *(9)*.

1.7 Antibiotic Resistance of *Campylobacter* spp. Colonizing Turkeys

The National Antimicrobial Resistance Monitoring System (NARMS) publishes an annual report focusing on enteric bacteria. Prior to establishing NARMS, the CDC monitored antimicrobial resistance of enteric bacteria, including *Campylobacter*, through periodic surveys of isolates from a panel of sentinel sites *(15)*. NARMS, which began at the CDC in 1996, began testing *Campylobacter* for resistance in 1997. According to the most recent report, both *C. jejuni* and *C. coli* are increasingly resistant to several types of antibiotics, each species displaying a different resistance pattern *(15, 31)*.

The most recent NARMS report details the antimicrobial resistance of 2,136 *Campylobacter* isolates, only 13 (<1%) of which came from ground turkey *(31)*. This highlights a major lack of antimicrobial resistance data for *Campylobacter* from turkey products, considering the frequency with which turkey flocks are colonized with *Campylobacter (4)*. The limited data available in the report show that *Campylobacter* isolates from turkey were frequently resistant to tetracycline (80% of *C. jejuni*, 100% of *C. coli*), erythromycin (14% of *C. coli*), and quinolones (40% of *C. jejuni*, 57% of *C. coli*) *(31)*. More data are needed to determine the significance of these rates, as the number of turkey-derived *Campylobacter* isolates is low.

Among *Campylobacter* isolates, resistance to tetracycline is common *(34, 54)*. Resistance to both tetracycline and kanamycin can be transmitted between organisms through plasmids *(48, 82)*. An excess of 74% of *Campylobacter* spp. isolates have been shown to be multidrug resistant (MDR, simultaneously resistant to three or more antimicrobials), with MDR rates being higher among conventionally-raised birds *(19, 34, 54)*.

Prevalence of antimicrobial resistance among *Campylobacter* isolates from turkeys is greatly influenced by whether the farm practices conventional or organic production. Luangtongkum et al. found that while less than 2% of *Campylobacter* isolates from organically-raised turkey flocks were resistant to antimicrobials, resistance in conventional isolates was 67% *(54)*. Both organic and conventional flocks were colonized at similar rates. In particular, *Campylobacter* isolates from conventionally-raised turkeys showed higher resistance to quinolones, erythromycin, clindamycin, kanamycin, tetracycline, and ampicillin than *Campylobacter* isolated from organically-raised poultry (both chicken and turkey) *(54)*. The study by Luangtongkum et al. also observed that the highest rates of MDR *Campylobacter* were from conventional turkey operations; both *C. jejuni* and *C. coli* isolates from 6 out of 10 conventional turkey farms were MDR, resistant to tetracycline, kanamycin, clindamycin, erythromycin, and fluoroquinolones *(54)*.

Campylobacter antimicrobial resistance patterns differ by source and species. Compared to chicken, *Campylobacter* isolated from turkeys is more likely to be resistant to ciprofloxacin and erythromycin *(33)*. Ciprofloxacin and erythromycin resistance are also more common in *C. coli* than *C. jejuni (33, 54)*.

In a study of *C. jejuni* isolates from Eastern North Carolina conventional turkey farms, 45% of isolates were shown to be resistant to four or five antimicrobials, but none were resistant to erythromycin *(34)*. Isolates were commonly resistant to ampicillin, tetracycline, streptomycin, and quinolones (naladixic acid and ciprofloxacin). Kanamycin resistance was frequently associated with resistance to tetracycline. However, the reverse was not true *(34)*.

Analysis of *C. jejuni* and *C. coli* isolated from skin-on retail turkey meat in Ontario demonstrated that resistance to one or more antibiotics was common in both *C. jejuni* and *C. coli* (78% and 92%, respectively) *(17)*. Most commonly, isolates were resistant to tetracycline alone. The next most common resistance pattern was tetracycline and ampicillin *(17)*.

There is currently no explanation for the differing resistance profiles between *C. jejuni* and *C. coli*. Possible explanations include species differences in the stability of mutations that lead to antibiotic resistance or different capacities to exchange DNA by natural transformation *(8)*. It is possible that erythromycin-resistance imposes a fitness cost to *C. jejuni (2, 37)*. In a recent study erythromycin-resistant mutants of *C. jejuni* showed reductions in fitness compared to their erythromycin-susceptible parental strains *(2)*. These included reduced adhesion and invasion characteristics to intestinal epithelial cells, shorter intracellular survivability within macrophages, and lower colonization upon co-inoculation into mice and chickens compared to the susceptible strains *(2, 97)*. A laboratory competition experiment showed that the erythromycin-resistant strain grew significantly slower than the susceptible parent strain, which suggests that the mutation responsible for erythromycin resistance imposes a fitness cost to the mutant*(2)*. Similar results have been noted in other laboratory competition studies of *C. jejuni*, but no differences have been noted in *C. coli (37, 97)*.

Fluoroquinolones

Fluoroquinolones, such as ciprofloxacin and Baytril™, are antimicrobials that inhibit the activity of DNA gyrase *(24)*. Acquisition of fluoroquinolone resistance through DNA transformation seems to occur frequently in various environmental niches, including a large range of CO_2 concentrations and temperatures *(47)*. Resistance to fluoroquinolones occurs at a rate of 22.0% in C. *jejuni* strains and 31.3% of *C. coli* strains are resistant to fluoroquinolones *(15)*.

Erythromycin

Erythromycin is a macrolide, and is typically the drug of choice for treating human campylobacteriosis *(74)*. DNA transformation resulting in erythromycin resistance occurs optimally at 42°C, the approximate body temperature of turkeys *(47)*. Resistance to erythromycin most frequently occurs in *C. coli;* it is uncommon in *C. jejuni* isolates *(15, 24, 34, 90, 92)*.

Correlation Between Introduction of Drugs and Antibiotic Resistance in Turkeys

Antibiotics are used in food animals for three major purposes: therapy (treatment of an identified bacterial infection), prevention of bacterial infections, and as feed additives to enhance growth performance *(88)*. Fluoroquinolones were approved for use in poultry production in the United States between 1995 and 2005, and evidence suggests a positive correlation between antibiotic use in poultry production and antibiotic resistance in bacteria

(31, 77). One study looked at quinolone resistance from 1992-1998, which is both before and after the introduction of veterinary fluoroquinolones (such as sarafloxacin and enrofloxacin) for use in U.S. poultry production. The study showed a sharp increase in antibiotic resistance from 1996-1998, a strong implication of the role of antibiotic use in resistance *(77)*. Similar patterns have emerged in the United Kingdom *(85)*.

FDA Ban on Antibiotics

Antibiotic-resistant *Campylobacter* can spread to humans through direct contact with a colonized animal or indirectly through consumption of meat products *(88)*. In 2005, FDA banned the use of the fluoroquinolone enrofloxacin, produced by the Bayer Corporation under the trade name Baytril™, in poultry production *(29, 31)*. Prior to 2005, Baytril™ was used to treat bacterial infections in poultry. However, the use of Baytril™ in poultry production may have contributed to resistance in *Campylobacter* spp. *(24, 29, 77)*. Such resistance can pose public health challenges, as it can lead to prolonged infection and difficulty in treating human campylobacteriosis when treatment is indicated *(29)*.

1.8 *Campylobacter* spp. in Retail Turkey Meat

It is known that turkey meat is contaminated with *Campylobacter* from the processing stage through retail *(5, 53, 66, 67)*. As previously stated, studies of processing plants including carcass swabs in the Midwestern United States and fresh meat samples in Germany have found *Campylobacter* contamination rates of 34% and 29%, respectively *(5, 53)*. In addition to processing plants, the German study examined *Campylobacter* rates in fresh retail turkey meats. *Campylobacter* rates in fresh retail turkey meat (including breast meat, cutlets, thighs, and drumsticks) were as high as 28% *(5)*. This suggests that the prevalence of *Campylobacter* contamination at processing is similar to the rate at retail.

A study of retail meats in the United States between 2002 and 2007, conducted at active CDC FoodNet sites, showed only 1.6% of ground turkey samples to be contaminated with *Campylobacter (98)*. This rate is dramatically lower than rates of *Campylobacter* in turkey retail meats in other countries such as Germany (28%), Canada (46%), and Turkey (46%) *(5, 12, 17)*. The primary difference between these studies and the study conducted in the U. S. is that the U. S. examined ground turkey, while each of the other countries examined turkey meat including breasts and legs. The findings may reflect a difference in *Campylobacter* survival in the different types of meat products.

Campylobacter that contaminates retail turkey meats in the United States has been shown to be resistant to several antibiotics, regardless of the geographic region *(33, 83)*. A study conducted in Iowa showed that *Campylobacter* isolates from retail ground turkey were more likely than *Campylobacter* from other types of meat to be resistant to ciprofloxacin, erythromycin, and doxycycline *(83)*. *Campylobacter* isolates from retail turkey collected near Washington, D. C. found high resistance rates to ciprofloxacin and erythromycin, with 26% coresistance *(33)*. *C. coli* was more likely than *C. jejuni* to be resistant to both antibiotics *(33)*.

According to the 2011 NARMS report on retail meat, only 9 of 634 isolates from poultry (1%) were resistant to 3 or more antimicrobials *(30)*. As with the previous NARMS reports, ground turkey was the only turkey retail meat included in the study. Most of the *Campylobacter* isolates obtained from retail meat came from chicken, including breasts; only

31 of 603 retail poultry isolates (5%) came from turkey *(30)*. This is likely because the NARMS data only include ground turkey. Prevalence of *Campylobacter* would likely be higher if different types of turkey meat, including those with skin, were included in the study *(5, 12, 17)*. Currently, *Campylobacter* in retail turkey is likely to be significantly underrepresented in NARMS data.

1.9 Control and Interventions

Little research has been done with turkeys, but information from broilers suggests that water can be a persistent source of bacteria in a poultry farm *(11)*. Chlorination of drinker water can reduce the risk of colonization *(4, 22, 26, 35)*. Other interventions that are successful in the reduction of *Campylobacter* on farms include water and line disinfection, replacement and vigorous cleaning of header water tanks, and cleaning the water distribution system *(65)*.

Limited research indicates that feed composition can have an impact on *Campylobacter* spread and colonization in broiler chickens *(58, 86)*. Moen et al. fed birds a diet supplemented with 15% oat and barley hulls for structure, in order to stimulate the gizzard *(58)*. Compared to the control diet, birds that were fed oat and barley hulls showed a delayed spread of *C. jejuni* throughout the flock and had a reduced amount of *C. jejuni* in their ceca *(58)*. In a separate study of diet formulations containing different protein sources, Udayamputhoor et al. found that birds fed plant-based protein were colonized with *C. jejuni* at significantly lower rates than birds fed animal protein and those fed a combination of animal and plant protein *(86)*. Research on feed composition and *Campylobacter* colonization has not yet been conducted with turkeys.

Additional research on broiler chickens has shown that using caprylic acid as a feed supplement can reduce *Campylobacter* colonization in market-age birds by 2-3 logs *(79)*. Similar results have been noted in day-old and ten-day old broiler chicks *(78, 80)*. Again, such research has not been conducted with turkeys.

1.10 Summary

Thermophilic *Campylobacter* spp., including *C. jejuni* and *C. coli*, are a leading cause of human gastroenteritis. Most cases of campylobacteriosis occur following consumption of raw or undercooked poultry. *Campylobacter* is commonly isolated from the intestines of livestock, including poultry, which is considered the main foodborne source of human campylobacteriosis. While *Campylobacter* transmission has been studied in broilers, its transmission in turkey flocks needs further examination.

Horizontal transmission is the likely method of pre-harvest *Campylobacter* transmission within turkey flocks. Once a bird becomes colonized, *Campylobacter* spreads quickly throughout the flock. Turkeys can become contaminated at any point during the production and processing cycle; even those flocks that remain *Campylobacter*-free until they reach market age can become contaminated during transport or processing.

Most of the current knowledge of *Campylobacter* strain types comes from either PFGE or MLST. PFGE is a phenotypic method of “DNA fingerprinting” that assists epidemiologists in distinguishing between outbreaks and sporadic cases of campylobacteriosis by comparing the separation of DNA fragments. MLST is a genotypic subtyping method that relies on DNA sequences rather than fragment size. MLST is used to determine the epidemiology of campylobacteriosis by defining *Campylobacter* clonal groups.

Campylobacter isolated from conventionally-raised turkey shows an increasing resistance to antibiotics, notably quinolones, erythromycin, clindamycin, kanamycin, tetracycline, and ampicillin. Isolates are commonly resistant to more than one antibiotic simultaneously. When present in a turkey flock, antibiotic-resistant *Campylobacter* isolates can be present on the final retail product, exposing the public to risk of antibiotic-resistant campylobacteriosis.

Resistance to macrolides such as erythromycin and fluoroquinolones is important to public health as these drugs are first-line medications in the treatment of campylobacteriosis. Antibiotic resistance patterns differ by geographic region and species. Currently there is no explanation for the differing resistance profiles between *C. jejuni* and *C. coli*, but it is possible that the differences arise from differences in the genetic code of the two species.

1.11 Conclusions and Further Research

The *Campylobacter* colonization trends of turkeys should be studied more in-depth, as they might differ from those of broiler chickens. Turkey is a significant food source in the American diet, thus it is worthwhile to define, explore, and further understand these potential differences. A better and more thorough understanding of *Campylobacter* colonization and strains specific to turkey production can inform efforts to reduce pre-harvest transmission of this pathogen. Further research should focus on the *Campylobacter* strain types that colonize turkeys, their characteristics, and mechanisms for survival and transmission outside their host.

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CHAPTER 2: Survival of *Campylobacter jejuni* and *C. coli* in a Fecal Composite and Drinker Water Suspension Stored at 4°C

ABSTRACT

Campylobacter jejuni and *Campylobacter coli* are leading bacterial agents of human foodborne illness in the United States and other industrialized nations. There is a high prevalence of both species in conventionally grown turkeys pre-harvest, and many strains exhibit resistance to multiple antibiotics. However, little is known about relative survival of different *Campylobacter* strains outside their animal host, in vehicles such as feces and water that are of high relevance for pre-harvest transmission.

The purpose of this study is to examine the differential survival of turkey-derived *Campylobacter* in vehicles of special relevance for pre-harvest transmission. Feces from *Campylobacter*-positive turkey flocks and a suspension of such feces in water were stored at 4°C and total *Campylobacter* populations were enumerated on CCDA at 48-hour intervals. Isolates from each time point (total, n = 548) were purified on Mueller Hinton Agar. For each isolate, species designations were determined by multiplex PCR and antibiotic susceptibility profiles were determined for five antibiotics: tetracycline (T), streptomycin (S), erythromycin (E), kanamycin (K), and ciprofloxacin (Q).

Overall, *Campylobacter* was isolated in feces and water between 12 and 16 days. There was no significant difference in survival between feces and water. *C. jejuni* survived longer than *C. coli* in feces ($p = 0.0005$), whereas *C. coli* survived longer than *C. jejuni* in water ($p < 0.0001$). The longest-surviving strains included *C. jejuni* with resistance profiles TSKQ and TKQ ($p = 0.0077$, fecal composite) and *C. coli* with profiles TK, TQ and TKQ (p

< 0.01, feces, p < 0.01, water). *C. coli* resistant to all five antibiotics (TSEKQ) appeared unable to survive longer than 4 days in feces.

This study provides data that can be used to monitor relative survival of different species and strains of *Campylobacter* outside their avian host. Results will inform efforts to reduce transmission of this pathogen in turkeys pre-harvest.

2.1 INTRODUCTION

Each year in the United States, *Campylobacter* spp. are responsible for 0.8 million cases of foodborne illnesses, accounting for approximately 9% of all foodborne illness from major pathogens and 15% of foodborne hospitalizations *(38)*. The two species of *Campylobacter* implicated in human disease are *C. jejuni* and *C. coli*. *C. jejuni* is more commonly implicated (>85% of cases) compared to *C. coli* (up to 15% of cases) *(13)*. Consumption of undercooked poultry is one of the main risk factors for contracting campylobacteriosis *(8, 11, 24)*. Ingesting as few as 400-800 bacteria has been shown to cause gastroenteritis in humans *(4, 22, 32)*. For a consumer, this means that accidentally ingesting as little as one drop of contaminated raw chicken juice can easily constitute an infectious dose *(22)*. Poultry including chickens and turkeys are particularly good reservoirs for thermophilic campylobacters, as the avian gut temperature is 42°C, allowing optimal growth conditions *(11)*. These bacteria are often shed in the feces of asymptomatic animals, providing additional sources for environmental contamination and routes for horizontal transmission to birds *(11, 29)*.

Knowledge of *Campylobacter* survival outside its avian host is not well-documented. *Campylobacter* cells are unable to grow below 30°C, but they are able to survive for a certain period of time, with survival dramatically better below room temperature *(33)*. The optimal temperature for *Campylobacter* survival is 4°C *(22)*. Prolonged survival at 4°C has been observed in *Campylobacter* isolates has been noted *(18, 37)*. In one study of isolates held at 4°C, *C. jejuni* isolated from either retail chicken or human clinical cases were able to survive for 14 days *(6)*. Isolates from human clinical cases survived longer than those from retail chicken meat *(6)*.

Water appears to increase survival of *Campylobacter (41, 44)*. Although few studies have investigated this phenomena, it is likely due to association with protozoans such as freshwater amoebae, which are believed to have a protective effect on *Campylobacter* cells *(44)*. In one study of *C. jejuni* and waterborne protozoa in broiler drinker water, bacteria were differentially stained according to whether or not they had been internalized by protozoa (through the process of phagocytosis) or remained extracellular *(41)*. *C. jejuni* that had been internalized by protozoa showed prolonged survival compared to *C. jejuni* that remained extracellular. Furthermore, *C. jejuni* inside waterborne protozoa exhibited increased resistance to disinfection *(41)*. It is unclear why internalized *Campylobacter* is more resistant to disinfection, but might be related to inactivation of disinfectants by the protozoa, thereby protecting *Campylobacter* from the effects of the disinfectant.

There has been relatively little research conducted on *Campylobacter* survival in animal feces; an important vehicle for transmission in animals pre-harvest. *Campylobacter* has been shown to survive for short periods of time in feces from various animals *(15, 30, 31, 39)*. *Campylobacter* was inactivated quickly in artificially-inoculated fecal pats from Canada geese and cows on pasture *(31, 39)*. *Campylobacter* from the Canada goose fecal samples could not be recovered after 2 days in the summer and 4 days in the winter *(31)*. Similarly, *Campylobacter* from cow pats could not be recovered after 4 days, except in the winter when it survived up to 16 days *(39)*. These same trends were found in naturally-occurring *Campylobacter* isolated from sheep and cow pats, where survival in feces on pasture lasted only a few days *(15, 30)*. Comparatively, *Campylobacter* might survive much better in compost compared to pats on pasture. One study of bovine compost used centrifugation in combination with ethidium monoazide to extract DNA from intact *Campylobacter* cells over a 10 month period *(20)*. This difference in survival between cow pats and compost is likely due to differences in temperature, moisture, and UV exposure between the two samples types.

Studies of chicken feces have shown limited survival of *Campylobacter (1, 5, 37)*. In particular, *C. jejuni* isolated from laying hen feces survived up to 6 days when stored at 20°C under controlled laboratory conditions, including 40-60% relative humidity *(1)*. Survival was longer for naturally-occurring *C. jejuni* isolates compared to isolates from artificially-inoculated feces, and survival differed by strain as determined by *flaA* typing. A separate study of isolates from broiler chicken ceca also found that *Campylobacter* can survive for at least 6 days when stored at 4°C *(37)*. The study examined the population decline of *C. jejuni* aliquots containing different levels of cells in phosphate buffer solution. Over the 6 day study, there was little decline in high level aliquots (10^8, 10^6, and 10^4 CFU/mL). However, low levels of *Campylobacter* (10^2 CFU/mL) were not conducive to survival, as *C. jejuni* was not detectable in these aliquots after day 2 of the study *(37)*. Similarly, a study of chicken fecal swabs stored in sterile water at room temperature used reverse transcriptase quantitative real-time PCR to find that *C. jejuni* was viable for up to 5 days *(5)*. In this study, *C. jejuni* populations started on day 1 at a maximum of 10^7 CFU/mL and declined approximately 2 logs between enumeration points (2 days apart), until day 7 when *Campylobacter* was not detected *(5)*. Survival in turkey feces has been especially understudied. Most *Campylobacter* survival studies in poultry, such as the ones listed above, have focused on chickens.

These conflicting data on the survival of *Campylobacter* in animal and poultry feces highlight the need for more research on *Campylobacter* survival outside the host. The purpose of the current study is to enumerate *Campylobacter* survival in turkey feces held at 4°C.

2.2 MATERIALS AND METHODS

2.2.1 Turkey flocks

The turkey flocks used for this study were being monitored for *Campylobacter* prevalence as part of a different ongoing project in our laboratory. As such, flocks were selected on the basis of being *Campylobacter* positive.

A total of seven turkey flocks from conventional grow-out farms in Eastern North Carolina were selected for this study. All turkeys were toms with an average age of 89 days (approximately 12.8 weeks). Flocks ranged in size from 3,400 to 7,616 birds (average flock size was approximately 4,500 birds), which is typical of conventional commercial turkey production in Eastern North Carolina.

The first four samples (flocks 1-3) were monitored for survival, species, antibiotic resistance, and pulsed-field gel electrophoresis (PFGE). One flock was sampled twice (at ages 106 and 133 days) in order to monitor *Campylobacter* profile changes within the same flock at different ages. Four additional flocks, samples 5-8, were monitored only for survival for the sake of comparing death curves to the first four samples. See Table 2.1 for details regarding each sample.

2.2.2 Sample collection and bacterial isolation

Eight individual turkey droppings, two from each quarter of the house, and approximately 50 mL of drinker water were collected from each farm. Fresh fecal material was collected and brought to the laboratory on ice within 3 hours of excretion. Samples were collected and processed on the same day.

2.2.3 Sample processing and *Campylobacter* enumeration

Two sample types were made from each farm visit: one fecal composite and one drinker water suspension. The fecal composite was made in single 50 mL sterile plastic Falcon tube (BD, San Jose, CA) by combining all eight individual turkey droppings in their entirety. The droppings were manually mixed using a sterile metal spatula until well combined. The drinker water suspension was made in a 15 mL sterile plastic Falcon tube (BD, San Jose, CA) by adding one gram of the fecal composite sample into 9 mL drinker water and vortexing to mix. Samples were stored in the dark at 4°C.

Beginning on Day 0 (the day samples were retrieved from the farm), dilutions of both the fecal composite sample and the water suspension were plated (100 µl) on mCCDA (Oxoid, Ogdensburg, NY) for enumeration. Plating for enumeration continued in two-day intervals until the number of *Campylobacter* colonies declined to near or below detection limit for reliable counts (2.0×10^3/g).

At every time point, presumptive *Campylobacter* colonies were isolated and purified on Mueller Hinton Agar (MHA) (BD, San Jose, CA). Colonies were selected based on their characteristic glossy grey appearance. Ten colonies per time point were attempted for isolation and purification in the first farm visit; 16 per time point were attempted for farm visits 2-4. Four additional farm samples, samples 5-8, were enumerated for total *Campylobacter* in order to construct death curves. Isolates from these samples were not characterized regarding species, antibiotic resistance, or strain types. A list of farm samples and their corresponding flocks are listed in Table 2.1. All plates were incubated at 42°C for 48 hours under microaerobic conditions using Campy GasPaks (BD, San Jose, CA).

Each isolate attempted for purification was given a name according to a specific algorithm. For example, isolate 3MT4-7 comes from the 3rd farm sample (the first number denotes the sample number, except for sample 1 where no number was used). The M denotes that the isolate was obtained from the fecal composite (a W would indicate the water suspension). The number after T refers to the day on which the isolate was taken (in this example, Day 4), and the number after the dash is the isolate number (of a possible total of 10 isolates per day in sample 1, or 16 possible isolates in samples 2-4). Therefore, the example isolate, 3MT4-7 is from the sample 3 fecal composite, was taken on Day 4 of enumeration, and is the 7th isolate of a possible 16 that were taken that day.

Table 2.1: Samples and corresponding farms, flocks, and molecular tests conducted for this study.

Sample Number	Farm	Flock	Bird Age (days)	Date Collected	Tests
1	A	1	131	8/29/2012	Enumeration AB resistance Species confirmation Subtyping
2	B	2	106	10/11/2012	Enumeration AB resistance Species confirmation Subtyping
3	B	2	133	11/7/2012	Enumeration AB resistance Species confirmation Subtyping
4	A	3	91	11/28/2012	Enumeration AB resistance Species confirmation Subtyping
5	C	4	41	2/25/13	Enumeration only
6	D	5	59	2/25/13	Enumeration only
7	E	6	56	3/20/13	Enumeration only
8	F	7	98	4/11/13	Enumeration only

2.2.4 Species determination

Multiplex polymerase chain reaction (PCR) was used to determine species of all *Campylobacter* isolates. PCR utilized the *C. jejuni*-specific *hip* primers (5'- ATG ATG GCT TCT TCG GAT AG-3' and 5'-GCT CCT ATG CTT ACA ACT GC-3') and the *C. coli*-specific *ceu* primers CC1 (5'-GAT TTT ATT ATT TGT AGC AGC G-3') and CC2 (5'-TCC ATG CCC TAA GAC TTA ACG-3') *(19, 28)*. Reactions used X-*Taq* DNA polymerase (Fisher, Fair Lawn, NJ) and were carried out in 21 µL with 2 µL of cells (1 colony suspended in 100 µL of sterile dI water) used as a DNA template. The conditions were 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 5 min.

2.2.5 Antibiotic resistance

All *Campylobacter* isolates were tested for resistance to six antimicrobials: tetracycline, streptomycin, erythromycin, kanamycin, nalidixic acid, and ciprofloxacin. Antibiotic plates were made for each compound individually by adding the minimum inhibitory concentration to MHA as listed in Table 2.2 *(16)*. Antimicrobials were purchased from Sigma Chemical Co. (St. Louis, MO), except kanamycin, nalidixic acid, and ciprofloxacin, which were purchased from Fisher Biotech (Fair Lawn, NJ). *C. jejuni* ATCC 33560 (purchased from the American Type Culture Collection) was used as the quality control strain. All isolates were also grown on MHA plates (Oxoid) to ensure viability.

Table 2.2: Minimum inhibitory concentration of antibiotics used for resistance testing.

Antibiotic	**Concentration (µg/mL)**
Tetracycline	16
Streptomycin	64
Erythromycin	8
Kanamycin	64
Nalidixic Acid	32
Ciprofloxacin	4

2.2.6 Pulsed-field gel electrophoresis

Genomic DNA fingerprinting was determined by Pulsed-field gel electrophoresis (PFGE) using the *Sma*I restriction enzyme according to established protocol *(12, 16, 34, 42)*. Using antibiotic resistance profiles to define strains, a representative panel of isolates was selected for each time point with each sample (*C. jejuni* isolates were selected from samples 1 and 4 while *C. coli* isolates were selected from samples 2 and 3). PFGE results were analyzed using BioNumerics software (Applied-Maths, Kortrijk, Beljium). Banding patterns were compared by using Dice coefficients with a 1.5% band position tolerance.

2.2.7 Statistical Analysis

Chi-square and Fishers Exact Tests were used to determine significance between survival of *Campylobacter* spp. in feces and water. Statistical analyses were conducted using SAS software (version 9.3, SAS Institute, Cary, NC).

2.3 RESULTS

2.3.1 Species

The prevalence of *C. jejuni* and *C. coli* per time point is shown for all four samples in Fig. 2.1. Overall, *C. coli* was isolated more often (60%) than *C. jejuni* (40%). *C. jejuni* only represented 50% or more of isolates on Days 2, 6, and 16. At all other time points, *C. coli* represented the majority of isolates for all samples.

One predominant species was identified in each of the four flocks studied. *C. jejuni* accounted for the majority of isolates from samples 1 and 4 (54 and 59%, respectively), whereas *C. coli* was the predominant species in isolates from samples 2 and 3 (90 and 71%, respectively). Samples 1 and 4 were obtained from two different flocks which were housed on the same grow-out farm at different time periods, while samples 2 and 3 were obtained at two different times from the same flock (Table 2.1).

Similar trends were noted when comparing prevalence of *C. jejuni* vs. *C. coli* in fecal samples and water suspensions, except that in sample 1, *C. coli* was isolated more frequently than *C. jejuni* in the water suspension (56% vs. 44%, respectively). While sample 3 was a predominantly *C. coli* sample, *C. jejuni* was isolated more frequently from the fecal composite than the water suspension (45% vs. 5%, respectively).

Species prevalence for individual samples is shown in Figs. 2.2-2.5. Sample 1 yielded predominantly *C. jejuni* (Fig. 2.2). *C. jejuni* accounted for approximately 50% or more of isolates from sample 1 at every time point except Days 4 and 8. At Days 14 and 16, 90% of sample 1 isolates were *C. jejuni*.

Sample 2 yielded predominantly *C. coli* (Fig. 2.3). *C. jejuni* was isolated infrequently, representing less than 20% of sample 2 isolates on Days 0-8. All isolates on Days 10-16 were *C. coli*.

Sample 3 also yielded predominantly *C. coli* (Fig. 2.4). On Day 0, well over 90% of isolates were *C. coli*. However, on Day 2 *C. coli* represented only ca. 30% of the isolates. Only a single colony (*C. coli*) was recovered on day 4 for this sample, preventing further monitoring of *C. jejuni* or *C. coli*.

Sample 4 yielded predominantly *C. jejuni* (Fig. 2.5). *C. jejuni* was especially prevalent on Days 0 and 2, representing approximately 90% of isolates. Except for Day 10, *C. jejuni* was the majority of isolates at all time points for this sample.

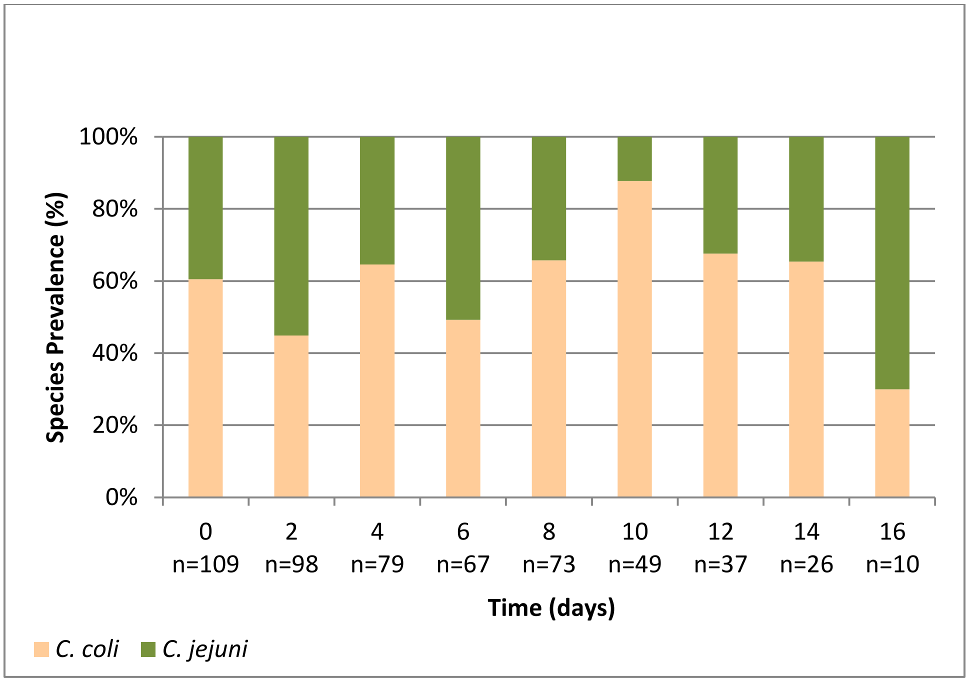


Fig. 2.1: Prevalence of *Campylobacter* spp. for all samples 1-4 by time point.

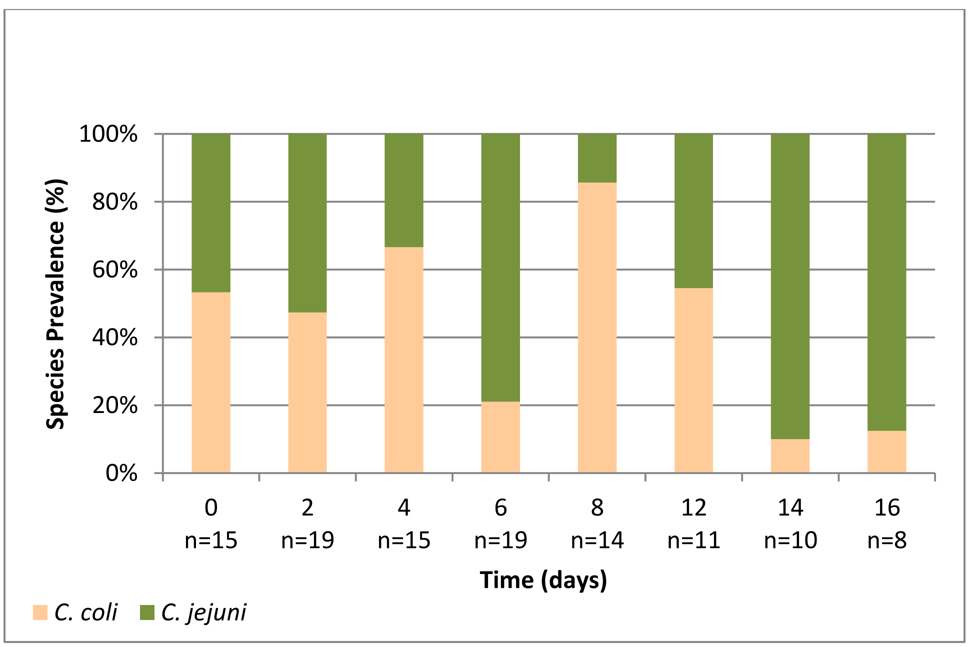


Fig. 2.2: Prevalence of *Campylobacter* spp. for sample 1 by time point.

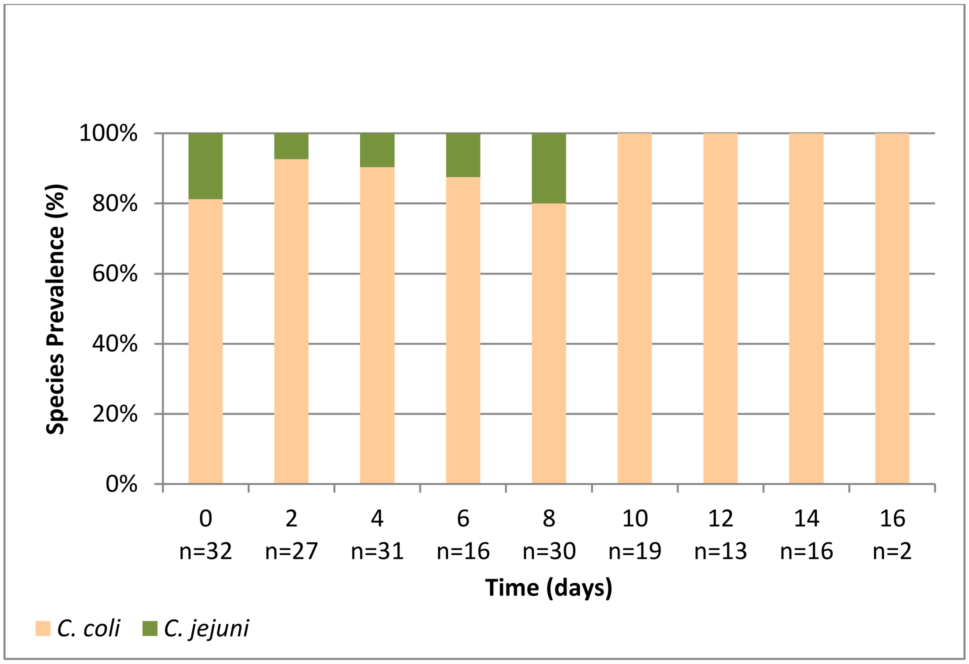


Fig. 2.3: Prevalence of *Campylobacter* spp. for sample 2 by time point.

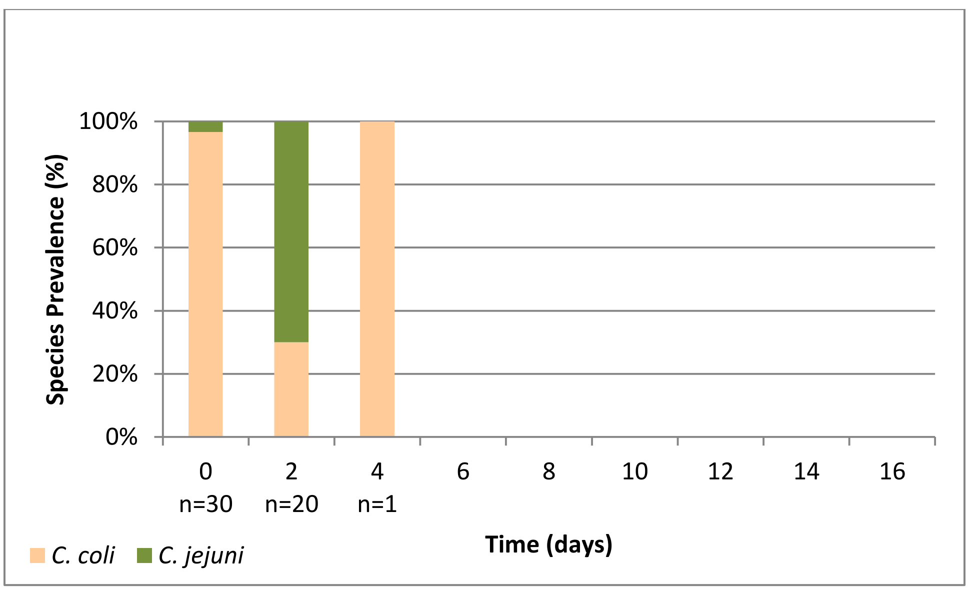


Fig. 2.4: Prevalence of *Campylobacter* spp. for sample 3 by time point.

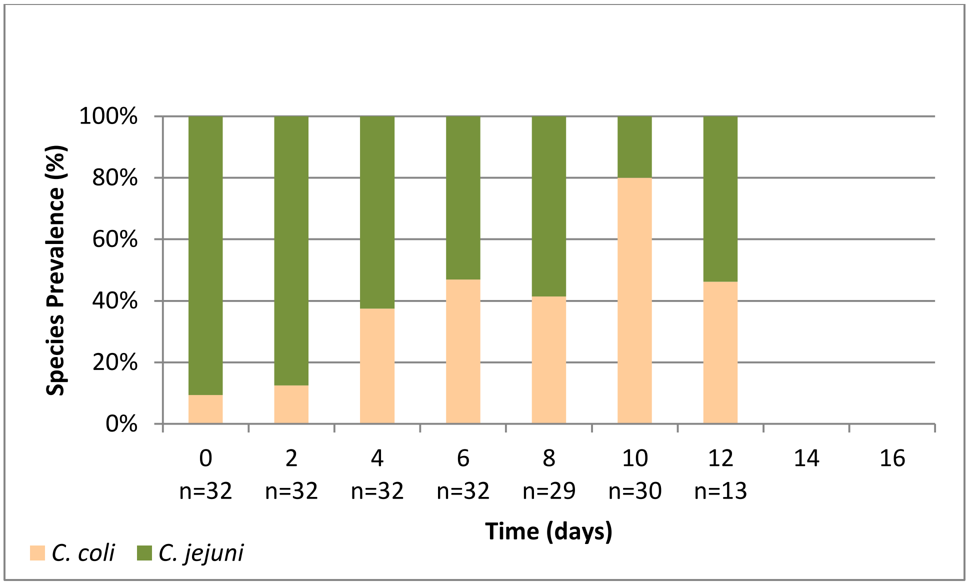


Fig. 2.5: Prevalence of *Campylobacter* spp. for sample 4 by time point.

2.3.2 Antibiotic resistance

Regardless of time point or sample type (feces or water suspension), the majority of *C. jejuni* had antibiotic resistance profiles TSKQ and TKQ while the majority of *C. coli* had profiles TK, TKQ, TQ, and TSEKQ (Table 2.3). Antibiotic resistance profile testing revealed several trends. For instance, *C. jejuni* with profile TSKQ and *C. coli* with profile TK were the most commonly isolated profiles throughout the entire study (28 and 26%, respectively, of all isolates tested). Profiles TK and TQ were commonly encountered in *C. coli* (42 and 19%, respectively, of all *C. coli*), but were rare (TK) or not detected (TQ) in *C. jejuni* (Table 2.3). Of all profiles that were identified, TKQ was the only profile that was commonly found in both *C. jejuni* and *C. coli* (26 and 30%, respectively). Erythromycin resistance was not detected in any *C. jejuni* isolates throughout the study (Table 2.3).

Isolation of antibiotic resistance profiles for *C. jejuni* and *C. coli* by time point are presented in Tables 2.4-2.6. Overall, isolation by time point reflects the same frequency patterns represented in Table 2.3. The most commonly isolated profile in sample 1, which yielded predominantly *C. jejuni*, was TSKQ *C. jejuni*, which was isolated steadily up to Day 16 in feces (Table 2.4). *C. coli* TKQ isolates were common in feces and water in sample 1, but dwindled in both after Day 12. In sample 2, which yielded predominantly *C. coli*, *C. coli* TK was isolated most frequently at nearly all time points in both feces and water (Table 2.5). Like sample 1, sample 4 yielded predominantly *C. jejuni* and its most common isolate at all time points was TSKQ *C. jejuni* (Table 2.6). sample 3 is not presented here due to its short survival results.

Table 2.3: Prevalence of specific antibiotic resistance profiles among *Campylobacter* isolates characterized in this study.

AB Resistance Profile	***C. jejuni* isolates (%)**	***C. coli* isolates (%)**
TSKQ	148 (67.9)	1 (0.3)
TKQ	57 (26.1)	98 (29.7)
TSQ	7 (3.2)	0
TK	3 (1.4)	139 (42.1)
PS	0	1 (0.3)
TQ	0	59 (17.9)
TSEKQ	0	26 (7.9)
T	3 (1.4)	2 (0.6)
TEKQ	0	3 (0.9)
K	0	1 (0.3)
Total	**218**	**330**

Table 2.4: Frequency of antibiotic resistance profiles found in *C. jejuni* and *C. coli* isolates obtained from sample 1 feces and water, presented by time point.

Fecal Composite								
	C. jejuni			***C. coli***				
	TSKQ	**TKQ**	**Other**	**TSEKQ**	**TK**	**TKQ**	**TQ**	**Other**
T0	4					4		
T2	3	1			1	4		
T4	4				2	2		
T6	4	3	1			2		
T8	2				3	4	1	
T10								
T12	3		2		2	2		
T14	7		2		1			
T16	5	1	1		1			
Water Suspension								
	C. jejuni			***C. coli***				
	TSKQ	**TKQ**	**Other**	**TSEKQ**	**TK**	**TKQ**	**TQ**	**Other**
T0	3					4		
T2		5	1		2	2		
T4	1				1	5		
T6	5	2			1	1		
T8					1	2		
T10								
T12					1	1		

Table 2.5: Frequency of antibiotic resistance profiles found in *C. jejuni* and *C. coli* isolates obtained from sample 2 feces and water, presented by time point.

Fecal Composite								
	C. jejuni			***C. coli***				
	TSKQ	**TKQ**	**Other**	**TSEKQ**	**TK**	**TKQ**	**TQ**	**Other**
T0	2	1	1	7	2	2	1	
T2			1	3	6	1		1
T4		3			7	2	4	
T6			1		9	1	1	
T8	5		1		4	3	1	
T10					8	3		
Water Suspension								
	C. jejuni			***C. coli***				
	TSKQ	**TKQ**	**Other**	**TSEKQ**	**TK**	**TKQ**	**TQ**	**Other**
T0	1	1		6	5		3	
T2			1	5	8		1	
T4				1	5	4	5	
T6				1	1			1
T8					8	3	3	2
T10					2	3	3	
T12					4	6	3	
T14					8	1	7	
T16					1		1	

Table 2.6: Frequency of antibiotic resistance profiles found in *C. jejuni* and *C. coli* isolates obtained from sample 4 feces and water, presented by time point.

Fecal Composite								
	C. jejuni			***C. coli***				
	TSKQ	**TKQ**	**Other**	**TSEKQ**	**TK**	**TKQ**	**TQ**	**Other**
T0	13			2		1		
T2	8	5			3			
T4	10	1			1	4		
T6	2	9			3	2		
T8	3	6	1		2	3		1
T10	3	2			6	5		
T12	7				3	2		1
Water Suspension								
	C. jejuni			***C. coli***				
	TSKQ	**TKQ**	**Other**	**TSEKQ**	**TK**	**TKQ**	**TQ**	**Other**
T0	16							
T2	6	9				1		
T4	8	1			3	4		
T6	3	3			7	3		
T8	5	2			4	2		
T10		1		1	7	5		

2.3.3 Survival of total *Campylobacter*

Throughout the study, *Campylobacter* was enumerated as CFU/g for feces and CFU/mL for the water suspension. By nature, the water suspension is a more dilute sample than the fecal composite, because it consists of 1g of fecal composite combined with 9mL of drinker water (hence, CFU/ml would correspond to CFU/0.1g of the original sample). For the sake of comparison, both the fecal and water *Campylobacter* numbers are presented here in CFU/g.

Overall, for samples 1, 2 and 4 *Campylobacter* was present in both the fecal composite samples and the water suspensions at levels of 10^6 CFU/g on Day 0. These numbers slowly declined until between Day 12 and Day 16, when *Campylobacter* was no longer detected in the samples. Sample 3 showed unusually low levels of *Campylobacter* on Day 0, with only 10^4 CFU/g in both the fecal and water samples, and *Campylobacter* in this sample 3 was undetectable by Day 4. Compared to the other samples, Sample 3 was unusually dry. As such, it was possibly mishandled, which would explain its relatively low *Campylobacter* prevalence. *Campylobacter* death curves for the individual Samples 1-4 are shown in Figs. 2.6-2.9.

In samples 1, 2, and 4, total *Campylobacter* displayed certain differences in survival between the fecal composite and the water suspension. At Day 0, the total *Campylobacter* population in sample 1 was higher in the fecal composite (1.35 x 10^6 CFU/g) compared to the water suspension (7.16 x 10^5 CFU/g) (Fig. 2.6). *Campylobacter* from the water suspension of this sample declined to the detection limit for reliable counts by Day 12, whereas *Campylobacter* isolated from the fecal composite did not decline to the limit until Day 16. Prevalence of total *Campylobacter* in sample 2 was similar in both the fecal and water samples until Day 8, when more *Campylobacter* was isolated from water than feces (6.8 x 10^5 CFU/g vs. 8.35 x 10^4 CFU/g, respectively) (Fig. 2.7). Unlike the trends in sample 1, *Campylobacter* from sample 2 approached the limit on Day 12 in feces and Day 16 in water. *Campylobacter* in sample 3 declined quickly compared to the other samples tested (Fig. 2.8). In sample 4, total *Campylobacter* levels were similar on Day 0 for both the fecal and water samples (10^6 CFU/g), but prevalence declined more rapidly in water than in feces Fig. 2.9). Despite these observed differences, however, overall there was no evidence of statistically significant differential survival of total *Campylobacter* between fecal and water conditions.

Four additional samples, samples 5-8 (Table 2.1), were collected for enumeration of total *Campylobacter* to provide further information on the population declines and for comparison with the death curves noted in Samples 1-4. Some slight differences in death curves were noted for samples 5-8, but overall the decline of *Campylobacter* was comparable to that seen in samples 1-4 (Fig. 2.10). In particular, sample 8 was similar to the typical death curve encountered in samples 1, 2 and 4: *Campylobacter* counts in this sample approached the limit on Day 12 (water) and Day 16 (fecal). *Campylobacter* in sample 7 survived in the water suspension longer than observed in any of the samples, including samples 1-4 (24 days vs. a maximum of 16 days in samples 1-4). However, *Campylobacter* spp. in the fecal composite approached the limit and was not enumerated after Day 16. In samples 5 and 6, *Campylobacter* did not survive past Day 8 in feces (sample 6) and in either water or feces (sample 5), which is shorter than the survival noted in samples 1-4 (Fig. 2.10).

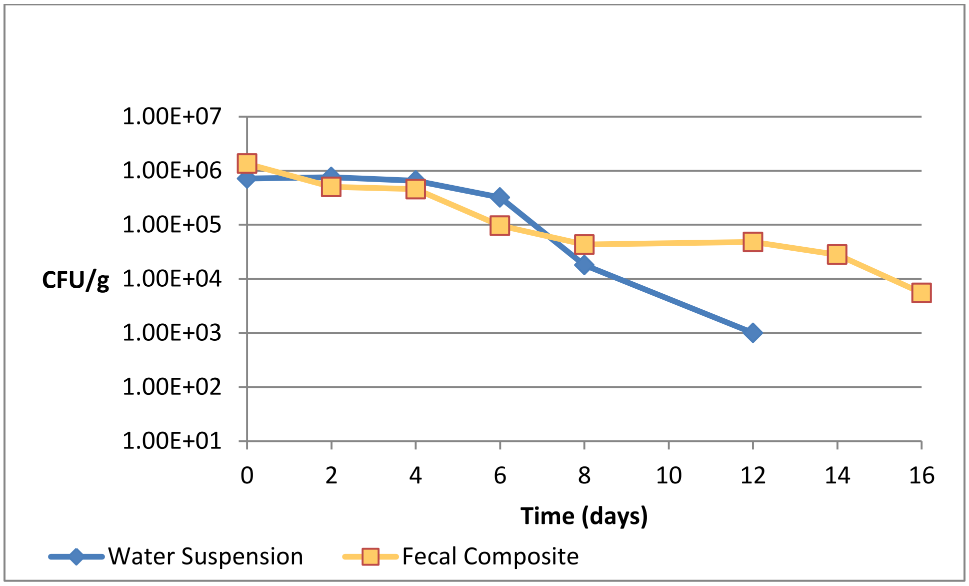


Fig. 2.6: Total *Campylobacter* death curve for sample 1 isolates.

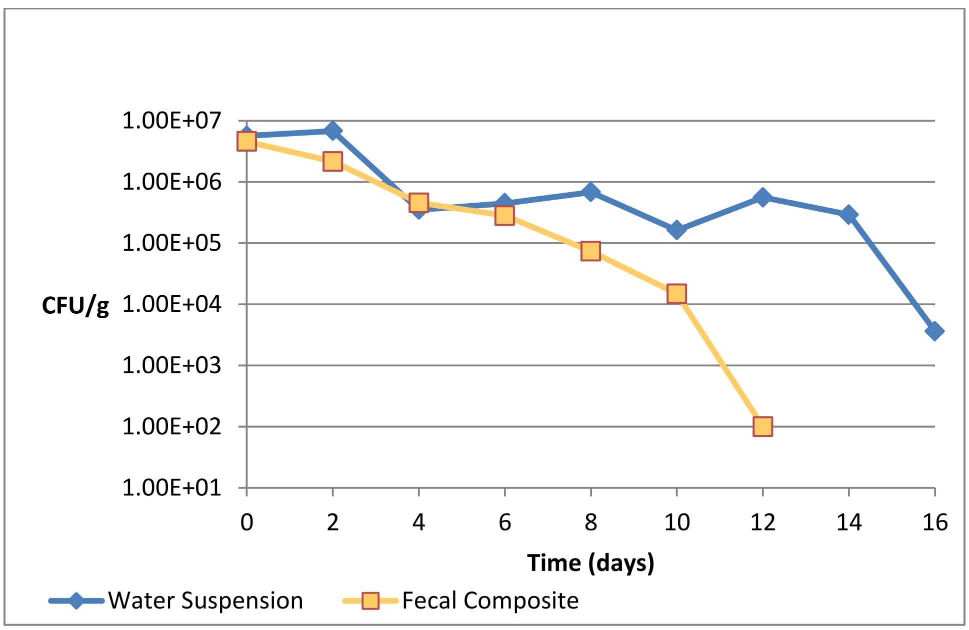


Fig. 2.7: Total *Campylobacter* death curve for sample 2 isolates.

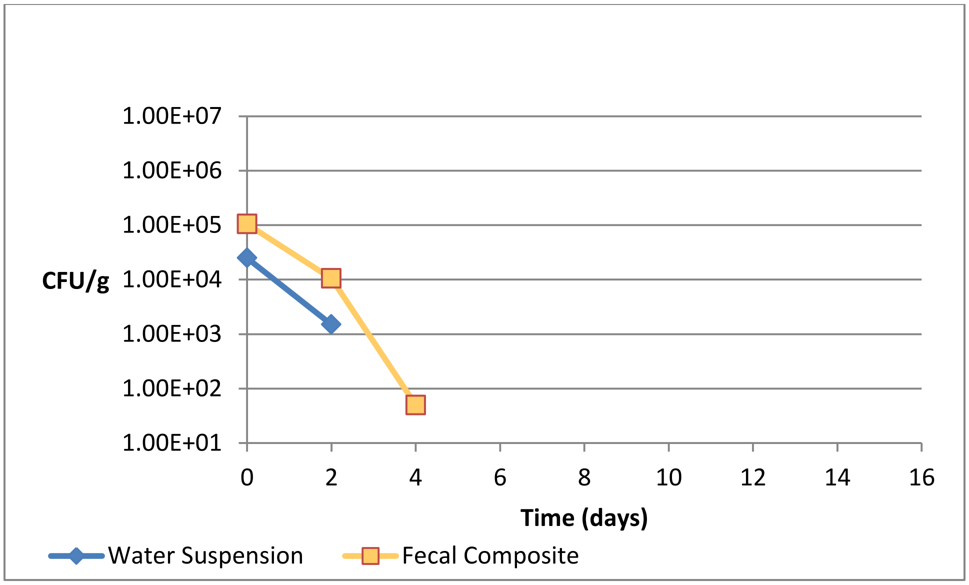


Fig. 2.8: Total *Campylobacter* death curve for sample 3 isolates.

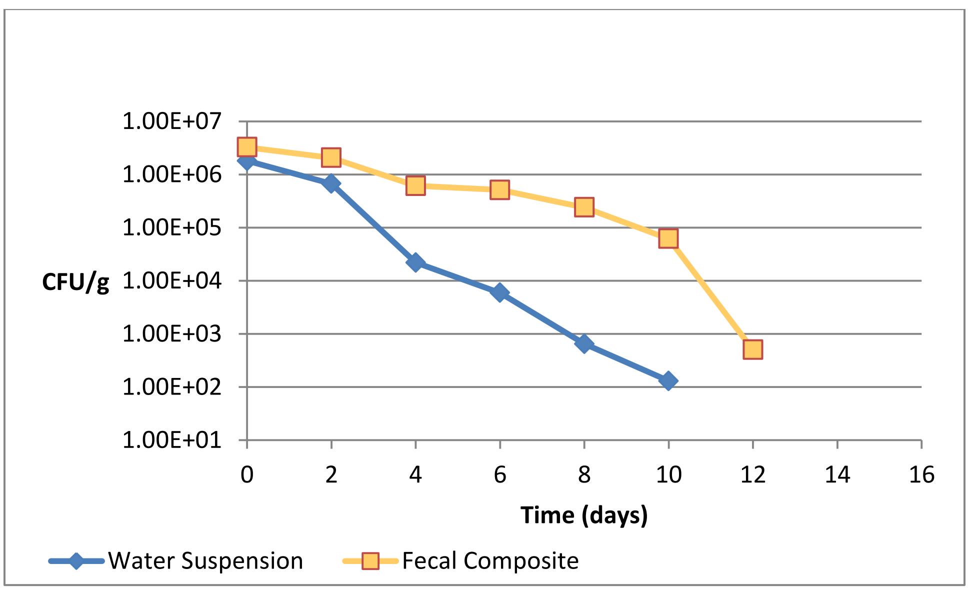


Fig. 2.9: Total *Campylobacter* death curve for sample 4 isolates.

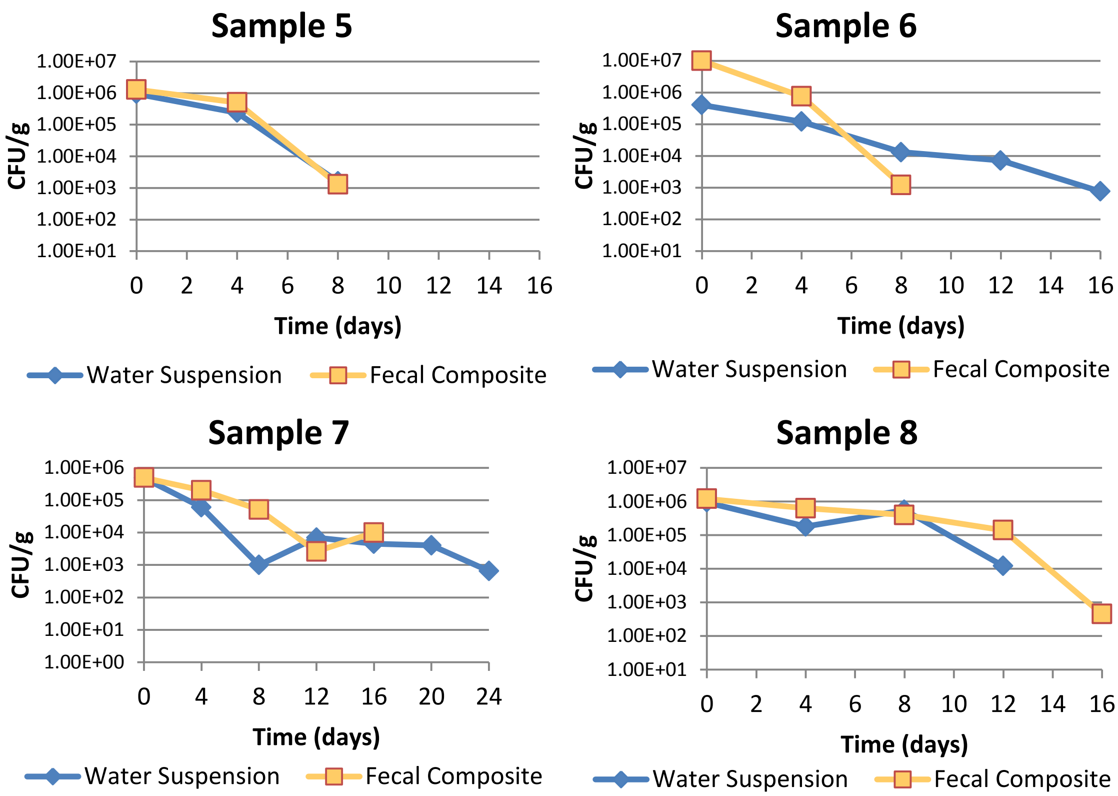


Fig. 2.10: Total *Campylobacter* death curves for samples 5-8. *Campylobacter* populations in these samples were enumerated for the sake of comparison to samples 1-4.

2.3.4 Survival of *C. jejuni*

When all isolates from samples 1-4 were combined for analysis, *C. jejuni* was revealed as surviving significantly longer in the fecal composite samples than *C. coli* ($p < 0.0005$) (Figs. 2.11-2.14). In particular, samples 1 and 3 contributed greatly to this significance, as *C. jejuni* accounted for the majority of isolates in the later time points from these samples (Fig. 2.11 and Fig. 2.13, respectively). To a lesser extent, sample 4 also contributed to the observed survival of *C. jejuni* at later time points (Fig. 2.14).

At all time points, the antibiotic resistance profile TSKQ was encountered in the majority of *C. jejuni* isolates (Fig. 2.15). TKQ was the second most common *C. jejuni* antibiotic resistance profile, and all other profiles were encountered only rarely. *C. jejuni* isolates with antibiotic resistance profiles TSKQ and TKQ were noteworthy for their relatively long survival in the fecal composite samples. Among all isolates encountered beyond Day 4 in the fecal composite, TSKQ and TKQ *C. jejuni* were more prevalent compared to other isolates (p = 0.0077).

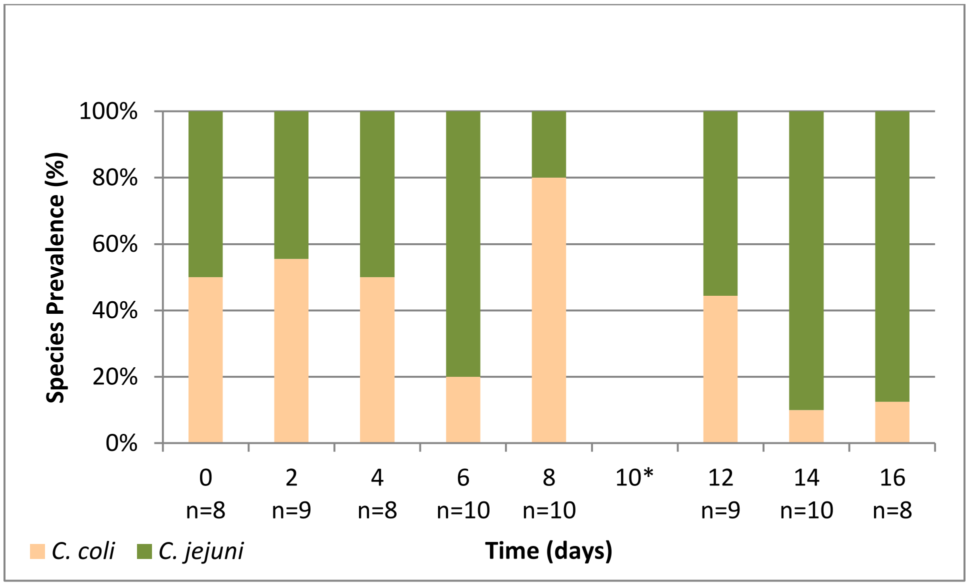


Fig. 2.11: Prevalence of *C. jejuni* relative to *C. coli* recovered from sample 1 fecal composite at different time points.
* Sample was not analyzed on Day 10.

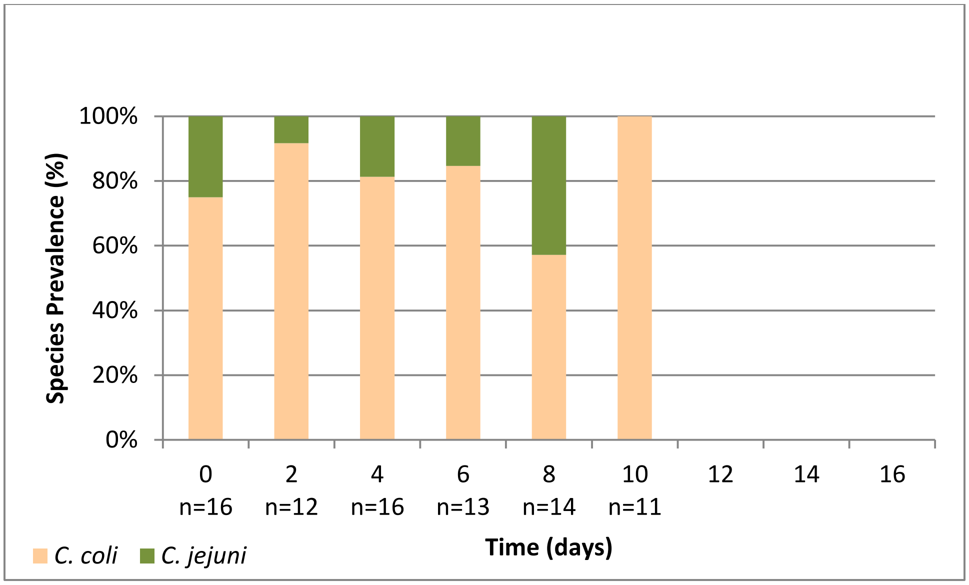


Fig. 2.12: Prevalence of *C. jejuni* relative to *C. coli* recovered from sample 2 fecal composite at different time points.

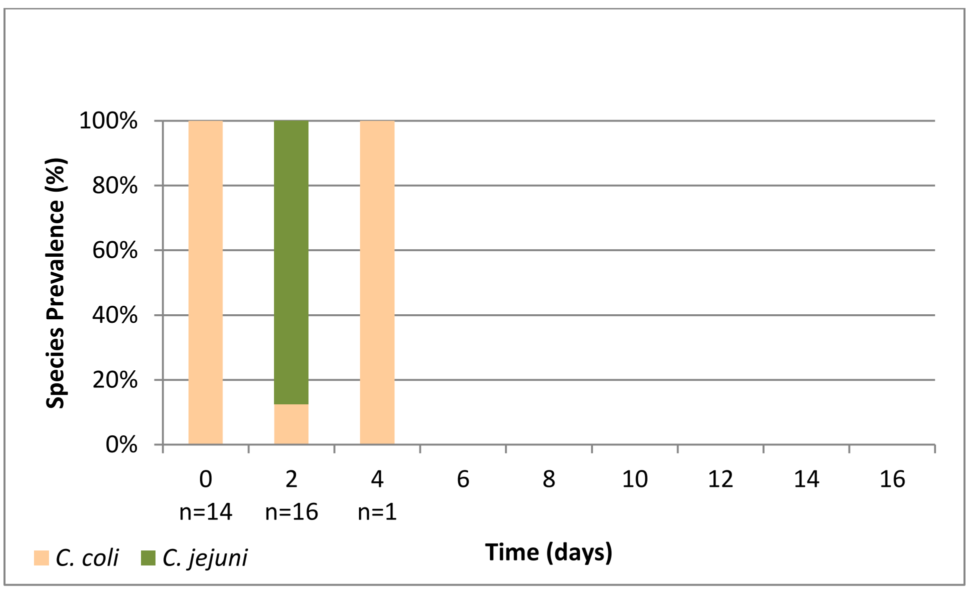


Fig. 2.13: Prevalence of *C. jejuni* relative to *C. coli* recovered from the sample 3 fecal composite sample at different time points.

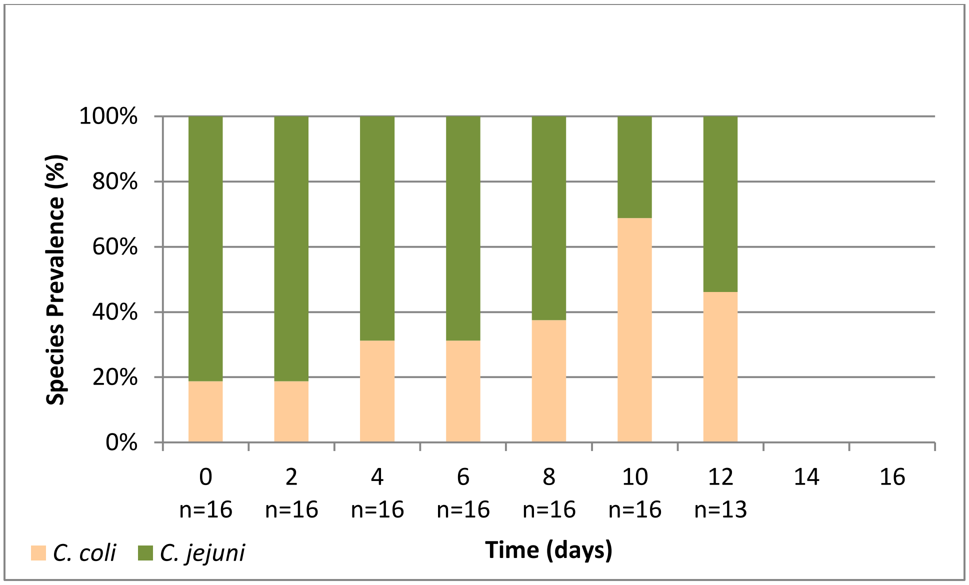


Fig. 2.14: Prevalence of *C. jejuni* relative to *C. coli* recovered from the sample 4 fecal composite sample at different time points.

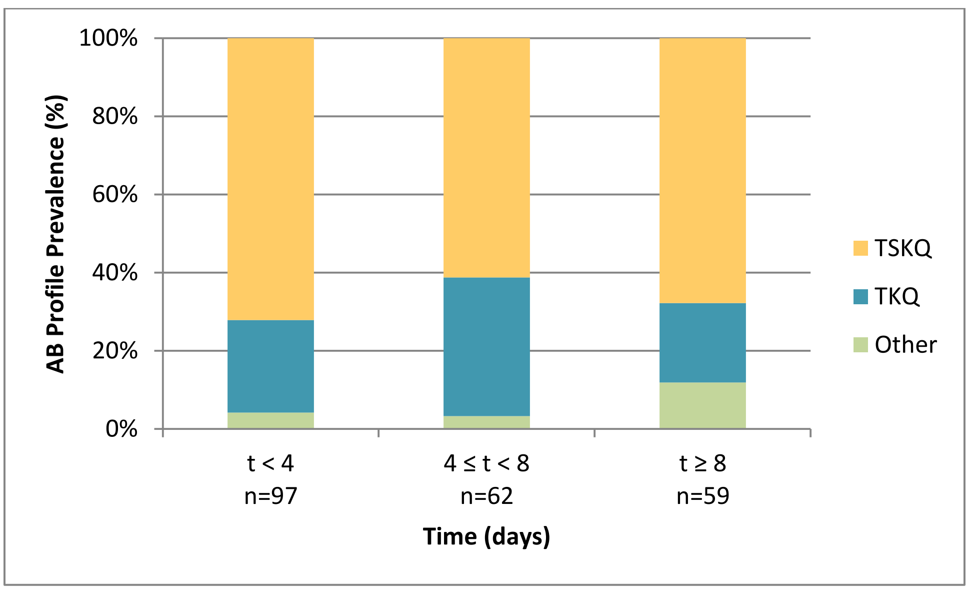


Fig. 2.15: Prevalence of *C. jejuni* antibiotic resistance profiles at different time points. Data are combined for isolates from the fecal composite samples and the water suspensions.

2.3.5 Survival of *C. coli*

C. coli survived significantly longer in the water suspensions than *C. jejuni* ($p = 0.0005$) (Figs. 2.16-2.19). Sample 1, which yielded predominantly *C. jejuni*, showed better survival of *C. coli* in the water suspension than *C. jejuni* (Fig. 2.16). All six of the isolates obtained on Days 8 and 12 (the final two days of enumeration) were *C. coli*. Even in sample 4, which was also yielded predominantly *C. jejuni*, *C. coli* was isolated more frequently in the later time points (Fig. 2.19). At the early time points of sample 4, *C. jejuni* was the majority of isolates recovered (100% on Day 0, over 90% on Day 2). However, after Day 6 approximately 50% or more of isolates were *C. coli*. By the final day of enumeration, Day 10, over 90% of the 14 isolates from this sample were *C. coli* (Fig. 2.19).

Among all *C. coli* isolates recovered beyond Day 4, both from the fecal and water samples, TK and TKQ were more prevalent compared to other profiles (Fig. 2.20). On the other hand, *C. coli* with the multidrug resistance (MDR) profile TSEKQ exhibited relatively limited ability for prolonged survival, as prevalence of TSEKQ isolates was lower after Day 4 ($p = 0.00000011$). On Days 0 and 2, TSEKQ represented approximately 20% of *C. coli* isolates obtained from all samples. However, by Day 4 and after, TSEKQ *C. coli* was isolated infrequently (Fig. 2.20).

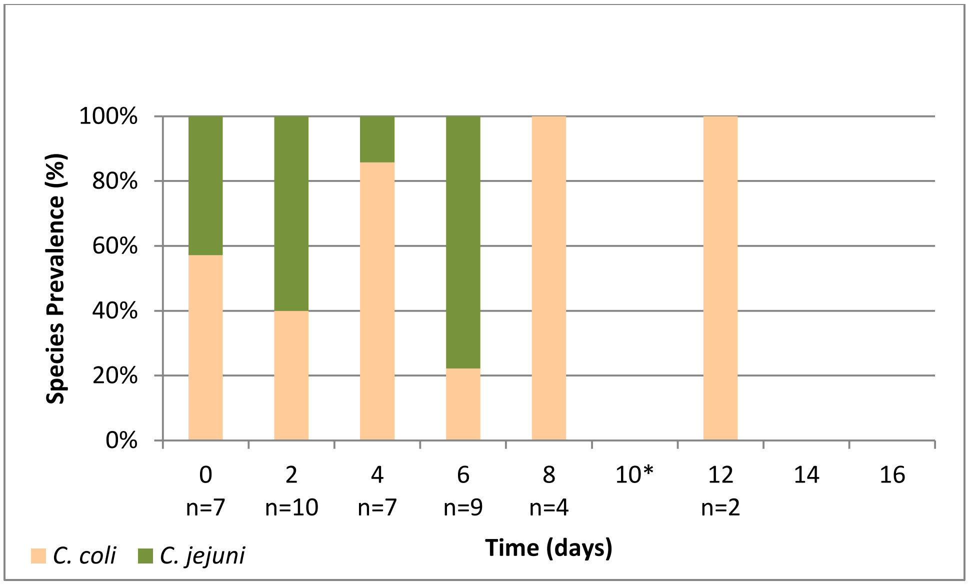


Fig. 2.16: Prevalence of *C. jejuni* relative to *C. coli* recovered from sample 1 water suspensions at each time point.
*Sample was not analyzed on Day 10.

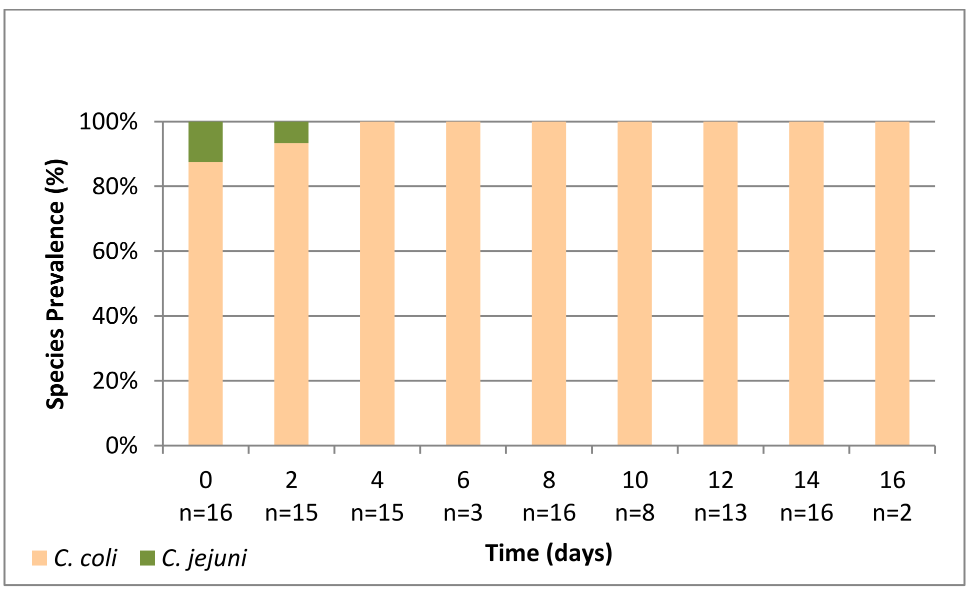


Fig. 2.17: Prevalence of *C. jejuni* relative to *C. coli* recovered from sample 2 water suspensions at each time point.

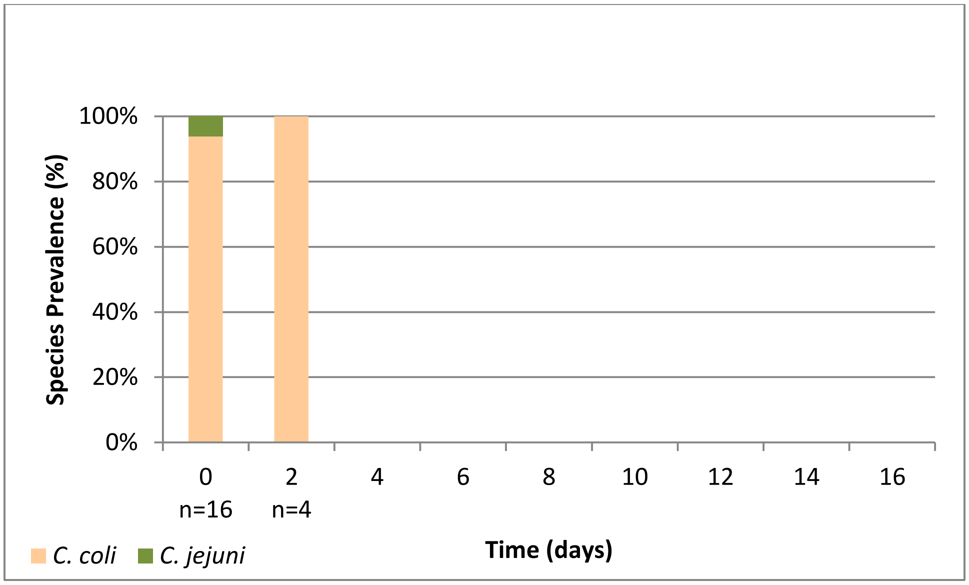


Fig. 2.18: Prevalence of *C. jejuni* relative to *C. coli* recovered from sample 3 water suspensions at each time point.

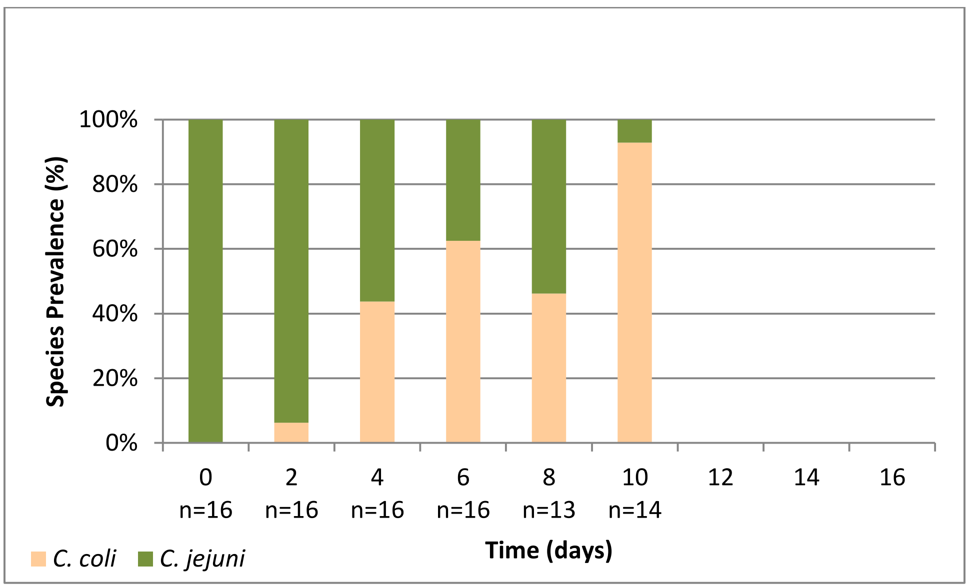


Fig. 2.19: Prevalence of *C. jejuni* relative to *C. coli* recovered from sample 4 water suspensions at each time point.

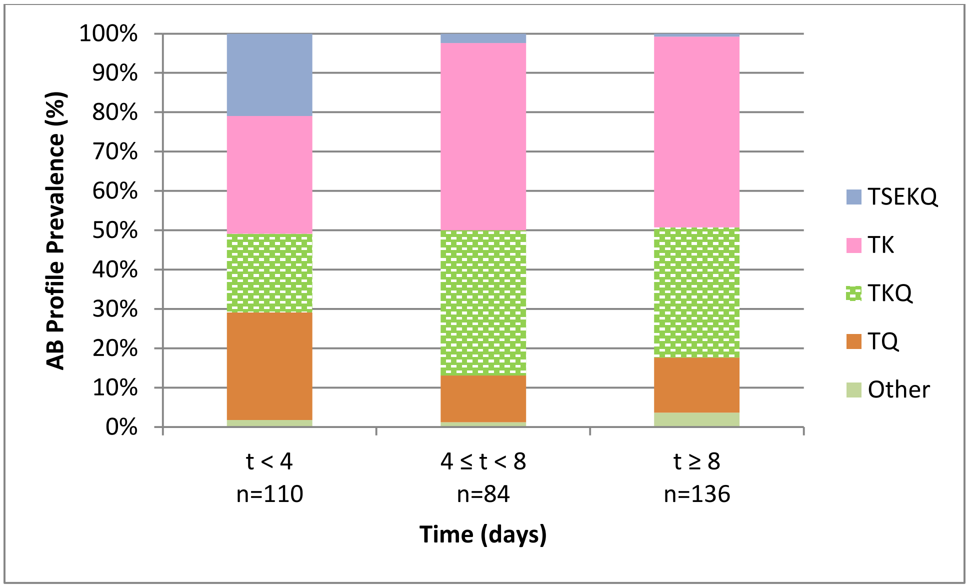


Fig. 2.20: Prevalence of *C. coli* antibiotic resistance profiles at different time points. Data are combined for isolates from the fecal composite samples and the water suspensions.

2.3.6 PFGE

Using antibiotic resistance profiles to define isolates, representative isolates of *C. jejuni* (samples 1 and 4) and *C. coli* (samples 2 and 3) were subtyped using PFGE. Banding patterns observed for each of the 4 samples are shown in Figs. 2.21-2.24. Table 2.7 groups banding patterns observed in *C. jejuni* into common PFGE profiles. A total of 3 different profiles were observed in *C. jejuni*. Isolates with the TSKQ antibiotic resistance profile displayed all 3 types of PFGE profiles found in *C. jejuni*. These types differed mostly in their lowest band (ca. 48.5 kilobases) (Fig. 2.21). At the 48.5 kb level, type 1 displayed a clean double band, type 2 displayed a clean single band, and type 3 displayed a single band with partial digestion underneath. Another key difference between types 1 and 2 occurred near the 242.5 kb marker, where type 1 has a double band and type 2 has a single band. All 3 types had a bright band near the top of the gel (ca. 727.5 kb), but type 3 was unique in that it displayed 4 evenly-spaced bands at 727.5, 679.0, 630.5, and 582.0 kb.

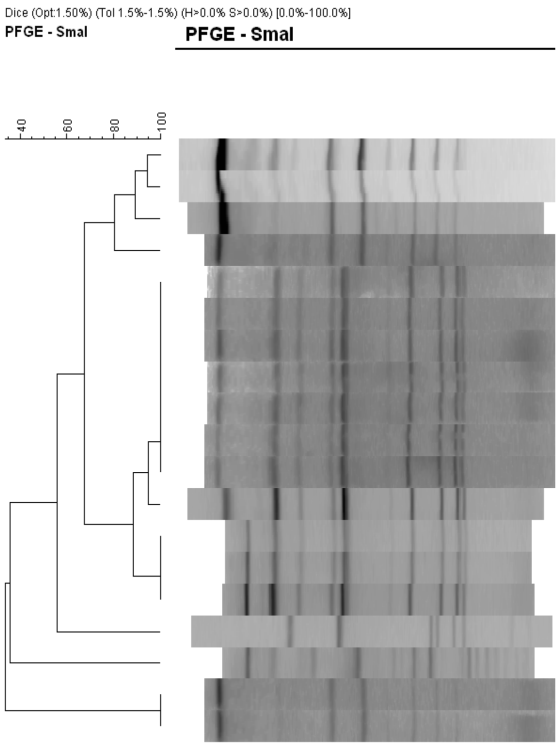
Table 2.8 groups banding patterns observed in *C. coli* into common PFGE profiles. A total of 4 different profiles were observed in *C. coli* isolates. *C. coli* with PFGE profiles A-1 and A-2 appear to be hearty *C. coli* isolates, as they are commonly isolated at all time points in Samples 2 and 3 (Figs. 2.23 and 2.24). The other two *C. coli* PFGE profiles, B and C, were rarely encountered in Sample 3 (Fig. 2.24). In both *C. jejuni* and *C. coli*, isolates with different antibiotic resistance profiles were shown to have overlapping PFGE profiles.

Table 2.7: PFGE profiles of representative *C. jejuni* isolates taken from samples 1 and 4 at different time points.

Isolate Name	AB Profile	PFGE Profile
MT0-2	TSKQ	1
MT2-4	TSKQ	1
MT8-5	TSKQ	2
MT16-2	TSQ	2
4MT4-3	TSKQ	2
4MT8-8	TSKQ	2
WT4-4	TSKQ	3
WT6-3	TKQ	(unassigned)
WT2-1	TSKQ	2
MT6-3	TSQ	1
MT14-4	TSQ	2
MT12-5	TSQ	2
4MT6-1	TSKQ	2
4MT2-3	TSKQ	2
4MT8-7	TSKQ	2
4MT10-3	TSKQ	2
4WT8-1	TSKQ	1
4WT10-12	TK	2

Table 2.8: PFGE profiles of representative *C. coli* isolates taken from samples 2 and 3 at different time points.

Isolate Name	AB Profile	PFGE Profile
2MT0-3	TKQ	A-1
2MT2-8	TK	A-1
2MT4-2	TK	A-1
2MT4-3	TQ	A-1
2MT6-1	TK	A-1
2MT6-4	TKQ	A-1
2MT4-9	TKQ	A-2
2MT6-14	TQ	A-2
2MT0-1	TSEKQ	B
2MT2-3	TKQ	C
2MT2-4	TEKQ	C



Strain	AB Profile	PFGE Profile
MT16-1	TSKQ	2
MT16-2	TSQ	2
WT2-1	TSKQ	2
MT8-5	TSKQ	1
MT0-2	TSKQ	1
MT12-2	TSKQ	1
MT14-2	TSKQ	1
MT2-1	TSKQ	1
MT2-4	TSKQ	1
MT4-1	TSKQ	1
MT6-1	TSKQ	1
WT0-2	TSKQ	1
MT6-3	TSQ	3
WT2-2	TSKQ	3
WT6-1	TSKQ	3
WT6-3	TKQ	(unassigned)
WT4-4	TSKQ	3
MT12-5	TSQ	2
MT14-4	TSQ	2

Fig. 2.21: PFGE profiles of select *C. jejuni* isolates obtained from sample 1. In these figures, “Key” refers to the isolate name as described in the Materials and Methods section.

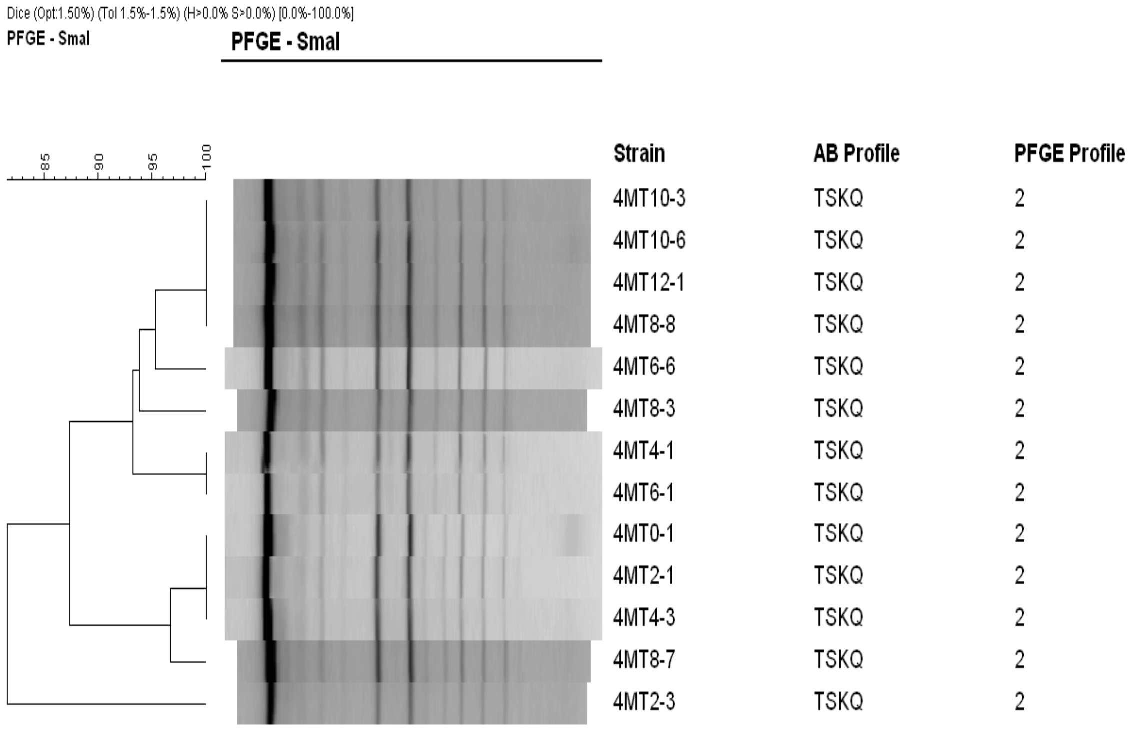


Fig. 2.22: PFGE profiles of select *C. jejuni* isolates obtained from sample 4. In these figures, "Key" refers to the isolate name as described in the Materials and Methods section.

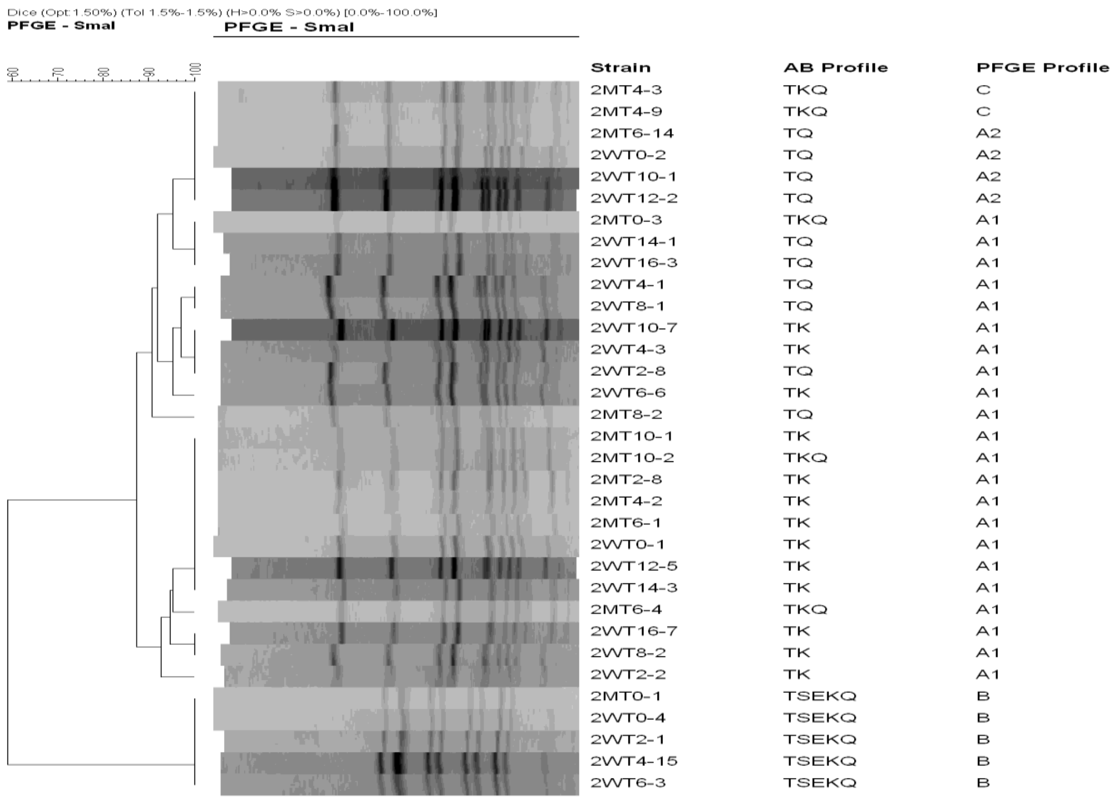


Fig. 2.23: PFGE profiles of select *C. coli* isolates obtained from sample 2. In these figures, “Key” refers to the isolate name as described in the Materials and Methods section.

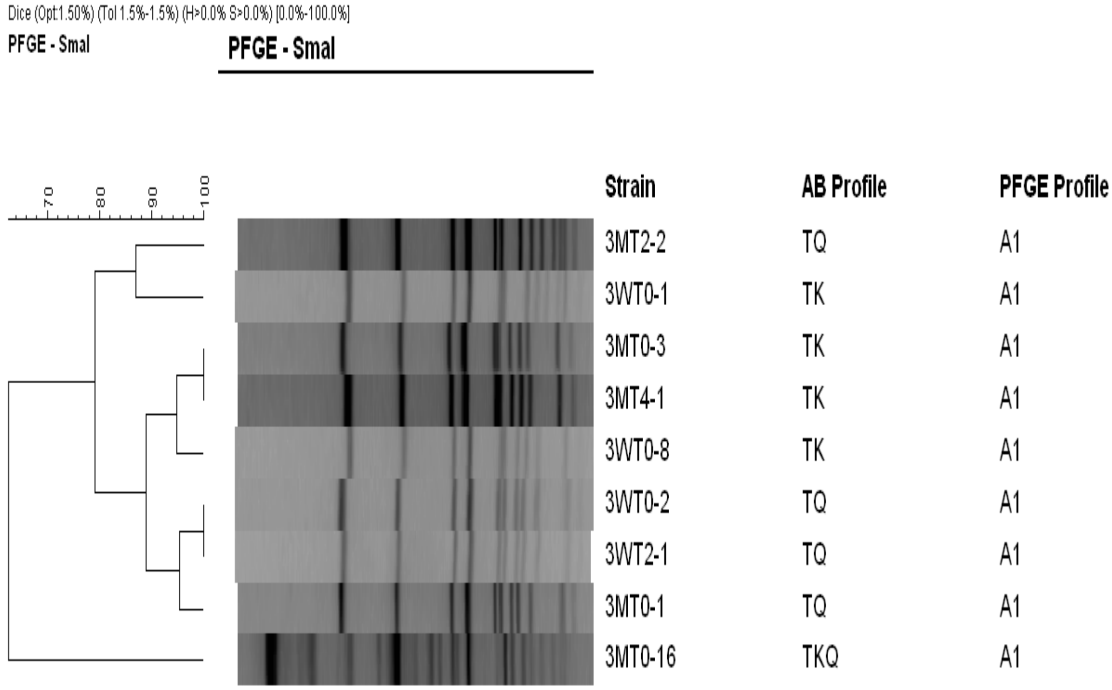


Fig. 2.24: PFGE profiles of select *C. coli* isolates obtained from sample 3. In these figures, "Key" refers to the isolate name as described in the Materials and Methods section.

2.4 DISCUSSION

This study assessed the survival of naturally-occurring *Campylobacter* isolates in turkey feces or in a suspension of feces in drinker water. As samples were stored at 4°C under controlled laboratory conditions, this study is intended to present a model for survival. The typical pattern of survival within the 8 farm samples was that *Campylobacter* was present at approximately 10^6 CFU/g (feces) or 10^5 CFU/mL (water) on Day 0. Total *Campylobacter* would gradually decline until between Day 12-16, when *Campylobacter* approached the limit and was no longer enumerated. This 12-16 day survival of *Campylobacter* in turkey feces is longer than the 6 day survival of naturally-occurring *C.*

jejuni noted by Ahmed et al. in laying hen feces and the 5 day survival of *C. jejuni* from chicken samples noted by Bui et al. *(1, 5)*.

This difference in *Campylobacter* survival is likely the result of differing sample storage conditions. Both Ahmed and Bui stored their fecal samples at room temperature, which likely explains why their survival was shorter than the present study *(1, 5)*. Fecal samples in the present study were held at 4°C, the ideal temperature for *Campylobacter* survival *(18, 22)*. Another possible explanation is that the differences in *Campylobacter* survival could be indicative of an inherent difference between the fecal samples from chickens vs. turkeys, as well as differences in the species and strains of *Campylobacter* in the samples. For instance, the present study followed both *C. jejuni* and *C. coli* for survival, whereas the Ahmed et al. and Bui et al. studies followed only *C. jejuni (1, 5)*. *C. coli* contributed significantly to prolonged survival in the present study, and is likely part of the reason the present study found longer survival compared to Ahmed et al. and Bui et al. *(1, 5)*.

This study did not find a significant difference between total *Campylobacter* survival in feces vs. water. This lack of significance was surprising, considering that water is known to have a protective effect on *Campylobacter* cells, and can prolong its survival *(41, 44)*. One possible explanation is that storing fecal samples in a capped tube at 4°C also provided a protective effect on *Campylobacter* within the fecal composite. In combination with the low temperature of 4°C, the dark environment and little airflow within the capped tube might have allowed the composite to remain at a relative humidity level high enough to prolong survival. Factors such as relative humidity should be taken into account for future survival studies.

In the present study, *C. coli* was a significantly longer survivor in the drinker water suspension than *C. jejuni* ($p = 0.0005$). While the reasons for this species-related difference has not been noted or defined in the literature, it has been established that water provides a protective effect to *Campylobacter* cells *(41, 44)*. This is likely due to interaction between *Campylobacter* and waterborne protozoa. The Snelling et al. study examined the phagocytosis of both *C. jejuni* and *C. coli* isolated from broiler cloacal swabs into the waterborne protozoa *A. castellanii* and *T. pyriformis*, both of which were present in broiler drinker water *(41)*. Phagocytosis into both of these protozoa strains increased the viability of *C. jejuni*, but not *C. coli*, which indicates that *Campylobacter* interaction with protozoa is species-specific.

The prolonged viability of *C. jejuni* in water noted in the Snelling et al. study is different than the prolonged survival of *C. coli*, but not *C. jejuni*, in the present study of *Campylobacter* isolated from turkey feces *(41)*. Again, this could indicate a difference in survival capabilities of *Campylobacter* isolated from turkey vs. chicken, or it could indicated a difference in waterborne protozoa present between the Snelling et al. study and the present study. Waterborne protozoa were not defined or examined in the present study.

C. jejuni survived longer in feces than *C. coli* ($p = 0.0005$). This is significant from a public health perspective because *C. jejuni* accounts for over 85% of human campylobacteriosis, thus its presence and persistence on poultry farms can result in unsafe food for the consumer if the bacteria are able to survive throughout processing and be present in the retail product *(3, 13, 26)*. As a result, it is crucial to study and thoroughly understand the survival of *Campylobacter* in feces as well as water. The most common *C. jejuni* antibiotic resistance profile noted in the present study was the multidrug resistant profile TSKQ (n = 148 isolates), followed by TKQ (n = 57). This is similar to other drug resistance profiles noted by Gu et al. in a study of *C. jejuni* from eastern North Carolina turkey farms *(16)*.

Significantly long surviving isolates included *C. jejuni* with antibiotic resistance profiles TSKQ and TKQ in feces (p = 0.0077), and *C. coli* with profiles TK, TQ, and TKQ in both feces and water (p = 0.00000011 in feces, p = 0.00011 in water). With the exception of *C. coli* with a TK profile, these long-surviving isolates all have resistance to fluoroquinolones in common, suggesting that the mutation responsible for this resistance provides enhanced fitness to the organism *(27)*. Similarly, all of these isolates are also resistant to tetracycline and kanamycin. There is no current research into the fitness effects of these resistances on *Campylobacter*.

Future research should focus on which of these resistance mutations, if any, provides the most significant benefit for fitness and survival in feces and water. This knowledge is important for a few reasons: First, length of survival is directly related to the ability of *Campylobacter* to persist on a farm. *Campylobacter* strains have been shown to persist on farms and colonize successive turkey flocks *(23)*. Within the turkey farm, *Campylobacter* can be easily spread throughout the flock via fecal contact, as birds are copraphagic. Once present in the environment, factors including nearby cattle or other farm animals, excessively moist litter, farm workers' boots, and transport crates can contribute to persistence and horizontal spread of *Campylobacter* within both turkey and broiler farming operation *(9, 10, 21, 25, 35, 36, 40, 46)*. The longer *Campylobacter* is able to survive, the more opportunity exists for it to be transmitted via one of the aforementioned vehicles. Second, a better understanding of which mutations protect *Campylobacter* will allow future researchers to develop effective molecular interventions, such as vaccines, in order to control this pathogen pre-harvest. The ability to reduce the amount of *Campylobacter* that persists long enough to be spread on a turkey farm will make it less likely that *Campylobacter* is present on retail turkey meat, making food safer for consumers.

C. coli with resistance profile TSEKQ comprised 20% of *C. coli* isolates on Days 0 and 2, but was infrequently isolated after Day 4 ($p = 0.00000011$). These findings seem to support the hypothesis that erythromycin imposes a fitness cost that makes it less competitive than an organism that is susceptible to erythromycin, as has been suggested in the literature *(2, 17, 45)*. Additionally, isolates resistant to erythromycin were infrequent in this study (5% of the total isolates). As such, interventions aimed at controlling *Campylobacter* pre-harvest would not be wise to focus on fitness and survival of erythromycin-resistant strains.

PFGE was used to define *Campylobacter* subtypes according to each isolate's genetic fingerprint. This allows us to see which isolates are genetically related to one another based on their banding separation patterns. This study found no clear correlation between antibiotic resistance profile and PFGE profile. There are mixed results regarding this phenomenon in the literature *(14, 43)*. However, we would expect that these profiles would match within isolates obtained from the same farm *(7)*. The lack of correlation presented by the current study could be the result of a few different things. For example, what we thought was the pure culture used for testing could have in fact been a mixed culture. It is therefore possible that in some cases, the predominant *Campylobacter* that was initially tested for antibiotic resistance was different than the predominant *Campylobacter* that grew when cultured from the -80°C freezer stock and tested for PFGE. Another possibility is that of laboratory error during the antibiotic resistance testing process.

This study used a culture-based detection method to enumerate *Campylobacter,* which has some distinct advantages. For example, culture-based detection methods allow for clear evidence of cell viability, and they produce isolates that can be purified and further characterized. One major limitation of using culture-based methods is the relatively high detection limit (in this study, 2.0×10^3 CFU/g for reliability of counts). In order to utilize a lower detection limit, future studies might prefer to use more sensitive methodologies such as enrichment or quantitative PCR.

The findings of the present study offer some new insight into the survival characteristics of *C. jejuni* and *C. coli* outside their avian host. It is important to understand the survival trends and characteristics of *Campylobacter* in order to design effective interventions to better protect public health. More detailed research is needed in order to better understand these survival characteristics and how they impact pre-harvest transmission of *Campylobacter*. Future research should focus on survival trends of *Campylobacter* within the turkey grow-out farm, including the effects of temperatures and ambient conditions (e.g. relative humidity) commonly encountered in turkey grow-out houses. This study presents a model system for survival of *Campylobacter* outside its avian host; survival in the field will likely be different.

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CHAPTER 3: Relative Fitness of *C. jejuni* and *C. coli* in Sterile Water Suspensions Stored at 4°C

ABSTRACT

Campylobacter jejuni and *C. coli* are increasingly resistant to several types of antibiotics, and each species displays a characteristically different resistance pattern. Antibiotic resistance, particularly to macrolides and fluoroquinolones, could impact fitness for survival.

In the present study, three *Campylobacter* strains were selected for study, each representing a different resistance profile: TSKQ *C. jejuni* (2MT8-3); TKQ *C. coli* (2MT6-4); and TSEKQ *C. coli* (2WT0-4). Sterile water suspensions were created by transferring strains from a broth culture into water both individually and in mixed-strain pairs. Strains were tested in pairs (*C. jejuni* 2MT8-3 TSKQ vs. *C. coli* 2WT0-4 TSEKQ and *C. jejuni* 2MT8-3 TSKQ vs. *C. coli* 2MT6-4 TKQ) for each of seven independent trials.

Each strain (*C. jejuni* 2MT8-3 TSKQ, *C. coli* 2WT0-4 TSEKQ, and *C. coli* 2MT6-4 TKQ) was able to survive equally up to 8 days in individual suspensions. In mixed-strain culture, TSKQ *C. jejuni* outcompeted TSEKQ *C. coli* on and after Day 2. In mixed-strain culture, TSKQ *C. jejuni* outcompeted TSEKQ *C. coli* on and after Day 2. Further research is needed in order to attribute differential survival of *Campylobacter* to specific antibiotic resistance genes.

3.1 INTRODUCTION

According to the most recent NARMS report, both *Campylobacter jejuni* and *C. coli* are increasingly resistant to several types of antibiotics, and each species displays a different resistance pattern *(2, 3)*. Resistance to macrolides (e.g. erythromycin) and fluoroquinolones (e.g. ciprofloxacin) are of particular concern, as these are the drugs most commonly used to treat campylobacteriosis *(5)*. Resistance to antibiotics does not seem to impact *Campylobacter's* ability to colonize a host, but there is evidence that antibiotic resistance contributes to the organism's overall fitness even in the absence of antibiotics *(1, 4, 6, 9, 10)*.

Erythromycin-resistance seems to impose a fitness cost on *C. jejuni cells (1, 4)*. In a recent study erythromycin-resistant mutants of *C. jejuni* showed reductions in fitness compared to their erythromycin-susceptible parental strains *(1)*. These included reduced adhesion and invasion characteristics to intestinal epithelial cells, shorter intracellular survivability within macrophages, and lower colonization upon co-inoculation into mice and chickens compared to the susceptible strains *(1, 9)*. A laboratory competition experiment showed that the erythromycin-resistant strain grew significantly slower than the susceptible parent strain, which suggests that the mutation responsible for erythromycin resistance imposes a fitness cost to the mutant*(1)*. Similar results have been noted in other laboratory competition studies of *C. jejuni*, but no differences have been noted in *C. coli (4, 9)*.

Although erythromycin resistance seems to impose a fitness cost on *C. jejuni*, resistant strains have been shown to be equally tolerant of chilling treatments compared to their susceptible parent strains *(4)*. This suggests that although they have a reduced ability to compete with erythromycin-susceptible strains *in vivo*, erythromycin-resistant strains of *C. jejuni* are equally capable of persisting in poultry products and impacting public health. However, in a study of strains on chicken skin, erythromycin-resistant *C. jejuni* mutants survived for a shorter time than their susceptible parental strains (3-5 days vs. over 18 days, respectively) *(9)*. This type of conflicting information demonstrates the need for further research into the fitness of macrolide-resistant *Campylobacter* strains, including *C. coli*, which have yet to be studied.

Information regarding fitness is similarly conflicting for fluoroquinolone-resistant *Campylobacter*. In one study of fluoroquinolone-susceptible *C. jejuni* and its naturally transformed resistant mutant, fluoroquinolone-resistant *Campylobacter* outcompeted its susceptible parent strain when co-inoculated into a chicken in the absence of antibiotic selection pressure *(6)*. Using the same type of co-inoculation study in chickens, Zeitouni and Kempf found the opposite to be true: fluoroquinolone-susceptible *Campylobacter* outcompeted the resistant strain *(10)*. As observed with macrolide-resistance, fluoroquinolone-resistant *Campylobacter* was found to survive for a shorter time on chicken skin than the susceptible strain (3-5 days vs. over 13 days, respectively) *(10)*.

Current literature does not definitively state whether resistance to antibiotics such as macrolides and fluoroquinolones leads to enhanced fitness or imposes a fitness cost in comparison to susceptible strains of *Campylobacter*. No studies have looked at the fitness implications of aminoglycoside resistance in *Campylobacter.* Additional research is needed in order to explore this, as fitness is directly relevant to the persistence and spread of *Campylobacter* both pre-harvest and at retail. The purpose of the present study was to determine the relative fitness in sterile deionized water at 4°C of *Campylobacter* isolates which had different antibiotic resistance profiles and also differed in their relative ability to survive in feces or water over prolonged periods of time.

3.2 MATERIALS AND METHODS

Three strains were selected for competition trials: long-surviving *C. jejuni* 2MT8-3 (TSKQ), isolated from a fecal composite sample stored at 4°C for 8 days; long-surviving *C. coli* 2MT6-4 (TKQ), from the same fecal composite sample stored at 4°C for 6 days; and short-surviving *C. coli* 2WT0-4 (TSEKQ), isolated on day 0 from a water suspension of the same fecal composite sample and (Table 3.1). For this study, long-surviving strains were those that were isolated from feces or a suspension of feces in water on or after Day 4 while short-surviving strains were those that were not isolated beyond Day 4. Seven independent trials were run, examining the survival differences between *C. coli* 2WT0-4 TSEKQ and *C. jejuni* 2MT8-3 TSKQ (Trials 1, 2, and 6) or *C. jejuni* 2MT8-3 TSKQ and *C. coli* 2MT6-4 TKQ (Trials 3, 4, 5, and 7).

Actively growing cells were cultured on MHA plates then transferred to MHB and grown in 24 hour broth culture. These broth cultures were used as inoculum to prepare water suspensions using sterile dI water as a diluent.

Water suspensions were created by mixing 1 mL of inoculum into 9 mL of sterile dI water. For each trial, three suspensions were made: one each of individual strains in dI water, and one 50/50 mix of the two strains in dI water (i.e., 0.5 mL of each of the two strains being studied).

Water suspensions (two with a single culture each, and one with the mixture) were enumerated by plating dilutions on MHA on Day 0, and every 4 days (Trial 1) or every 2 days (Trials 2-7) thereafter until *Campylobacter* was near or below the detection limit. During enumeration, prevalence of *C. jejuni* relative to *C. coli* in mixed-strain suspensions was determined by colony morphology. On MHA, *C. jejuni* colonies appear glossy and translucent, whereas *C. coli* colonies have a characteristic yellow appearance. In rare cases where colony morphology was ambiguous, multiplex PCR was used in order to definitively determine species. On Day 0, 24 hour broth cultures of each of the two individual strains were also diluted and plated for inoculum enumeration. The detection limit for all competition trials is 1 x 10^1 CFU/mL.

Table 3: Definition of *Campylobacter* isolates used in each of Trials 1-7.

Isolate Name	Species	Antibiotic Profile	Isolation Source	Survival (days)*
2WT0-4	*C. coli*	TSEKQ	Water	≤ 4
2MT8-3	*C. jejuni*	TSKQ	Feces	≥ 4
2MT6-4	*C. coli*	TKQ	Feces	≥ 4

* The survival times are defined by survival trends observed in the fecal composite or water suspension, as discussed in the previous chapter.

3.3 RESULTS

3.3.1 Trial 1: TSEKQ vs. TSKQ

This was the first of the competition trials, examining the survival of *C. coli* 2WT0-4 TSEKQ compared to *C. jejuni* 2MT8-3 TSKQ. In our study of survival in a fecal composite sample and a suspension of the feces in water, *C. coli* 2WT0-4 TSEKQ proved to be a short survivor (≤ 4 Days), and TSKQ a long survivor (≥ 4 Days). Because we expected longer survival in water at 4°C, we enumerated every 4 days. As shown in Fig. 3.1, *Campylobacter* was present in all three suspensions at a similar level on Day 0. Although cell counts in the mixed suspension were one log CFU/Ml higher than those for the individual strains at Day 4, *Campylobacter* was undetectable in all three suspensions by Day 8, which was the third enumeration point. In this first trial, broth culture inocula of *C. coli* 2WT0-4 TSEKQ and *C. jejuni* 2MT8-3 TSKQ were not enumerated separately from strain suspensions on Day 0 due to an oversight.

Fig. 3.2 shows the prevalence of each strain at each enumeration point. At both Days 0 and 4, *C. jejuni* was the predominant strain in the 50/50 mixed-strain culture.

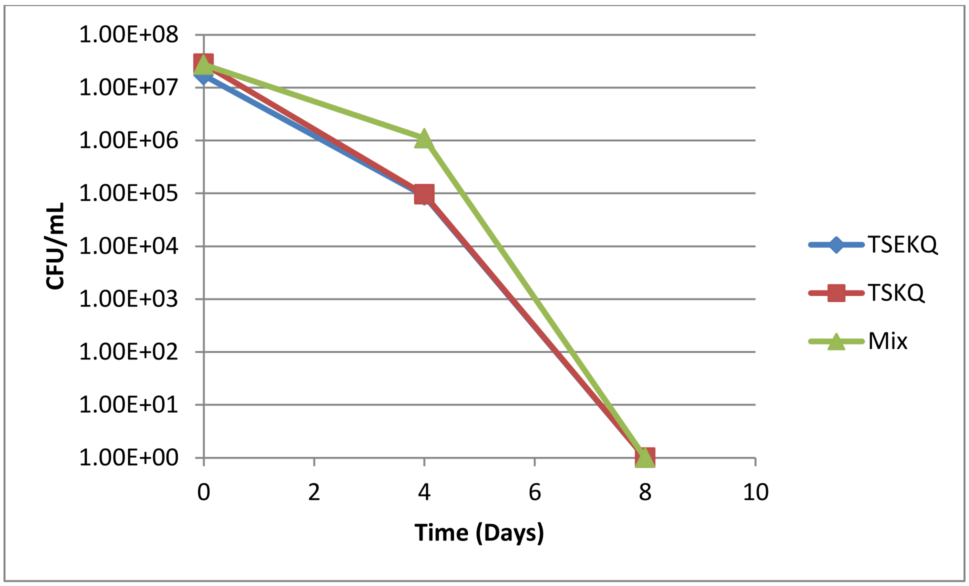

Fig. 3.1: Death curves for each of the three water suspensions in Trial 1: *C. jejuni* 2MT8-3 TSKQ, *C. coli* 2WT0-4 TSEKQ, and mixed-strain.

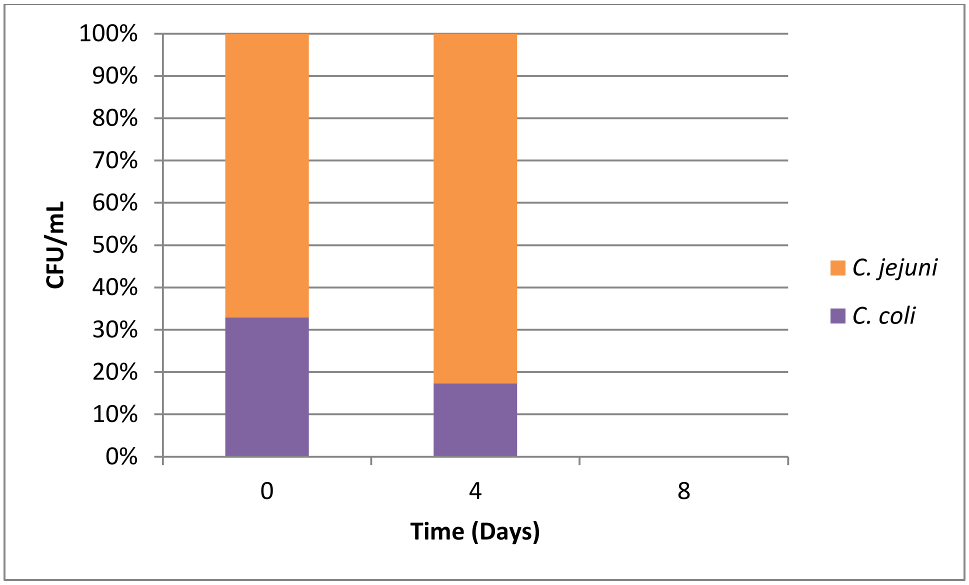

Fig. 3.2: Percent of *C. jejuni* 2MT8-3 TSKQ relative to *C. coli* 2WT0-4 TSEKQ present in the mixed strain suspension at each point of enumeration during Trial 1.

3.3.2 Trial 2: TSEKQ vs. TSKQ

This is a replicate of Trial 1, utilizing the same strains. For this trial, enumeration was done every 2 days in order to better capture *Campylobacter* population decline. Fig. 3.3 shows the decline in CFUs in each of the three water suspensions. On Day 0, inoculum amounts for each culture were 4.7 x 10^7 CFU/mL (*C. coli* 2WT0-4 TSEKQ) and 5.1 x 10^6 CFU/Ml (*C. jejuni* 2MT8-3 TSKQ). By Day 4, *C. jejuni* 2MT8-3 TSKQ was present in lower numbers than *C. coli* 2WT0-4 TSEKQ, although *C. jejuni* survived longer in the mixed-strain suspension than *C. coli* (see Fig. 3.4).

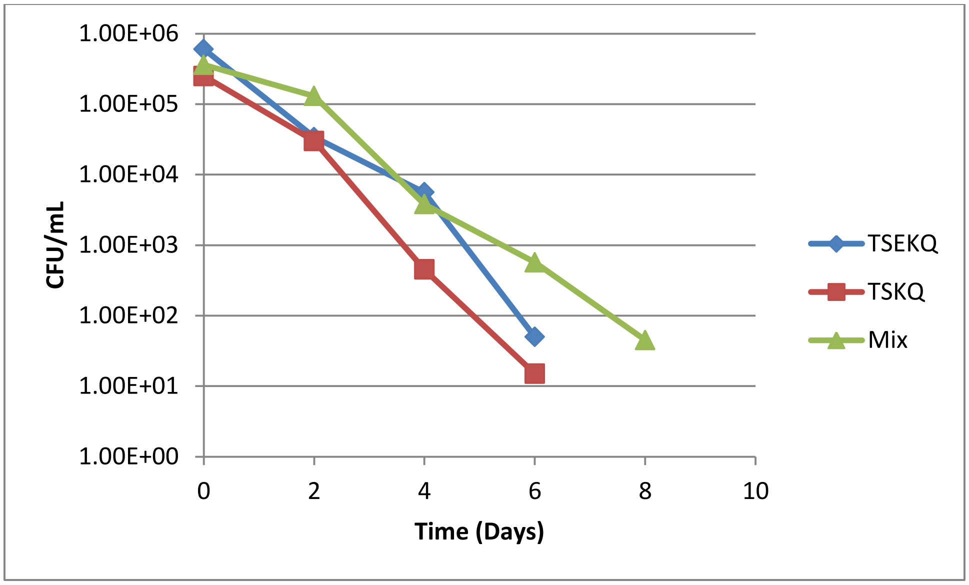

Fig. 3.3: Death curves for each of the three water suspensions in Trial 2: *C. jejuni* 2MT8-3 TSKQ, *C. coli* 2WT0-4 TSEKQ, and mixed-strain.

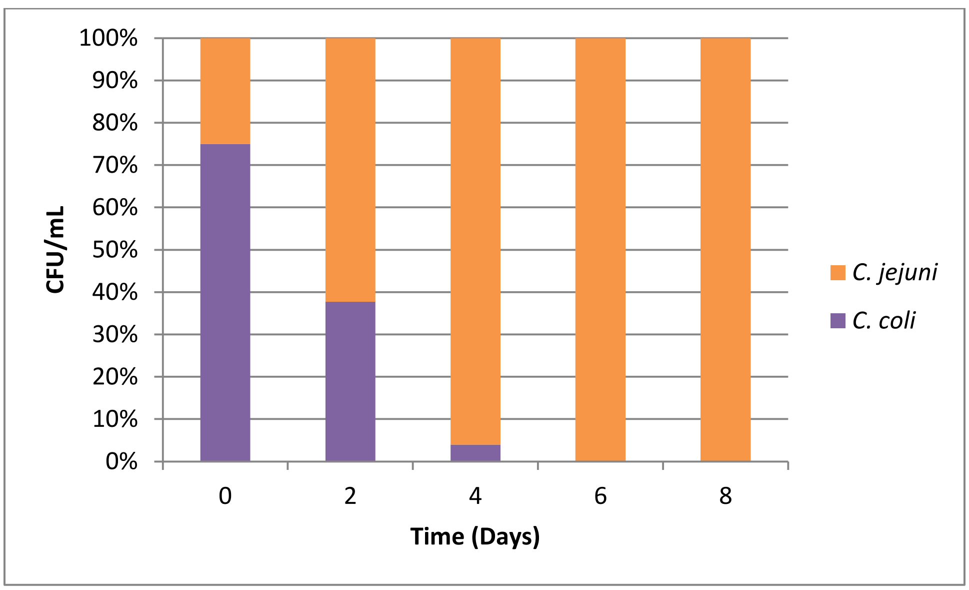

Fig. 3.4: Percent of *C. jejuni* 2MT8-3 TSKQ relative to *C. coli* 2WT0-4 TSEKQ present in the mixed strain suspension at each point of enumeration during Trial 2.

3.3.3 Trial 6: TSEKQ vs. TSKQ

This is a replicate of Trial 2, utilizing the same strains. As in Trial 2, enumeration was done every 2 days in order to capture *Campylobacter* decline over time. Fig. 3.5 shows the decline of *Campylobacter* in all three water suspensions. On Day 0, inoculum levels were 8.4 x 10^8 CFU/mL (*C. coli* 2WT0-4 TSEKQ) and 1.8 x 10^8 CFU/mL (*C. jejuni* 2MT8-3 TSKQ). Again, the short-surviving *C. coli* (2WT0-4 TSEKQ) was outlived by the long-surviving *C. jejuni* (2MT8-3 TSKQ). *C. jejuni* 2MT8-3 TSKQ survived in water approximately 4 days longer than *C. coli* 2WT0-4 TSEKQ (Fig. 3.5).

By Day 4, *C. coli* 2WT0-4 TSEKQ had declined nearly below detection both in the individual strain water suspension and in the mixed-strain water suspension. On Day 8, *C. jejuni* 2MT8-3 TSKQ was the only detectable strain in the mixed-strain cell suspension (see Fig. 3.6).

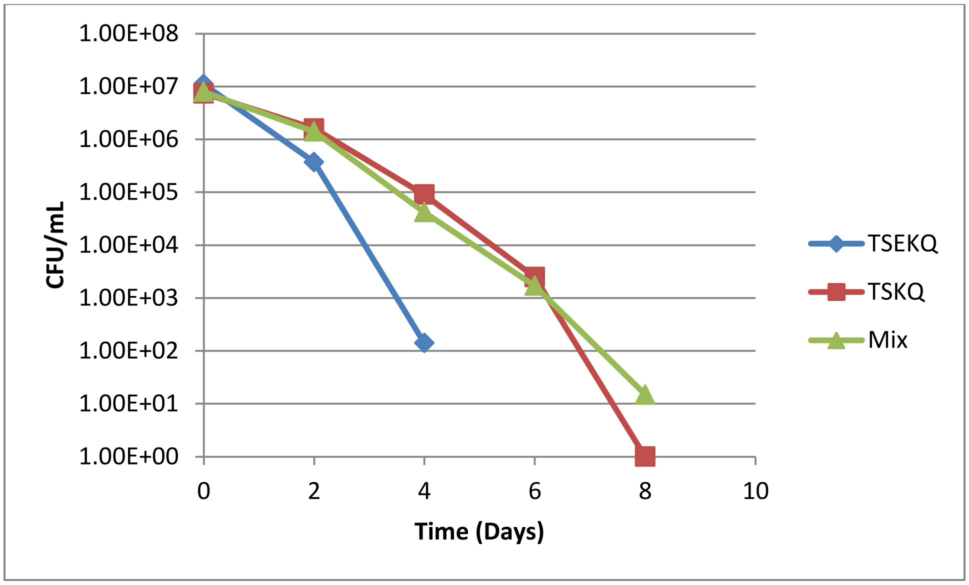

Fig. 3.5: Death curves for each of the three water suspensions in Trial 6: *C. jejuni* 2MT8-3 TSKQ, *C. coli* 2WT0-4 TSEKQ, and mixed-strain.

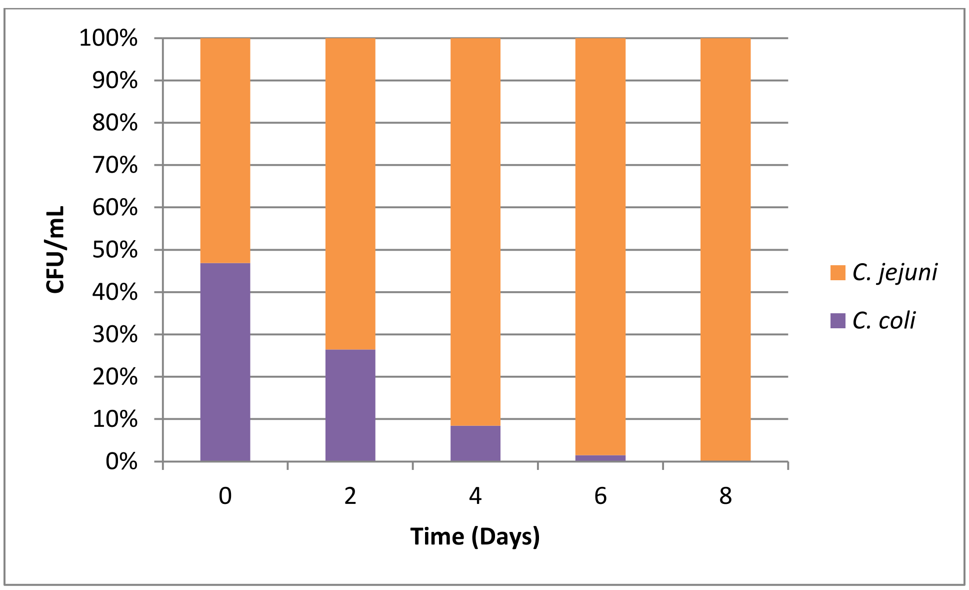

Fig. 3.6: Percent of *C. jejuni* 2MT8-3 TSKQ relative to *C. coli* 2WT0-4 TSEKQ present in the mixed-strain water suspension at each point of enumeration during Trial 6.

3.3.4 Trial 3: TSKQ vs. TKQ

This trial was to determine the differential survival of a long-surviving *C. jejuni* (2MT8-3 TSKQ) (≥ 4 days; the same strain used for Trials 1 and 2) vs. a long-surviving *C. coli* (2MT6-4 TKQ) (≥ 4 days).

C. coli 2MT6-4 TKQ inoculum did not grow to expected levels. On Day 0, *C. coli* 2MT6-4 TKQ inoculum showed *Campylobacter* cells present at only 1 x 10^5 CFU/mL, significantly lower than *C. jejuni* 2MT8-3 TSKQ inoculum, which was 3.4 x 10^8 CFU/mL (see Fig. 3.7). Due to this drastic difference, *C. coli* 2MT6-4 TKQ was not detectable in the mixed-strain water suspension, and only the *C. jejuni* 2MT8-3 TSKQ water suspension was enumerated beyond Day 2. *C. jejuni* 2MT8-3 TSKQ was undetectable at Day 6.

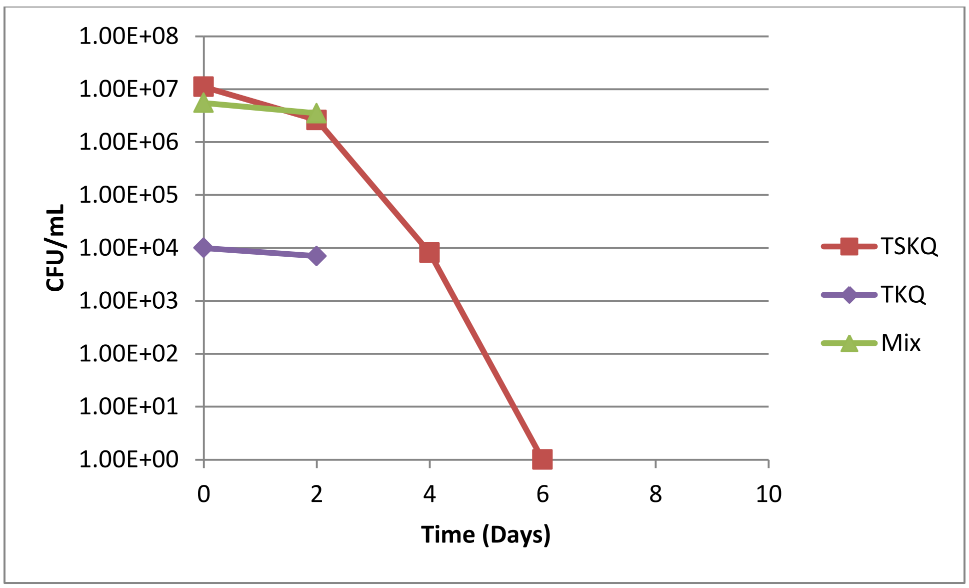

Fig. 3.7: Death curves for each of the three water suspensions in Trial 3: *C. jejuni* 2MT8-3 TSKQ, *C. coli* 2MT6-4 TKQ, and mixed-strain.

3.3.5 Trial 4: TSKQ vs. TKQ

This is a replicate of Trial 3, using the same strains. As shown in Fig. 3.8, we did not have the same issue with *C. coli* 2MT6-4 TKQ growth that we had in Trial 3. This time, *C. coli* 2MT6-4 TKQ inoculum was approximately 1 log CFU/mL higher than *C. jejuni* 2MT8-3 TSKQ inoculum (5.2 x 10^8 and 4.2 x 10^7 CFU/mL, respectively). At Day 0, *C. coli* 2MT6-4 TKQ was also present in the water suspension at approximately 1 log CFU/mL higher than *C. jejuni* 2MT8-3 TSKQ.

In this trial, *C. jejuni* 2MT8-3 TSKQ declined more rapidly in the water suspension than *C. coli* 2MT6-4 TKQ, although *C. coli* 2MT6-4 TKQ was the only strain present in the mixed-strain water suspension by Day 6, the final point of enumeration (see Fig. 3.9).

Trial 4 showed several anomalies throughout the course of enumeration. On Day 0, colonies from inoculum plates were normal, but colonies from all three water suspensions were tiny pinpoint colonies that were barely visible. On Day 0, the ratio of *C. jejuni* to *C. coli* was determined by subculturing 38 colonies from a mix plate onto MHA in order to view their morphology. On MHA, *C. jejuni* and *C. coli* can easily be distinguished based on color: *C. jejuni* colonies have a glossy translucent appearance; *C. coli* cultures appear more darkly yellow. The ratio of species determined from that subculture was applied to the total mixed-strain count. As a quality assurance measure, species were verified using multiplex PCR for 2 of these subcultures based on morphology (1 presumed *C. jejuni* and 1 presumed *C. coli*) to ensure that these species could be distinguished visually on MHA. In both cases, PCR results confirmed the presumptive species results.

At Day 4, both *C. coli* 2MT6-4 TKQ and the mixed-strain water suspensions produced more than one dilution with plates that had similar numbers of colonies (*C. coli* 2MT6-4 TKQ range was $1.1 \times 10^4 - 5.5 \times 10^4$ CFU/mL; mixed-strain range was $6.2 \times 10^2 - 3.9 \times 10^3$ CFU/mL). Because there is no way to definitively determine the correct CFU/mL in this scenario, Day 4 was omitted for both the *C. coli* 2MT6-4 TKQ and mixed-strain suspensions in Fig. 3.8.

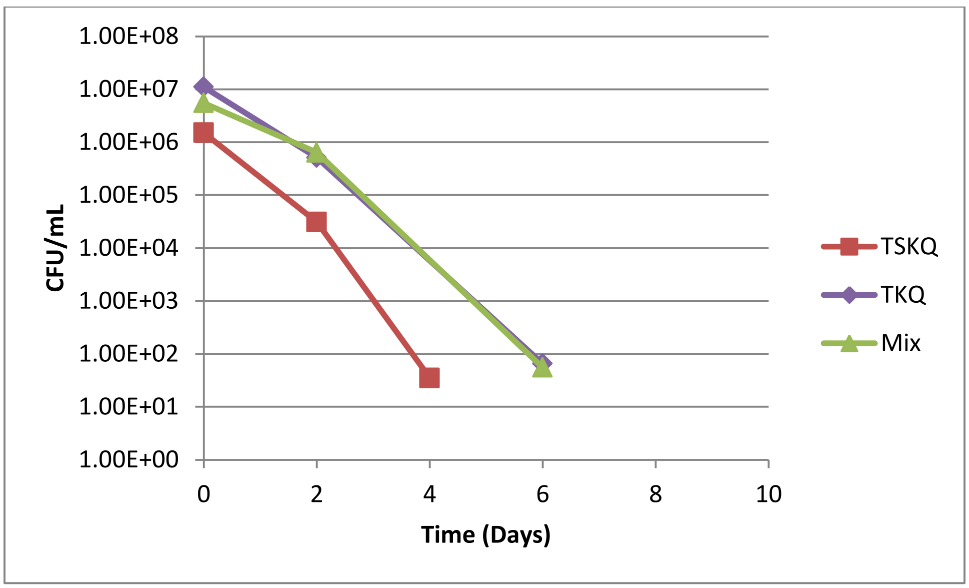

Fig. 3.8: Death curves for each of the three water suspensions in Trial 4: *C. jejuni* 2MT8-3 TSKQ, *C. coli* 2MT6-4 TKQ, and mixed-strain. Note that on Day 4, both *C. coli* 2MT6-4 TKQ and mixed-strain water suspension contained more than one dilution with similar colony counts. This time point has been omitted for those two suspensions, as it is not possible to determine the most correct enumeration.

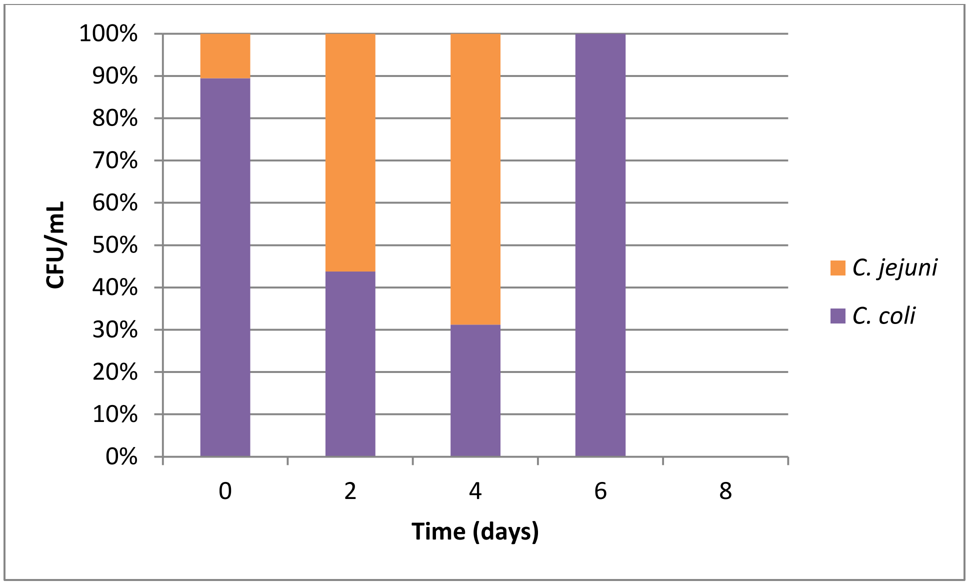

Fig. 3.9: Percent of *C. jejuni* 2MT8-3 TSKQ relative to *C. coli* 2MT6-4 TKQ present in the mixed-strain water suspension at each point of enumeration during Trial 4.

3.3.6 Trial 5: TSKQ vs. TKQ

This is a replicate of Trials 3 and 4, utilizing the same strains. In this replicate, both *C. jejuni* 2MT8-3 TSKQ and *C. coli* 2MT6-4 TKQ inoculum grow to the same level, as shown in Fig. 3.10. Inocula levels were 8.7 x 10^7 CFU/mL (*C. jejuni* 2MT8-3 TSKQ) and 5.9 x 10^7 CFU/mL (*C. coli* 2MT6-4 TKQ). All three water suspensions showed similar cell counts at Day 0. *C. jejuni* 2MT8-3 TSKQ was present in the water suspension at higher levels than *C. coli* 2MT6-4 TKQ on Days 4 and 6, but *C. coli* 2MT6-4 TKQ levels were higher on Day 8.

C. coli 2MT6-4 TKQ and the mixed-strain suspension showed similar rates of decline throughout the trial. As we saw in Study 4, *C. coli* 2MT6-4 TKQ was the only strain present in the mixed-strain water suspension on the final Day of enumeration, Day 8 (see Fig. 3.11).

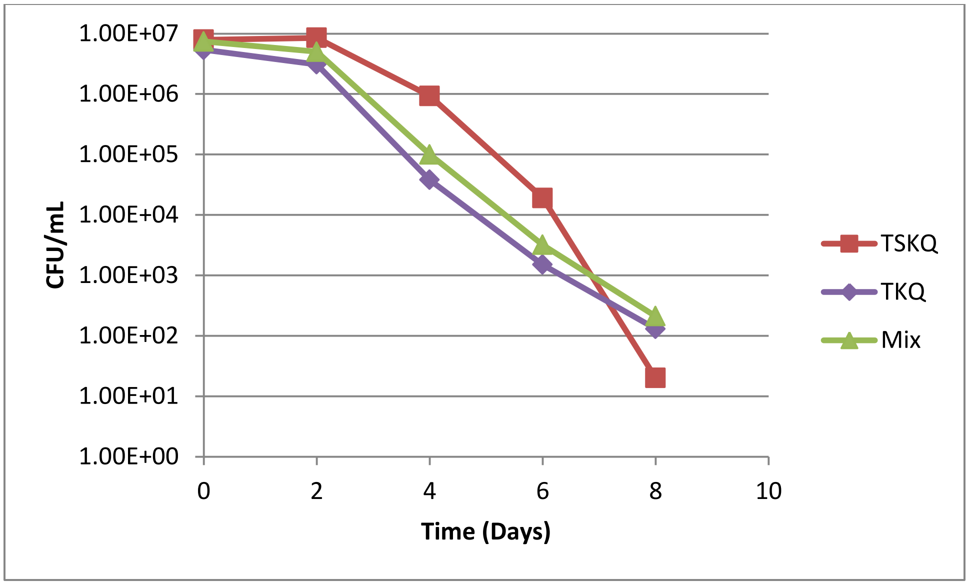

Fig. 3.10: Death curves for each of the three water suspensions in Trial 5: *C. jejuni* 2MT8-3 TSKQ, *C. coli* 2MT6-4 TKQ, and mixed-strain.

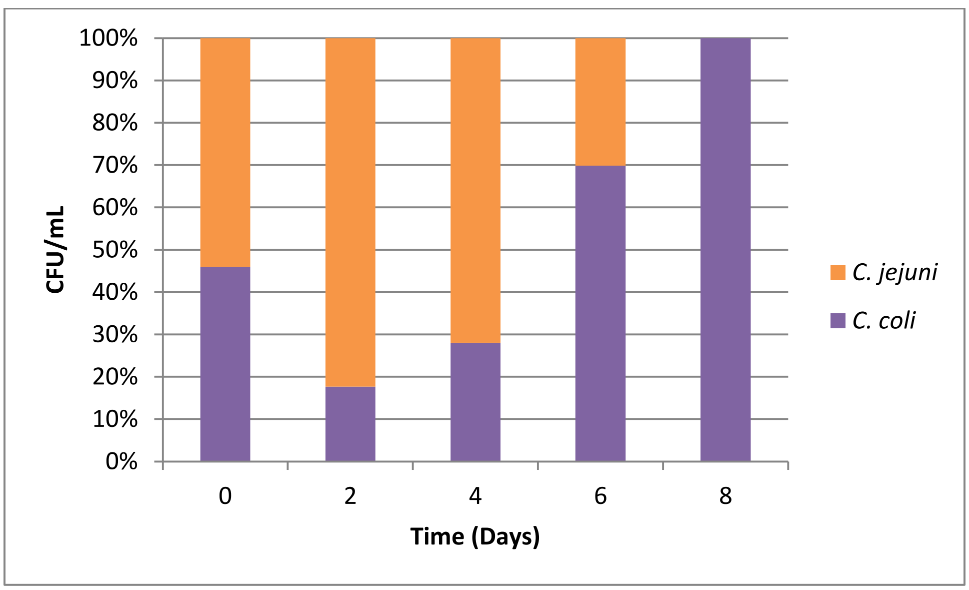

Fig. 3.11: Percent of *C. jejuni* 2MT8-3 TSKQ relative to *C. coli* 2MT6-4 TKQ present in the mixed-strain water suspension at each point of enumeration during Trial 5.

3.3.7 Study 7: TSKQ vs. TKQ

This is a replicate of Trials 3, 4, and 5, utilizing the same strains. Inocula levels in this trial were 1.8 x 10^8 CFU/mL (*C. jejuni* 2MT8-3 TSKQ) and 6.9 x 10^8 CFU/mL (*C. coli* 2MT6-4 TKQ). As in Trials 4 and 5, *C. jejuni* 2MT8-3 TSKQ and *C. coli* 2MT6-4 TKQ were detectable in their individual water suspensions for the same length of time (8 days) (see Fig. 3.12). Unlike Trials 4 and 5, where only *C. coli* 2MT6-4 TKQ was detectable in the mixed-strain culture at Day 8, *C. jejuni* 2MT8-3 TSKQ and *C. coli* 2MT6-4 TKQ were approximately equally represented on Day 8 (see Fig. 3.13).

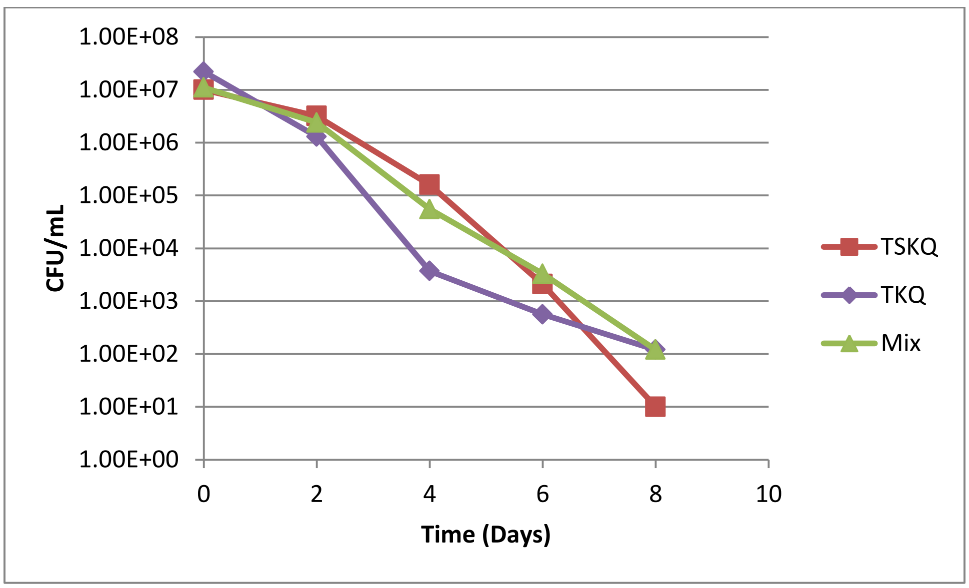

Fig. 3.12: Death curves for each of the three water suspensions in Trial 7: *C. jejuni* 2MT8-3 TSKQ, *C. coli* 2MT6-4 TKQ, and mixed-strain.

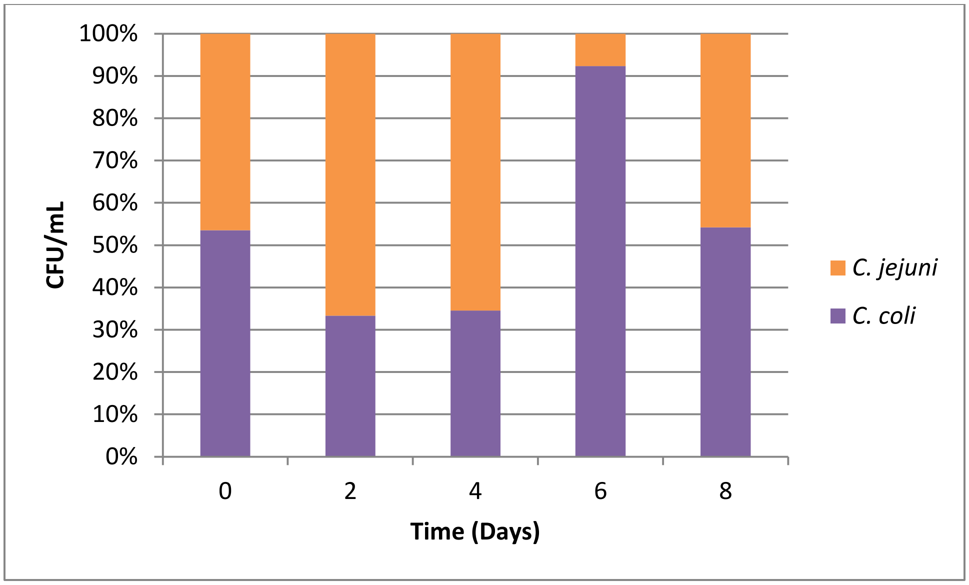

Fig. 3.13: Percent of *C. jejuni* 2MT8-3 TSKQ relative to *C. coli* 2MT6-4 TKQ present in the mixed-strain water suspension at each point of enumeration during Trial 7.

3.4 DISCUSSION

To our knowledge, this is the first study to examine competitive survival of *Campylobacter* isolates, as previous work has focused on competitive growth *(1, 4, 9, 10)* In Trials 1 and 2, long-surviving *C. jejuni* isolate (2MT8-3 TSKQ) and short-surviving *C. coli* isolate (2WT0-4 TSEKQ) persisted for similar lengths of time within their individual water suspensions. However, on Days 4 and beyond, *C. jejuni* accounted for the majority of isolates recovered from the water suspension. In Study 2, *C. jejuni* counts were approximately 1 log CFU/mL lower than *C. coli* counts on Day 4, yet *C. jejuni* accounted for over 95% of the isolates recovered from the mixed-strain water suspension. These findings could indicate that erythromycin indeed imposes a fitness cost that makes it less competitive than an organism that is susceptible to erythromycin, as has been suggested in the literature

(1, 4, 9). Before this conclusion can be definitively made, it must be noted that previous studies of the fitness effects of erythromycin-resistance have focused on isogenic mutants, whereas our study examined naturally occurring unrelated isolates of different species. There could be significant differences in the fitness trends between these two types of isolates. For this reason, it is difficult to attribute these differences solely to erythromycin resistance. Further research will need to be conducted on the genetic mutation responsible for erythromycin resistance, and how it affects fitness.

The effects of streptomycin resistance on the fitness of *Campylobacter* have not been documented in the literature. In Trials 4, 5, and 7, we examined the competitive survival of a long-surviving *C. jejuni* (2MT8-3 TSKQ) and a long-surviving *C. coli* (2MT6-4 TKQ). In these trials, *C. coli* consistently outcompeted *C. jejuni* in the mixed-strain suspension at later time points. On the final days of Trials 4 and 5 (Day 6 and Day 8, respectively), *C. coli* represented 100% of isolates in the mixed-strain suspension. In Trials 5 and 7, Day 6 is particularly interesting, as *C. jejuni* is present at approximately 1 log CFU/mL higher in its individual suspension compared to *C. coli*, yet *C. coli* accounts for the majority of colonies formed from the mixed-strain suspension (70% in Study 5 and over 90% in Study 7). This could indicate that resistance to streptomycin imposes a fitness cost compared to susceptibility. As noted above, this study examined naturally occurring unrelated isolates of different species rather than isogenic mutants, therefore survival differences cannot be attributed solely to streptomycin resistance. Additional research is needed in order to conclude whether streptomycin resistance plays a role in *Campylobacter* fitness for survival.

Of particular interest is the fact that on Day 0 of Study 4, all colonies from each of the three water suspensions grew as pinpoint colonies that were not distinguishable by color to the naked eye. This was likely due to cell injury. Specifically, it is likely that *Campylobacter* cells were injured when transferred from broth culture into sterile water on Day 0, which is why colonies did not grow to be very large. By Day 2, colonies from Study 4 resumed growing to a normal size, indicating that injured cells had either died or recovered.

In this study of competitive survival between strains, the survival of strains *C. jejuni* 2MT8-3 TSKQ, *C. coli* 2MT6-4 TKQ, and *C. coli* 2WT0-4 TSEKQ in sterile water at 4°C differed slightly compared to the survival we noted in the previous chapter. For example, *C. jejuni* 2MT8-3 TSKQ was only recovered through Day 8 in this study. In feces, TSKQ *C. jejuni* strains were isolated up through Day 16, as noted in the previous chapter. Survival in water was the same for both this competition study and the previous survival study (8 days). This is not surprising, as *C. jejuni* was a better survivor in feces compared to water ($p = 0.0005$).

Both *C. coli* 2MT6-4 TKQ and *C. coli* 2WT0-4 TSEKQ also survived in sterile water at 4°C for a maximum of 8 days in this study. In the previous study of survival, TKQ *C. coli* strains were recovered longer in both feces and drinker water (12 days vs. 14 days, respectively). However, compared to survival in sterile water at 4°C, TSEKQ *C. coli* was recovered for less time in feces (2 days) and more time in drinker water (10 days).

Reasons for the differences in *C. jejuni* TSKQ and *C. coli* TKQ survival between this study and the survival study presented in the previous chapter are unclear. One distinct difference between the two studies is the presence of organic material in the culture.

Campylobacter in the present study was cultured from freezer stock and transferred into sterile water. In the previous study of survival, fresh fecal samples were made into a composite and a drinker water suspension, and *Campylobacter* extracted from the samples. The fecal material present in both the composite and drinker water suspension provided *Campylobacter* cells with nutrients that likely prolonged their survival compared to that noted in the present study. Additionally, protozoa were likely present in the drinker water used in the previous study of survival, whereas the present study used sterile water which would not have harbored protozoa. As previously noted, waterborne protozoa can have a protective effect on *Campylobacter* survival *(7, 8)*.

C. coli 2WT0-4 TSEKQ was isolated up to 8 days in the present study, but was not isolated in feces beyond Day 2 of the previous survival study. This could indicate that TSEKQ *C. coli* is a poor competitor compared to other *Campylobacter* strains. Specifically, when present in a fecal composite with several other *Campylobacter* cells, TSEKQ *C. coli* was not isolated beyond Day 2. However, when purified and transferred into sterile dI water stored at 4°C, TSEKQ *C. coli* could be isolated up to 8 days. This competitive disadvantage is supported by the mixed-strain cultures examined in the present study, where *C. coli* 2WT0-4 TSEKQ was consistently outcompeted by *C. jejuni* 2MT8-3 TSKQ on and after Day 2 (Figs. 3.2, 3.4, and 3.6).

The fitness of different strains of *Campylobacter* should be studied more in depth, as fitness for survival is of utmost importance to pre-harvest transmission of this important foodborne pathogen. Future research should focus on the fitness impact of antibiotic resistance including all antibiotics commonly used in turkey farming and treatment of human Campylobacteriosis.

3.5 REFERENCES

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CHAPTER 4: Implications and Conclusions

4.1 Implications of the Present Work

One predominant theme that arose from the present studies was the correlation between *Campylobacter* spp. antibiotic resistance profiles and survival. In the survival studies described in Chapter 2, *C. jejuni* with resistance profile TSKQ and *C. coli* with profile TKQ were among the longest surviving isolates in feces and a drinker water suspension ($p = 0.0077$ and $p = 0.00000011$, respectively). When select isolates were combined in a sterile water suspension for the competition studies as described in Chapter 3, TKQ *C. coli* consistently outcompeted TSKQ *C. jejuni* at late time points.

The survival study described in Chapter 2 identifies *C. coli* with the antibiotic resistance profile TSEKQ as a short survivor, as it comprised approximately 20% of *C. coli* isolates on Days 0 and 2, but was rarely isolated on Day 4 and beyond. When combined with long-surviving TSKQ *C. jejuni* in sterile water for competition studies (Chapter 3), TSEKQ *C. coli* was consistently outcompeted in late time points.

In both of the competition study cases described above, the *Campylobacter* isolate with more antibiotic resistance proved to be less fit for competition compared to a *Campylobacter* isolate with less resistance (*i.e.*, TKQ *C. coli* outcompeted TSKQ *C. jejuni* and TSKQ *C. jejuni* outcompeted TSEKQ *C. coli* in mixed-strain sterile water suspensions). This could indicate that the genetic mutations responsible for antibiotic resistance in *Campylobacter* impose a fitness cost to the cell, implying that as a *Campylobacter* cell acquires more resistance, its fitness to survive diminishes in comparison to *Campylobacter* cells with less resistance.

In order to efficiently distinguish the percent of each isolate present in the competition study mixed-strain sterile water suspensions, we compared a *C. jejuni* isolate to a *C. coli* isolate in each of the trials. The two species are easily distinguished visually on MHA based on their morphology, as *C. jejuni* appears glossy and translucent while *C. coli* appears yellowish. As competition studies were conducted with different species rather than isogenic mutants, the present findings alone cannot be used to support the hypothesis that genetic mutations responsible for antibiotic resistance impose a fitness cost to *Campylobacter* cells.

4.2 Conclusions and Future Research

Both the survival and competition studies presented here indicate a correlation between antibiotic resistance and the survival and fitness of *Campylobacter*. The relationship between resistance and survival and fitness of *Campylobacter* cells should be explored more in-depth in order to determine the presence or absence of a causal relationship. Future research on competitive survival in *Campylobacter* should focus on the specific gene mutations responsible for antibiotic resistance and whether they impose a fitness cost or a fitness benefit. Understanding the mechanisms of *Campylobacter* survival and fitness is essential to controlling its persistence and transmission during turkey production.

APPENDIX

Appendix: *Campylobacter* Isolates

Table A: Comprehensive list of *Campylobacter* isolates characterized in this study.

Sample #	Isolate	Species	Antibiotic Resistance
1	WT0-2	jejuni	TSKQ
1	WT0-3	jejuni	TSKQ
1	WT0-4	coli	TKQ
1	WT0-5	jejuni	TSKQ
1	WT0-7	coli	TKQ
1	WT0-8	coli	TKQ
1	WT0-10	coli	TKQ
1	MT0-1	coli	TKQ
1	MT0-2	jejuni	TSKQ
1	MT0-3	jejuni	TSKQ
1	MT0-4	coli	TKQ
1	MT0-5	jejuni	TSKQ
1	MT0-6	jejuni	TSKQ
1	MT0-8	coli	TKQ
1	MT0-10	coli	TKQ
1	WT2-1	jejuni	TSKQ
1	WT2-2	jejuni	TKQ
1	WT2-3	coli	TKQ
1	WT2-4	jejuni	TKQ
1	WT2-5	jejuni	TKQ
1	WT2-6	jejuni	TKQ
1	WT2-7	coli	TK
1	WT2-8	coli	TKQ
1	WT2-9	coli	TK
1	WT2-10	jejuni	TKQ
1	MT2-1	jejuni	TSKQ
1	MT2-2	coli	TK
1	MT2-3	coli	TKQ
1	MT2-4	jejuni	TSKQ
1	MT2-5	coli	TKQ
1	MT2-6	coli	TKQ
1	MT2-7	coli	TKQ
1	MT2-9	jejuni	TSKQ
1	MT2-10	jejuni	TSKQ
1	WT4-2	coli	TKQ

Table A (continued)

1	WT4-3	coli	TKQ
1	WT4-4	jejuni	TSKQ
1	WT4-5	coli	TK
1	WT4-6	coli	TKQ
1	WT4-8	coli	TKQ
1	WT4-10	coli	TKQ
1	MT4-1	jejuni	TSKQ
1	MT4-2	jejuni	TSKQ
1	MT4-3	jejuni	TSKQ
1	MT4-4	jejuni	TSKQ
1	MT4-5	coli	TK
1	MT4-7	coli	TK
1	MT4-9	coli	TKQ
1	MT4-10	coli	TKQ
1	WT6-1	jejuni	TSKQ
1	WT6-2	coli	TKQ
1	WT6-3	jejuni	TKQ
1	WT6-4	jejuni	TSKQ
1	WT6-5	jejuni	TSKQ
1	WT6-6	jejuni	TSKQ
1	WT6-7	jejuni	TSKQ
1	WT6-9	coli	TK
1	WT6-10	jejuni	TKQ
1	MT6-1	jejuni	TSKQ
1	MT6-2	jejuni	TSQ
1	MT6-3	jejuni	TSQ
1	MT6-4	jejuni	TKQ
1	MT6-5	jejuni	TSKQ
1	MT6-6	coli	TKQ
1	MT6-7	coli	TKQ
1	MT6-8	jejuni	TKQ
1	MT6-9	jejuni	TSKQ
1	MT6-10	jejuni	TSKQ
1	WT8-4	coli	TK
1	WT8-5	coli	TKQ
1	WT8-7	coli	PS
1	WT8-8	coli	TKQ
1	MT8-1	coli	TKQ

Table A (continued)

1	MT8-2	coli	TK
1	MT8-3	coli	TQ
1	MT8-4	coli	TK
1	MT8-5	jejuni	TSKQ
1	MT8-6	coli	TKQ
1	MT8-7	coli	TKQ
1	MT8-8	coli	TK
1	MT8-9	jejuni	TSKQ
1	MT8-10	coli	TKQ
1	WT12-7	coli	TK
1	WT12-9	coli	TKQ
1	MT12-1	coli	TK
1	MT12-2	jejuni	TSKQ
1	MT12-4	coli	TKQ
1	MT12-5	jejuni	TSQ
1	MT12-6	coli	TK
1	MT12-7	jejuni	TSQ
1	MT12-8	jejuni	TSKQ
1	MT12-9	coli	TKQ
1	MT12-10	jejuni	TSKQ
1	MT14-1	coli	TK
1	MT14-2	jejuni	TSKQ
1	MT14-3	jejuni	TSKQ
1	MT14-4	jejuni	TSQ
1	MT14-5	jejuni	TSKQ
1	MT14-6	jejuni	TSKQ
1	MT14-7	jejuni	TSQ
1	MT14-8	jejuni	TSKQ
1	MT14-9	jejuni	TSKQ
1	MT14-10	jejuni	TSKQ
1	MT16-1	jejuni	TSKQ
1	MT16-2	jejuni	TSQ
1	MT16-3	jejuni	TSKQ
1	MT16-5	jejuni	TKQ
1	MT16-7	jejuni	TSKQ
1	MT16-8	jejuni	TSKQ
1	MT16-9	coli	TK
1	MT16-10	jejuni	TSKQ

Table A (continued)

2	2WT0-1	coli	TK
2	2WT0-2	coli	TQ
2	2WT0-3	coli	TQ
2	2WT0-4	coli	TSEKQ
2	2WT0-5	coli	TK
2	2WT0-6	coli	TSEKQ
2	2WT0-7	coli	TQ
2	2WT0-8	coli	TSEKQ
2	2WT0-9	coli	TK
2	2WT0-10	coli	TK
2	2WT0-11	coli	TSEKQ
2	2WT0-12	coli	TK
2	2WT0-13	jejuni	TKQ
2	2WT0-14	jejuni	TSKQ
2	2WT0-15	coli	TSEKQ
2	2WT0-16	coli	TSEKQ
2	2MT0-1	coli	TSEKQ
2	2MT0-2	jejuni	TKQ
2	2MT0-3	coli	TKQ
2	2MT0-4	coli	TSEKQ
2	2MT0-5	jejuni	TSKQ
2	2MT0-6	jejuni	TSKQ
2	2MT0-7	coli	TSEKQ
2	2MT0-8	jejuni	T
2	2MT0-9	coli	TKQ
2	2MT0-10	coli	TK
2	2MT0-11	coli	TSEKQ
2	2MT0-12	coli	TSEKQ
2	2MT0-13	coli	TQ
2	2MT0-14	coli	TK
2	2MT0-15	coli	TSEKQ
2	2MT0-16	coli	TSEKQ
2	2WT2-1	coli	TSEKQ
2	2WT2-2	coli	TK
2	2WT2-3	coli	TK
2	2WT2-4	coli	TK
2	2WT2-5	coli	TK
2	2WT2-6	coli	TSEKQ

Table A (continued)

2	2WT2-8	coli	TQ
2	2WT2-9	coli	TSEKQ
2	2WT2-10	coli	TK
2	2WT2-11	jejuni	T
2	2WT2-12	coli	TSEKQ
2	2WT2-13	coli	TK
2	2WT2-14	coli	TK
2	2WT2-15	coli	TSEKQ
2	2WT2-16	coli	TK
2	2MT2-3	coli	TKQ
2	2MT2-4	coli	TEKQ
2	2MT2-5	coli	TSEKQ
2	2MT2-6	coli	TSEKQ
2	2MT2-7	jejuni	T
2	2MT2-8	coli	TK
2	2MT2-11	coli	TK
2	2MT2-12	coli	TK
2	2MT2-13	coli	TSEKQ
2	2MT2-14	coli	TK
2	2MT2-15	coli	TK
2	2MT2-16	coli	TK
2	2WT4-1	coli	TQ
2	2WT4-2	coli	TKQ
2	2WT4-3	coli	TK
2	2WT4-4	coli	TK
2	2WT4-5	coli	TKQ
2	2WT4-6	coli	TKQ
2	2WT4-7	coli	TK
2	2WT4-8	coli	TKQ
2	2WT4-9	coli	TQ
2	2WT4-11	coli	TQ
2	2WT4-12	coli	TK
2	2WT4-13	coli	TK
2	2WT4-14	coli	TQ
2	2WT4-15	coli	TSEKQ
2	2WT4-16	coli	TQ
2	2MT4-1	jejuni	TKQ
2	2MT4-2	coli	TK

Table A (continued)

2	2MT4-3	coli	TQ
2	2MT4-4	coli	TQ
2	2MT4-5	coli	TK
2	2MT4-6	coli	TQ
2	2MT4-7	coli	TK
2	2MT4-8	jejuni	TKQ
2	2MT4-9	coli	TKQ
2	2MT4-10	coli	TQ
2	2MT4-11	coli	TK
2	2MT4-12	coli	TK
2	2MT4-13	coli	TKQ
2	2MT4-14	jejuni	TKQ
2	2MT4-15	coli	TK
2	2MT4-16	coli	TK
2	2WT6-1	coli	TEKQ
2	2WT6-3	coli	TSEKQ
2	2WT6-6	coli	TK
2	2MT6-1	coli	TK
2	2MT6-2	coli	TK
2	2MT6-3	jejuni	TSKQ
2	2MT6-4	coli	TKQ
2	2MT6-5	coli	TK
2	2MT6-6	coli	TK
2	2MT6-7	coli	TK
2	2MT6-8	coli	TK
2	2MT6-11	coli	TK
2	2MT6-12	jejuni	TK
2	2MT6-13	coli	TK
2	2MT6-14	coli	TQ
2	2MT6-15	coli	TK
2	2WT8-1	coli	TQ
2	2WT8-2	coli	TK
2	2WT8-3	coli	TK
2	2WT8-4	coli	TK
2	2WT8-5	coli	T
2	2WT8-6	coli	TK
2	2WT8-7	coli	TKQ
2	2WT8-8	coli	TQ

Table A (continued)

2	2WT8-9	coli	TEKQ
2	2WT8-10	coli	TK
2	2WT8-11	coli	TKQ
2	2WT8-12	coli	TK
2	2WT8-13	coli	TQ
2	2WT8-14	coli	TK
2	2WT8-15	coli	TKQ
2	2WT8-16	coli	TK
2	2MT8-1	jejuni	TK
2	2MT8-2	coli	TQ
2	2MT8-3	jejuni	TSKQ
2	2MT8-4	jejuni	TSKQ
2	2MT8-6	jejuni	TSKQ
2	2MT8-7	jejuni	TSKQ
2	2MT8-8	coli	TKQ
2	2MT8-9	coli	TK
2	2MT8-10	coli	TKQ
2	2MT8-11	coli	TK
2	2MT8-12	coli	TK
2	2MT8-13	jejuni	TSKQ
2	2MT8-14	coli	TK
2	2MT8-16	coli	TKQ
2	2WT10-1	coli	TQ
2	2WT10-2	coli	TQ
2	2WT10-5	coli	TQ
2	2WT10-7	coli	TK
2	2WT10-10	coli	TKQ
2	2WT10-12	coli	TKQ
2	2WT10-13	coli	TK
2	2WT10-15	coli	TKQ
2	2MT10-1	coli	TK
2	2MT10-2	coli	TKQ
2	2MT10-3	coli	TK
2	2MT10-5	coli	TK
2	2MT10-7	coli	TK
2	2MT10-8	coli	TK
2	2MT10-10	coli	TKQ
2	2MT10-11	coli	TKQ

Table A (continued)

2	2MT10-12	coli	TK
2	2MT10-13	coli	TK
2	2MT10-16	coli	TK
2	2WT12-1	coli	TKQ
2	2WT12-2	coli	TQ
2	2WT12-4	coli	TKQ
2	2WT12-5	coli	TK
2	2WT12-7	coli	TKQ
2	2WT12-8	coli	TKQ
2	2WT12-9	coli	TK
2	2WT12-10	coli	TKQ
2	2WT12-11	coli	TQ
2	2WT12-12	coli	TQ
2	2WT12-13	coli	TK
2	2WT12-15	coli	TKQ
2	2WT12-16	coli	TK
2	2WT14-1	coli	TQ
2	2WT14-2	coli	TQ
2	2WT14-3	coli	TK
2	2WT14-4	coli	TK
2	2WT14-5	coli	TQ
2	2WT14-6	coli	TK
2	2WT14-7	coli	TK
2	2WT14-8	coli	TQ
2	2WT14-9	coli	TK
2	2WT14-10	coli	TKQ
2	2WT14-11	coli	TQ
2	2WT14-12	coli	TQ
2	2WT14-13	coli	TK
2	2WT14-14	coli	TQ
2	2WT14-15	coli	TK
2	2WT14-16	coli	TK
2	2WT16-3	coli	TQ
2	2WT16-7	coli	TK
3	3WT0-1	coli	TK
3	3WT0-2	coli	TQ
3	3WT0-3	coli	TQ
3	3WT0-4	coli	TQ

Table A (continued)

3	3WT0-5	coli	TQ
3	3WT0-6	coli	TQ
3	3WT0-7	jejuni	TSKQ
3	3WT0-8	coli	TKQ
3	3WT0-9	coli	TK
3	3WT0-10	coli	TQ
3	3WT0-11	coli	TQ
3	3WT0-12	coli	TQ
3	3WT0-13	coli	TQ
3	3WT0-14	coli	TQ
3	3WT0-15	coli	TQ
3	3WT0-16	coli	TKQ
3	3MT0-1	coli	TQ
3	3MT0-3	coli	TK
3	3MT0-4	coli	TQ
3	3MT0-5	coli	TK
3	3MT0-6	coli	TQ
3	3MT0-7	coli	TK
3	3MT0-8	coli	TQ
3	3MT0-9	coli	TQ
3	3MT0-10	coli	TQ
3	3MT0-11	coli	TQ
3	3MT0-13	coli	TQ
3	3MT0-14	coli	TQ
3	3MT0-15	coli	TK
3	3MT0-16	coli	TKQ
3	3WT2-1	coli	TQ
3	3WT2-2	coli	TQ
3	3WT2-3	coli	TQ
3	3WT2-4	coli	T
3	3MT2-1	jejuni	TSKQ
3	3MT2-2	coli	TQ
3	3MT2-3	jejuni	TSKQ
3	3MT2-4	jejuni	TSKQ
3	3MT2-5	jejuni	TSKQ
3	3MT2-6	jejuni	TSKQ
3	3MT2-7	jejuni	TSKQ
3	3MT2-8	jejuni	TSKQ

Table A (continued)

3	3MT2-9	jejuni	TSKQ
3	3MT2-10	jejuni	TSKQ
3	3MT2-11	coli	TQ
3	3MT2-12	jejuni	TSKQ
3	3MT2-13	jejuni	TSKQ
3	3MT2-14	jejuni	TKQ
3	3MT2-15	jejuni	TSKQ
3	3MT2-16	jejuni	TSKQ
3	3MT4-1	coli	TKQ
4	4WT0-1	jejuni	TSKQ
4	4WT0-2	jejuni	TSKQ
4	4WT0-3	jejuni	TSKQ
4	4WT0-4	jejuni	TSKQ
4	4WT0-5	jejuni	TSKQ
4	4WT0-6	jejuni	TSKQ
4	4WT0-7	jejuni	TSKQ
4	4WT0-8	jejuni	TSKQ
4	4WT0-9	jejuni	TSKQ
4	4WT0-10	jejuni	TSKQ
4	4WT0-11	jejuni	TSKQ
4	4WT0-12	jejuni	TSKQ
4	4WT0-13	jejuni	TSKQ
4	4WT0-14	jejuni	TSKQ
4	4WT0-15	jejuni	TSKQ
4	4WT0-16	jejuni	TSKQ
4	4MT0-1	jejuni	TSKQ
4	4MT0-2	coli	TSEKQ
4	4MT0-3	jejuni	TSKQ
4	4MT0-4	jejuni	TSKQ
4	4MT0-5	coli	TSEKQ
4	4MT0-6	jejuni	TSKQ
4	4MT0-7	jejuni	TSKQ
4	4MT0-8	jejuni	TSKQ
4	4MT0-9	jejuni	TSKQ
4	4MT0-10	jejuni	TSKQ
4	4MT0-11	jejuni	TSKQ
4	4MT0-12	jejuni	TSKQ
4	4MT0-13	coli	TKQ

Table A (continued)

4	4MT0-14	jejuni	TSKQ
4	4MT0-15	jejuni	TSKQ
4	4MT0-16	jejuni	TSKQ
4	4WT2-1	jejuni	TSKQ
4	4WT2-2	jejuni	TSKQ
4	4WT2-3	coli	TKQ
4	4WT2-4	jejuni	TSKQ
4	4WT2-5	jejuni	TKQ
4	4WT2-6	jejuni	TSKQ
4	4WT2-7	jejuni	TKQ
4	4WT2-8	jejuni	TKQ
4	4WT2-9	jejuni	TKQ
4	4WT2-10	jejuni	TKQ
4	4WT2-11	jejuni	TKQ
4	4WT2-12	jejuni	TKQ
4	4WT2-13	jejuni	TKQ
4	4WT2-14	jejuni	TKQ
4	4WT2-15	jejuni	TSKQ
4	4WT2-16	jejuni	TSKQ
4	4MT2-1	jejuni	TSKQ
4	4MT2-2	jejuni	TSKQ
4	4MT2-3	jejuni	TSKQ
4	4MT2-4	coli	TK
4	4MT2-5	jejuni	TSKQ
4	4MT2-6	jejuni	TKQ
4	4MT2-7	coli	TK
4	4MT2-8	jejuni	TKQ
4	4MT2-9	coli	TK
4	4MT2-10	jejuni	TSKQ
4	4MT2-11	jejuni	TKQ
4	4MT2-12	jejuni	TSKQ
4	4MT2-13	jejuni	TSKQ
4	4MT2-14	jejuni	TKQ
4	4MT2-15	jejuni	TSKQ
4	4MT2-16	jejuni	TSKQ
4	4WT4-1	coli	TKQ
4	4WT4-2	jejuni	TSKQ
4	4WT4-3	jejuni	TSKQ

Table A (continued)

4	4WT4-4	coli	TKQ
4	4WT4-5	coli	TK
4	4WT4-6	coli	TK
4	4WT4-7	jejuni	TSKQ
4	4WT4-8	jejuni	TSKQ
4	4WT4-9	jejuni	TSKQ
4	4WT4-10	jejuni	TSKQ
4	4WT4-11	jejuni	TKQ
4	4WT4-12	jejuni	TSKQ
4	4WT4-13	coli	TKQ
4	4WT4-14	coli	TKQ
4	4WT4-15	jejuni	TSKQ
4	4WT4-16	coli	TK
4	4MT4-1	jejuni	TSKQ
4	4MT4-2	jejuni	TSKQ
4	4MT4-3	jejuni	TSKQ
4	4MT4-4	jejuni	TSKQ
4	4MT4-5	jejuni	TSKQ
4	4MT4-6	jejuni	TSKQ
4	4MT4-7	coli	TKQ
4	4MT4-8	jejuni	TSKQ
4	4MT4-9	coli	TKQ
4	4MT4-10	coli	TKQ
4	4MT4-11	jejuni	TSKQ
4	4MT4-12	jejuni	TSKQ
4	4MT4-13	jejuni	TSKQ
4	4MT4-14	coli	TK
4	4MT4-15	jejuni	TSKQ
4	4MT4-16	coli	TKQ
4	4WT6-1	coli	TKQ
4	4WT6-2	jejuni	TKQ
4	4WT6-3	jejuni	TSKQ
4	4WT6-4	jejuni	TKQ
4	4WT6-5	coli	TK
4	4WT6-6	coli	TK
4	4WT6-7	coli	TK
4	4WT6-8	coli	TK
4	4WT6-9	jejuni	TSKQ

Table A (continued)

4	4WT6-10	coli	TK
4	4WT6-11	coli	TKQ
4	4WT6-12	jejuni	TSKQ
4	4WT6-13	coli	TK
4	4WT6-14	jejuni	TKQ
4	4WT6-15	coli	TK
4	4WT6-16	coli	TKQ
4	4MT6-1	jejuni	TSKQ
4	4MT6-2	jejuni	TKQ
4	4MT6-3	coli	TK
4	4MT6-4	jejuni	TKQ
4	4MT6-5	coli	TK
4	4MT6-6	jejuni	TSKQ
4	4MT6-7	jejuni	TKQ
4	4MT6-8	coli	TK
4	4MT6-9	coli	TKQ
4	4MT6-10	jejuni	TSKQ
4	4MT6-11	jejuni	TKQ
4	4MT6-12	jejuni	TKQ
4	4MT6-13	jejuni	TKQ
4	4MT6-14	jejuni	TKQ
4	4MT6-15	jejuni	TKQ
4	4MT6-16	coli	TKQ
4	4WT8-1	jejuni	TSKQ
4	4WT8-2	coli	TKQ
4	4WT8-3	jejuni	TKQ
4	4WT8-4	coli	TK
4	4WT8-5	jejuni	TSKQ
4	4WT8-6	jejuni	TSKQ
4	4WT8-7	coli	TKQ
4	4WT8-8	coli	TK
4	4WT8-9	jejuni	TSKQ
4	4WT8-10	coli	TK
4	4WT8-11	jejuni	TSKQ
4	4WT8-12	coli	TK
4	4WT8-13	jejuni	TSKQ
4	4MT8-1	coli	TKQ
4	4MT8-2	coli	K

Table A (continued)

4	4MT8-3	jejuni	TSKQ
4	4MT8-4	coli	TK
4	4MT8-5	jejuni	TSKQ
4	4MT8-6	jejuni	TSKQ
4	4MT8-7	jejuni	TSKQ
4	4MT8-8	jejuni	TSKQ
4	4MT8-9	coli	TK
4	4MT8-10	coli	TKQ
4	4MT8-11	jejuni	TKQ
4	4MT8-12	jejuni	TKQ
4	4MT8-13	jejuni	TKQ
4	4MT8-14	coli	TKQ
4	4MT8-15	jejuni	TKQ
4	4MT8-16	jejuni	TKQ
4	4WT10-1	coli	TK
4	4WT10-2	coli	TK
4	4WT10-3	coli	TK
4	4WT10-4	coli	TKQ
4	4WT10-5	coli	TK
4	4WT10-6	coli	TKQ
4	4WT10-7	coli	TKQ
4	4WT10-8	coli	TKQ
4	4WT10-10	coli	TK
4	4WT10-11	coli	TK
4	4WT10-12	jejuni	TK
4	4WT10-13	coli	TKQ
4	4WT10-14	coli	TSEKQ
4	4WT10-15	coli	TK
4	4MT10-1	coli	TKQ
4	4MT10-2	coli	TK
4	4MT10-3	jejuni	TSKQ
4	4MT10-4	jejuni	TKQ
4	4MT10-5	coli	TK
4	4MT10-6	jejuni	TSKQ
4	4MT10-7	jejuni	TSKQ
4	4MT10-8	jejuni	TSKQ
4	4MT10-9	coli	TKQ
4	4MT10-10	coli	TK

Table A (continued)

4	4MT10-11	coli	TK
4	4MT10-12	coli	TK
4	4MT10-13	coli	TKQ
4	4MT10-14	coli	TKQ
4	4MT10-15	coli	TKQ
4	4MT10-16	coli	TK
4	4MT12-1	jejuni	TSKQ
4	4MT12-3	coli	TKQ
4	4MT12-4	jejuni	TSKQ
4	4MT12-5	coli	TK
4	4MT12-6	jejuni	TSKQ
4	4MT12-7	jejuni	TSKQ
4	4MT12-8	coli	TK
4	4MT12-9	jejuni	TSKQ
4	4MT12-10	jejuni	TSKQ
4	4MT12-11	coli	TSKQ
4	4MT12-12	jejuni	TSKQ
4	4MT12-13	coli	TK
4	4MT12-14	coli	TKQ