FATE AND ECOLOGY OF CAMPYLOBACTERS IN A SOUTHEASTERN GEORGIA WATERSHED

by

ETHELL VEREEN, JR.

(Under the Direction of ERIN K. LIPP)

ABSTRACT

Campylobacters comprise a group of closely related gram-negative bacteria that primarily colonize the gastrointestinal tracts of a variety of host species and are the most common cause of bacterial enteritis globally. Although assumed to be a food-borne disease, the distinct seasonality in cases suggests that environmental exposures also may be important. In this study we show that environmental detection of waterborne campylobacter was highly associated with discharge from a wastewater treatment plant and moderately associated with run-off in agrarian reaches of the watershed. Despite a low persistence under warm temperatures *in vitro*, campylobacters were more frequently isolated and present in larger numbers during the summer months, which suggests that loading from both human and domestic animal waste may be high in this watershed. In summary, campylobacters were frequently present along agrarian and sewage impacted stretches of streams in southeastern Georgia and may be an underappreciated exposure source for clinical cases.

INDEX WORDS: *Campylobacter* spp., *Campylobacter jejuni*, campylobacteriosis, environmental transmission, waterborne pathogen, waterborne disease, Satilla River, Seventeen Mile River, water quality

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DEDICATION

To my grandparents,

Henry & Eva Vereen
Edward & Bertha-Mae Bennett

And whatsoever ye do in word or deed,

do all in the name of the Lord Jesus,

giving thanks to God and the Father by him.

Col 3:17

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CHAPTER 1

INTRODUCTION

Campylobacter species, particularly *Campylobacter jejuni* and *Campylobacter coli,* are the etiological agents for campylobacteriosis, which is recognized as one of the most frequent causes of acute diarrheal disease throughout the world (59, 65). As much as 1% of the population is thought to be infected with *Campylobacter* spp. every year in Europe and the United States (66). Most cases of campylobacteriosis occur as isolated, sporadic events, not as a part of large outbreaks (46). Despite limited surveillance, over 10,000 cases are reported to the Centers for Disease Control and Prevention (CDC) each year (51). Active surveillance in the United States indicates that the incidence of *Campylobacter* infections is decreasing (51, 12). However, it is estimated that *Campylobacter* still causes ~2 million cases of gastroenteritis each year (51) and undoubtedly, many more cases go undiagnosed and unreported.

Most campylobacteriosis cases in the United States and throughout the world are recorded in the summer months (58, 55, 12). This trend also holds for Georgia (USA), as the statewide incidence of monthly infections peaks between May and August (11). It has been suggested that changes in food handling and consumption in the summer months (i.e., outdoor barbecuing) may lead to this predictable seasonality (55); however, this does not always hold (41, 31) and does not explain the same pattern across different nations and cultures (59, 41, 23, 45).

This thesis is an investigation of the environmental prevalence and seasonality of campylobacters in a rural southeastern Georgia (USA) watershed and begins to relate

environmental loading and clinical case burden in the region. Chapter 2 provides an overview of the literature related to this topic and Chapter 3 presents the research approach and findings. The final section of this thesis, Chapter 4, provides an overview of the conclusions derived from this study.

CHAPTER 2

LITERATURE REVIEW

INTRODUCTION

Most campylobacters and *Campylobacter*-like organisms have been assigned to rRNA superfamily VI, which includes *Helicobacter*, the family Campylobacteraceae and a number of other taxa (44). The family Campylobacteraceae includes the genera *Arcobacter* and *Campylobacter*, characterized as fastidious Gram-negative, non-spore forming, motile, microaerophilic spiral-shaped organisms (36, 62). The genus *Campylobacter* comprises a group of closely related bacteria that primarily colonize the gastrointestinal tracts of a wide variety of host species. Some of these bacteria are commensals in birds (38), but many, particularly *Campylobacter jejuni*, and its close relative *Campylobacter coli*, are enteric pathogens of humans and wild, domestic, and domesticated animals (38, 54). Contaminated or untreated drinking water, raw milk, and poultry appear to be the most common vehicles of transmission (28, 40), but environmental waters may be a significant source of human infection, and contaminated surface waters have been responsible for a number of outbreaks of *C. jejuni* infection (8, 33, 52, 50). *Campylobacter* species, particularly *C. jejuni* and *C. coli*, are the etiological agents for campylobacteriosis, which is recognized as one of the most frequent causes of acute diarrheal disease throughout the world (59, 65). *Campylobacter* infections exhibit a marked seasonality with most reported cases occurring in the spring and peaking in the summer months (58, 55, 12).

TAXONOMY OF *CAMPYLOBACTER*

Campylobacter is derived from the Greek word "kampylos," which means curved (64, 27). The organism was given this name because of the spiral or S-shaped morphology of the cells (64). The first identification of *Campylobacter* was in 1909, when, because of morphological similarity with vibrios, these organisms were originally classified as *Vibrio fetus* (7). In the subsequent decades, similar organisms were found and included in the genus *Vibrio* as *Vibrio jejuni*, *Vibrio coli*, *Vibrio sputorum*, *Vibrio bubulus* and *Vibrio fecalis* (62). Because of their microaerophilic growth requirements and their nonfermentative metabolism, *V. fetus* and *V. bulbus* were included in a new genus, *Campylobacter*, by Sebald and Véron in 1963 (as cited in 62).

The genera *Campylobacter, Arcobacter, Helicobacter, Wolinella*, and "*Flexispira*" make up a separate eubacterial lineage identified as rRNA superfamily VI within the class *Proteobacteria* (63). This group is often referred to as the campylobacteria or campylobacters (18). Common genotypic and phenotypic features have been found to differentiate the genera *Campylobacter* and *Arcobacter* from the other members of this group and the family *Campylobacteriacaea* was proposed (62). New members of the genus *Campylobacter* and related genera are being identified with regularity and the genus *Campylobacter* presently contains 16 species (1, 68).

DESCRIPTION OF THE CAMPYLOBACTERIACEAE

Campylobacters are microaerophilic, very small, curved, thin, Gram-negative rods (1.5 - 5 μm) exhibiting corkscrew like motility. They usually possess a polar flagellum at one or both ends of the cell. They are slow-growing fastidious organisms, which are asaccharolytic and generally biochemically unreactive, unlike other enteric pathogens such as *Salmonella* and

Shigella (43, 49). All members of the genus *Campylobacter* are microaerophilic, generally requiring oxygen at lower tensions than in air for growth, but tolerance to oxygen varies greatly depending on the species and strain. The optimal growing conditions are a gas mixture of 10% CO_2, 8% N_2, and 10% H_2 (20).

Campylobacters differ with respect to their critical and optimum temperature requirements. *Arcobacter* can grow microaerobically and aerobically and has the ability to grow at 15^{o}C, which is a distinctive feature that differentiates *Arcobacter* species from *Campylobacter* species (30). *C. jejuni, C. coli, C. lari* and *C. upsaliensis* can grow well 37^{o} C and at temperatures up to 45°C and are often referred to as the thermophilic *Campylobacter* spp. (20).

Under natural conditions, *Campylobacter* growth is only achieved within a suitable host (38). The natural habitats of most *Campylobacter* species are the intestines of birds and other warm-blooded animals (65). The intestinal tracts of domestic animals such as pigs, cattle, dogs and cats have also been recognized as a reservoir (56); however, the favored environment appears to be the intestines of avian species, including wild birds, chickens, turkeys, quails, ducks, and ostriches (39). Campylobacters are usually commensal within their avian hosts, with the possible exception of ostriches (39). Survival outside of a host is limited and environmental exposures result in morphological and physiological changes to the cells. As *Campylobacter* cells begin to age, they become coccoid in shape (35). This is a common response for bacteria in the environment because it increases the surface area to volume ratio of the cell (32); however, this transition is also reported to indicate the cells' entrance into a viable but nonculturable (VBNC) state (34, 47). While this stage is believed to increase environmental persistence its significance in environmental transmission to humans (or other hosts) is not well understood.

CAMPYLOBACTERIOSIS

Campylobacter species, particularly *C. jejuni* and *C. coli,* are common causes of campylobacteriosis and are the most frequent causes of this acute diarrheal disease in humans throughout the world (59, 65). Other *Campylobacter* or *Campylobacter*-like species that have been associated with human disease include *C. fetus, C. lari, Helicobacter fennelliae, H. cinaedi, C. hyointestinalis, C. upsaliensis, C. jejuni* subsp. *doyleii, C. sputorum, Arcobacter cryaerophila,* and *A. butzleri* (1). Although it is widely assumed that campylobacteriosis is primarily a food-borne disease, the majority of infections are sporadic, and the sources of infection are rarely determined (55). Most people who become ill with campylobacteriosis experience diarrhea, cramping, abdominal pain, and fever within two to five days after exposure to the organism. *Campylobacter* can cause mild to severe diarrhea, with loose, watery stools often followed by bloody diarrhea and can be accompanied by nausea and vomiting (5). The illness typically lasts one week and some persons who are infected with *Campylobacter* are asymptomatic.

Campylobacter spp. are highly infective. The infective dose of *C. jejuni* ranges from 500 to 10,000 cells, depending on the strain, damage to cells from environmental stresses, and the susceptibility of the host (61, 4). In persons with compromised immune systems, *Campylobacter* occasionally spreads to the bloodstream and causes a serious life-threatening infection. The infections are manifested as meningitis, pneumonia, miscarriage, and a severe form of Guillain-Barré syndrome (5, 37).

Persons of all ages may be affected, but the pattern of age- and sex-specific distribution rates of *Campylobacter* is unique among enteric bacteria (59, 38, 51). The highest isolation rate occurs in the first year of life, but a large second peak occurs in the young adult years (59, 51). Isolation rates for males are higher than those for females with this predominance extended from

infancy to age 45, above which the infection rates are equal for both sexes (59). Some of the sex-related difference may be explained by gender-related differences in food-handling practices (51). Men report more unsafe food-handling, preparation, and consumption practices than do women (51), and the results of the FoodNet population survey in the United States suggested that men eat more foods known to be risky for disease than do women, including food that present a high for *Campylobacter* contamination (e.g., pink chicken, unpasteurized milk; 53, 51). However, the gender-related difference in incidence persists even among young children, which suggests that other factors may play a role. Boys aged <10 years, including infants, have a higher reported incidence of several infectious diseases, including *Salmonella* and *Shigella* infections, and it has been speculated that these differences may be due to greater susceptibility to infectious diseases among males (51).

GLOBAL BURDEN OF CAMPYLOBACTERIOSIS

Epidemiological surveys have suggested that in developed nations, such as Europe and the United States, as much as 1% of the population is infected with *Campylobacter* spp. every year (66). In 2003, a total of 15,600 laboratory-diagnosed cases of all foodborne infections (bacterial and parasitic) under surveillance were reported in the U.S., and of these 3,021 were *Campylobacter*, or approximately 19% (12). Active surveillance since 1996 (12) indicates that the incidence of *Campylobacter* infections in the United States is decreasing (12, 51); however, elsewhere reported cases are on the rise (14, 45). Since the early 1980s, the number of cases of *Campylobacter*-related diarrhea reported in England and Wales has increased nearly five-fold and has exceeded levels of *Salmonella* cases (19). In 1998 alone in Great Britain, there were 58,000 reported cases of campylobacteriosis (66). The incidence of human campylobacteriosis in Denmark has risen steadily since 1992, reaching 4,620 cases (86 cases per 100,000 people) in

2001 (2). *Campylobacter* is also the most frequently notified cause of enteric disease in New Zealand. New Zealanders suffer a very high rate of campylobacteriosis, with 14,786 cases (395.6 per 100,000 people) in 2003 (3).

Lesser developed nations generally do not have national surveillance programs for campylobacteriosis; therefore, incidence values often do not exist (14). Most estimates of incidence in these countries are based on laboratory surveillance of pathogens responsible for diarrhea. *Campylobacter* isolation rates in lesser developed nations range from 5 to 20% of cases of diarrhea (14). This is supported by a recent South African study, where it was reported that 22.3% of 10,538 stool specimens examined during an 84-month study belonged to the *Campylobacter* group (16). In all cases, these are likely conservative estimates of disease incidence, as enteric disease surveillance is known to underestimate incidence considerably (54). Contributing to underestimation of disease burden includes the fact that many clinical laboratories do not routinely culture for *Campylobacter* spp. and others may not use optimal culture methods (59). Additionally, the number of cases of campylobacteriosis may be underreported because the disease is self-limiting and affected persons may not seek treatment.

CAMPYLOBACTERIOSIS SEASONALITY

In lesser developed nations, *Campylobacter* enteritis (campylobacteriosis) has no distinct seasonal trend (60, 14); and such patterns are even less evident in tropical and subtropical countries, although higher incidences have been observed during rainy seasons (55). The lack of seasonal trends may be due to lack of extreme temperature variation as well as lack of adequate surveillance for epidemics (60, 14).

Conversely, the incidence of enteritis caused by campylobacters in developed nations exhibits a distinct seasonality. In industrialized countries in temperate climates there is a

consistent increase in isolation rate during the summer months and a distinct decrease during the winter months (1). In the United States an increase in cases is noted during the spring and peaks in the summer months of June or July (CDC, 2001). Similarly, human campylobacter infections peak in July and August in Denmark (45, 41). This seasonality is comparable to that of other European countries including Wales, Scotland, Finland and Sweden (41, 31).

Explanations for the rise in the number of cases during the summer include higher levels of poultry contamination in warmer weather and summer food-consumption patterns, including barbecuing and eating outdoors, which may result in food that is undercooked or cross-contaminated (51). Studies have also suggested a possible association between environmental factors such as temperature, humidity, and sunlight and *Campylobacter* carriage in broilers (42, 38, 41, 45). In both the United States and Denmark, seasonal variation in carriage-rate in market broilers at retail level was similar to the seasonal patterns in human cases (67, 41, 45). In Norway, however, the proportion of colonized flocks peaked in the autumn, after the summer peak in human cases (26, 41). Newell et al. (presented at the 10th International Workshop on *Campylobacter*, *Helicobacter*, and Related Organisms, Baltimore, Md. 1999) suggested that the seasonal variation in humans coincides with or even precedes, rather than follows, that in poultry, which may indicate seasonality in common not yet identified environmental sources (39).

CAMPYLOBACTERS IN THE ENVIRONMENT AND POTENTIAL ENVIRONMENTAL TRANSMISSION

Another rationale for seasonality is related to *Campylobacter* survival and prevalence in environmental sources (8, 24, 54). *Campylobacter* may enter environmental waters via discharged sewage, agricultural and storm water run-off, or through the feces of animals, birds,

or infected humans (48, 15). In a study of thermophilic campylobacters in liquid sewage effluent in the United Kingdom during 1988 and 1989, Jones et al. (1990) observed a prominent seasonality with distinct peaks in May and June. This seasonal variation coincided with campylobacter enteritis in the community, but because this sewage contained sewage effluent primarily from abbatoir and animal processing (with only minimal inputs from the community) it suggests that non-human, or environmental, reservoirs may be an important factor in this coincident seasonality. In addition, Hörman et al. (2004) has shown that in Finland *Campylobacter* spp. were detected less frequently during the winter than during the spring, summer, or autumn in surface waters. Outside of these reports, most environmental studies have shown seasonality in campylobacter detection that is different from that seen in clinical cases. *Campylobacter* numbers are frequently found to be higher in the winter than in the summer (8, 9, 25). Bolton et al. (8) conducted a study to determine the prevalence of thermophilic campylobacters at selected sites along a river system. While the greatest frequency of isolation and highest counts (>10-230 campylobacters 100 ml^{-1}) were associated with sites adjacent to or downstream of sewage works, the highest counts were recovered in the late autumn and winter, with the least isolations in spring and summer (8). Likewise, Carter et al. (9) recovered *Campylobacter* spp. at higher rates in the fall (55%) and winter (39%) as compared to lower rates in the spring (25%) and summer (30%) from a number of natural water sources. Studies have shown that *Campylobacter* spp. survive longer at lower temperatures (6, 29, 17, 57), therefore increasing the likelihood of isolation in winter months. Additionally, *Campylobacter* spp. are susceptible to photodegradation (8, 42) and the combination of high temperatures and longer hours of sunlight during the summer months may influence their survival in temperate climates (8, 42).

While traditionally associated with food, *Campylobacter* spp. have been associated with waterborne outbreaks around the world. Unlike the seasonality of campylobacteriosis in general, seasonal patterns among waterborne campylobacter infections are often variable. Tauxe (59) suggested that waterborne *Campylobacter* outbreaks tend to occur in spring or early fall in the United States, an association attributed to seasonality of surface water contamination. Other anecdotal studies have shown waterborne outbreaks to occur in various seasons and months in different countries including May in Canada (13, Walkerton Ontario), June in Norway (33), August in Finland (21), and August in the United States (10).

SUMMARY

Although it is widely assumed that campylobacteriosis is primarily a food-borne disease, the majority of infections are sporadic, and the sources of infection are rarely determined (55). Handling and consumption of poultry or poultry-related products are considered to be a primary source for *Campylobacter*-induced disease in humans (22). This suggestion is consistent with studies showing that the gastrointestinal tracts of birds are commonly colonized by campylobacters (38). However, water is potentially an important reservoir of the thermophilic campylobacters and is an established vehicle for the transmission of these organisms to humans and domestic animals (8, 24, 21). Studies have shown campylobacters to be common in natural waters such as streams, rivers, and lakes due to discharges from wastewater treatment plants, runoff from pastures after rain, and direct contamination by wild birds and animals (25, 21). In the developed world, *Campylobacter* isolations typically increase during the summer and fall months and although most cases appear to be sporadic, epidemics do occur. In the developing world, there is less seasonal variation and epidemics are not reported (60). Environmentally, campylobacter peaks in the fall and winter in contrast to the seasonality of campylobacter

enteritis. Although studies have begun to speculate on factors that may be attributed to the seasonality and differences of each (*Campylobacter* clinical cases and environmental detection) future studies should focus on environmental factors that may drive these relationships.

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CHAPTER 3

FATE AND ECOLOGY OF CAMPYLOBACTERS IN COASTAL PLAIN STREAMS (GEORGIA, U.S.A.) [1]

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ABSTRACT

Campylobacter is the leading cause of bacterial associated diarrhea in the United States and most developed countries. While considered a foodborne disease, many clinical cases cannot be linked to a food source. In rural and agrarian areas environmental transmission may be an important contributor to case loads. To address this issue, we investigated waterborne campylobacters in a mixed-use rural watershed in the coastal plain of southern Georgia (USA). Six sites representing varying degrees of agricultural and human influence were surveyed bi-weekly to monthly for one year for culturable thermophilic campylobacters and other measures of water quality. Campylobacters were frequently present along agrarian and sewage impacted stretches of streams in southeastern Georgia. Mean campylobacter counts were highest downstream from a wastewater treatment plant (WWTP) that handled both human and poultry slaughterhouse waste ($\leq$595 CFU ml^{-1}); concentrations were significantly higher than the other four upstream sites ($p<0.05$). Similarly, 93% of the samples from the site directly downstream of the WWTP were positive for campylobacter while only 15% of the samples from the control site were positive. Counts were significantly correlated with measures of water quality including fecal coliform bacteria, conductivity, pH, and nutrients (NO_3^-, PO_4^{3-}, and NH_3). Despite a low persistence under warm temperatures (25^o C) *in vitro*, campylobacters were more frequently isolated and present in larger numbers during the summer months and were significantly correlated with temperature and precipitation, which also peaked in the summer. This suggests that loading from both human and domestic animal waste may be high in this watershed during summer months. Mixed-use watersheds, supporting agriculture production, human populations and wildlife maybe at high risk for contamination by campylobacters.

INTRODUCTION

The family *Campylobacteraceae* includes the genera *Campylobacter* and *Arcobacter* (49). The campylobacteria (campylobacters) more commonly refers to a range of taxonomically related genera including *Campylobacter*, *Arcobacter, Helicobacter* and *Sutterella* (14). Campylobacters are characterized as Gram-negative, non-spore-forming, motile, microaerobic, and 'S' or spiral shaped organisms (49). Campylobacters, particularly *Campylobacter jejuni* and *Campylobacter coli,* are the etiological agents for campylobacteriosis, which is recognized as one of the most frequent causes of acute diarrheal disease throughout the world (47, 50). Within 2-5 days, most people who become ill with campylobacteriosis experience symptoms which include, mild to severe diarrhea, with loose, watery stools often followed by bloody diarrhea that may be accompanied by nausea and vomiting (4). The illness typically lasts one week and some persons who are infected with *Campylobacter* spp. do not show symptoms (4).

As much as 1% of the population is thought to be infected with *Campylobacter* spp. every year in Europe and the United States (52). Most cases of *Campylobacter* infection occur as isolated, sporadic events not as a part of large outbreaks (34). Despite limited surveillance, over 10,000 cases are reported to the Centers for Disease Control and Prevention (CDC) each year (26), with an estimated total number of cases of 2 million (26). Active surveillance since 1996 (7) indicates that the incidence of *Campylobacter* infections in the United States is decreasing (7, 41); however, elsewhere reported cases are on the rise (10, 33).

Most campylobacteriosis cases in the United States and throughout the world are recorded in the summer followed by declines in the fall and winter (46, 43, 7). In Georgia, the statewide incidence of monthly infections peaks between May and August (6). Handling and consumption of poultry or poultry-related products are considered to be a primary source for

Campylobacter infection (16). It has been suggested that changes in food handling and consumption in the summer months (i.e., outdoor barbecuing) may lead to this predictable seasonality (43); however, this assessment does not always hold (31, 25) and does not explain the same pattern across different nations and cultures (47, 31, 18, 33). Furthermore, the pathways involved in *Campylobacter* contamination of poultry flocks also remain unclear. Given that the majority of human infections are sporadic, sources other than food (poultry) may be important in disease transmission, especially in areas where foodborne disease burden has declined. Other reported sources of infection have included contaminated water and raw milk (22, 30); however, in most cases the exact source of disease is not determined (43).

The distribution of campylobacterosis outbreaks is bimodal, with peaks in May and October (46); however, reported clinical cases reach their peak in summer months, when outbreaks tend to occur infrequently (15). Given these contrasting patterns, it is speculated that campylobacter sources associated with outbreaks are quite different from sources responsible for non-outbreak cases (46, 15). Environmental loading and transmission may be one factor associated with non-outbreak cases, particularly related to contamination of ambient waters (5, 27, 42, 40).

Although they require a host for growth, campylobacters are not uncommon in natural waters such as streams, rivers, and lakes due to discharges from wastewater treatment plants, runoff from pastures after rain, and direct contamination by feces of wild birds and animals (38, 11, 21, 15). The survival or persistence of *Campylobacter* spp. outside of a suitable host depends on many factors, such as oxygen content, presence of nutrients, temperature, and pH (8). Recent research also indicates that campylobacters may survive within vacuoles of protozoa in environmental waters (3). Additionally, *Campylobacter* spp. may enter a viable but

nonculturable (VBNC) state (where organisms are not able to grow in culture but remain metabolically active), which was first described by Rollins and Colwell (1986). The VBNC state has been considered to play a role in prolonging *Campylobacter* spp. survival in the environment (38, 11).

Mixed use watersheds, supporting agriculture production, human populations and wildlife maybe at high risk for contamination by campylobacters. Determining environmental factors that contribute to both loading and persistence of campylobacters is important for controlling potential waterborne transmission. Here we describe the temporal and spatial distribution of thermotolerant campylobacters in a rural watershed in southeast Georgia (USA) and assess potential drivers for environmental loading, seasonality and persistence and begin to relate these to clinical burden in the region.

MATERIALS AND METHODS

Field Study

Sampling Area

Samples were collected within the Satilla River Basin, which drains 10,204 km^2 in southeast Georgia (USA). It is flanked by the Altamaha River basin to the north and the Suwannee and St. Mary's River basins to the south. Field sampling was focused in the Seventeen Mile River (4 of 6 stations), which is located in the headwaters of the Satilla River Basin near Douglas, Broxton, and Ambrose, Georgia (Fig. 1); the watershed is approximately 764 km^2. An upstream site was located near the headwaters of the Satilla River (site 5); a control station (site 6) was located in the Lower Ocmulgee watershed in the Broxton Rocks Preserve. The four remaining stations (sites 1– 4) were located along the Seventeen Mile River with site 4 being the most upstream and site 1 the most downstream (Fig. 1). Site 2 was immediately down-

stream from the municipal wastewater treatment plant (WWTP) for the city of Douglas that handles both human and poultry slaughterhouse waste. The other three stations along the Seventeen Mile River were primarily influenced by agriculture (Tbl. 1). Site 5 was located on the Satilla River (Fig. 1) and was not only the most upstream of all the sites sampled, but also had the highest percentage of agricultural land-use (Tbl. 1). Site 1, the most downstream of all stations, had slightly less agricultural influence (26% in its immediate area) but it received flow from the entire basin.

Sample Collection

Samples were collected bi-weekly June through August 2003 and monthly from September 2003 through May 2004; all samples were collected between 8:00 AM and 10:00 AM. Water was analyzed for conductivity (mS/cm), temperature (^{o}C), pH, dissolved oxygen (DO, mg L^{-1}), turbidity (NTU), oxidation reduction potential (ORP, mV), fluorescence and chlorophyll *a* (μg L^{-1}) with a YSI® 6600 Multiparameter Sonde (Yellow Springs, OH) on-site. The probe was placed into the deepest part of the stream channel and used to record instantaneous values. Water for nutrient and microbial analyses was collected in sterile 1-liter polypropylene bottles as discrete surface grabs and transported on ice to the USDA-Agricultural Research Services (USDA-ARS) Southeast Watershed Research Laboratory (SEWRL) in Tifton, GA. Samples were processed for microbial analysis within four hours of taking the first sample.

Nutrient Analyses

Water samples were filtered through Whatman 934 AH filters for determination of suspended sediment following standard methods (9). An aliquot of the filtrate was stored for nutrient analysis. An aliquot of the unfiltered sample was stored for analysis of total N and Total P in a digestate. The filtered sample was analyzed for nitrate-N, ammonium-N, dissolved

molybdate reactive P (DMRP), and chloride using EPA approved colorimetric techniques (9) on a Lachat Flow Injection Analyzer. The unfiltered sample was analyzed for Total Kjeldahl N (TKN) and Total P (TP) using digestion and colorimetric techniques adapted from EPA approved methods (9). Total N was calculated as the sum of unfiltered TKN and nitrate-N. Samples were analyzed for dissolved total carbon (DTC), dissolved inorganic carbon (DIC), and dissolved organic carbon (DOC) using a Shimadzu model 5050 TOC analyzer and for potassium using standard methods on a Perkin-Elmer atomic absorption spectrophotometer (9).

Microbiological Analyses

Water samples were screened for fecal coliform bacteria by membrane filtration and growth on mFC agar following Standard Methods (1). Plates were incubated for 24 $\pm$ 2 h in a 44.5 ^{o}C water bath. All blue colonies were counted as fecal coliform bacteria and enumerated as colony forming units (CFU) per 100 ml.

Thermotolerant campylobacters (*Campylobacter* and *Arcobacter* [14]) were detected and enumerated using a modified direct-plating method as described by Rosef et al. (13). Briefly, duplicate 100-µL aliquots of water from each sample were directly spread onto modified charcoal cefoperazone deoxycholate agar (mCCDA, Oxoid (Lenexa, Kansas)). Petri dishes were then placed in nonvented BBL^{TM} (Cockeysville, Maryland) GasPak Jar Systems, and microaerobic conditions were created and maintained using BBL^{TM} (Sparks, Maryland) CampyPak Plus Microaerophilic System Envelops with Palladium Catalyst ($H_2 + CO_2$). Plates were incubated for 48 $\pm$ 1 h at 42^{o}C and gray, mucoid colonies were counted as presumptive Campylobacters. Colonies were confirmed by observation of Gram-negative spiral bacteria, darting motility, positive oxidase reaction, positive catalase reaction, and growth only in a microaerobic atmosphere (32, 39). Campylobacters produce a spreading growth on selective

agar and it is difficult to determine species level discrimination by visual examination alone (5). The isolation techniques used confirmed presence of campylobacters without regard to species (32).

Other data

Basin-wide rainfall was estimated using data from a National Climatic Data Center (NCDC) weather station in Douglas, Georgia (COOPS 092783) (Fig. 1). Campylobacter concentrations at each site were investigated for their relationship to rainfall in the watershed. To best determine the response to precipitation events correlation analyses were performed for each site using several measures of precipitation: daily rainfall, rainfall on the day preceding the sampling, total rainfall in the 7 days before the day sampled, total rainfall in the 10 days prior to sampling, total rainfall for the month before sampling, total rainfall for the month of sample collection, total rainfall 30 days preceding the sampling and 7 day, 10 day, 30 day maximum daily rainfall preceding the sampling. Monthly data reports on flow and treatment standards were obtained from the wastewater treatment plant (WWTP) upstream of site 2. The treatment plant's capacity is 6.00 million gallons per day (MGD), and during times of increased rain, the influent flow may exceed this (plant operator, personal communication).

Statistical analyses

Data were analyzed using the SAS system for Windows, release 8.02 (SAS Institute, Inc., Cary, NC). Differences in means were evaluated with nonparametric analysis of variance procedures and correlations were determined with the Spearman's correlation coefficient test. For seasonal analyses July, August, and September were designated as Summer; October, November, and December as Fall; January, February, and March as Winter; and April, May, and June as Spring. In all cases, p values ≤ 0.05 were regarded as significant.

In vitro *survival studies*

A microcosm experiment using *Campylobacter jejuni* subsp. *jejuni* (ATCC 49943) was conducted to determine its persistence and survival in environmental waters under high and low temperatures. 500 mL of river water (collected from site 3; Seventeen Mile Creek) and 500 ml of deionized (DI) water (used as a control) were sterilized by autoclaving and added to each of four replicate 2-L flasks. Each flask was then inoculated with 1 ml of a log-phase culture of *C. jejuni*. Duplicate flasks for each water type were kept in the dark at 5^{o} and 25^{o} C for 30 days. One-ml aliquots were collected prior to inoculation, daily for one week and at days 10, 13, 16, 23 and 30; an additional aliquot of 1 mL was taken at each time interval and stored frozen (-20^{o}C).

Each aliquot was serially diluted and analyzed for culturable *C. jejuni* by spread plate counting on mCCDA as previously described. Total viable *C. jejuni* counts were determined by epifluorescence microscopy using a Live/Dead BacLight Kit (Molecular Probes, Eugene, OR). Briefly, samples were serially diluted, stained with 200 - 1000 µl of 2X Live/Dead staining reagent and then concentrated by filtering the entire volume through a 0.2 µm-pore size black isopore polycarbonate membrane filter and observed at 10 X – 40X magnification under fluorescent light. Final concentrations of viable cells (fluorescing green under UV illumination) were determined using the average counts from three random microscopic fields per filter.

Statistical analyses

Data were analyzed using the SAS system for Windows, release 8.02 (SAS Institute, Inc., Cary, NC). Inferences about population effects were made with the Proc Mixed procedure. In all cases p values ≤ 0.05 were regarded as significant. Survival curves were calculated using nonlinear regression (curve fit), one phase exponential decay with GraphPad Prism® Prism for Windows version 4.03 (GraphPad Software, Inc., San Diego, CA).

RESULTS

Water Quality

Physical and chemical parameters

In total 14 samples were collected from each of the six sites. Water temperature ranged from 4.8°C in January to 27.9°C in July. Average water temperature for each season was significantly different ($p < 0.001$). For summer, average water temperature was 23.8°C, 13°C for fall, 10.3°C for winter, and 19.4°C for spring. Dissolved oxygen levels varied inversely with water temperature ($r = -0.66$, $p < 0.001$) and were lowest in July with an average of 2.99 mg L^{-1}, and highest in February with an average of 11.88 mg L^{-1}.

Conductivity, pH, nitrogen, phosphorous and ORP were the only water quality parameters that varied significantly by site ($p<0.05$) (Tbl. 2). Conductivity measurements at site 2 (WWTP) were significantly higher than at all other sites. Additionally, NO_3-N, PO_4^{3-}, and NH_3-N levels measured at site 2 were also significantly higher than all other sites. Potassium levels were significantly lower at this station while DTC was not significantly different. Values of pH for site 6 (control) were significantly lower than all other sites. ORP levels at site 2 (WWTP) were the lowest (131 mV) and the highest (227 mV) was at site 6 (control). While none of the nutrients differed significantly between seasons, pH, ORP, and turbidity did show significant seasonal variation ($p<0.05$) (Tbl. 3). DO and pH were highest during the winter at 9.68 mg/L and 7.42 respectively (Tbl. 3). Turbidity was highest during the summer (4.4 NTU).

Fecal coliform concentrations were not significantly different by site; however, higher concentrations were detected most frequently at site 2, immediately downstream from the WWTP. The mean fecal coliform concentration at this site was 698 CFU 100 ml^{-1}. Concentrations were lowest at the control site 6 (mean of 55 CFU 100 ml^{-1}). Fecal coliform

concentrations did not show any significant seasonal variation; however, highest concentrations were detected in the summer at a mean concentration of 456 CFU 100 ml^{-1}. Mean fecal coliform concentrations were 137 CFU 100 ml^{-1} for fall, 108 CFU 100 ml^{-1} for winter and 148 CFU 100 ml^{-1} for spring. Fecal coliform concentrations were significantly correlated with conductivity ($r = 0.28$), DO ($r = -0.29$), Cl ($r = 0.28$), NH_3-N ($r = 0.23$), and DIC ($r = 0.27$) levels ($p<0.05$).

Distribution of thermotolerant campylobacters

Campylobacters were detected at all sites sampled (Tbl. 4). Mean concentrations were lowest at the control site (site 6, 2 CFU ml^{-1}) and highest downstream from the municipal wastewater treatment plant (WWTP) (Site 2, 158 CFU ml^{-1}). Campylobacter levels at site 2 were significantly greater than all of the upstream sites (3 – 6) but did not differ significantly from site 1, the most downstream station (Tbl. 4). The highest concentration detected at any sample time was also at site 2 (595 CFU ml^{-1}, August 2003). Ninety-three percent (13/14) of the samples from this site tested positive by culture, whereas only 15% (2/13) of the samples from the control site were positive (Tbl. 4). At the remaining upstream sites, the percentage of positive samples followed the percent of land used for agriculture (i.e., the higher percentage of agricultural lands also had the higher percentage of samples positive for campylobacters) (Tbl. 4).

Seasonal distribution of campylobacters

Campylobacters reached their greatest concentrations at all sites between July and September 2003 (Fig. 2). Although there was no significant difference in concentrations between seasons, mean levels were lowest during the fall (19 CFU ml^{-1}) and highest during the summer (74 CFU ml^{-1}). Levels at site 2, which were highest throughout the study, peaked in the summer and declined to lowest levels in the fall (Fig. 2). Levels at the more heavily agricultural

sites (sites 4 and 5) also peaked in the summer but reached their lowest levels in the spring (Fig. 2). Samples were only positive in summer at the control site (site 6) and were not detected during the rest of the year.

Relationship between campylobacters, fecal coliform bacteria, and water quality variables

Basin wide campylobacter concentrations were significantly correlated with fecal coliform levels, temperature, conductivity, and chlorophyll *a* ($p<0.05$; Tbl. 5). Concentrations were also significantly correlated to nutrients (NO_3^--N, Cl, PO_4^{3-}, potassium) and DIC levels (Tbl. 5). Because there were significant differences between campylobacter counts between sites ($p=0.0003$), separate correlations were calculated for each station. Of note, campylobacters were only significantly correlated with fecal coliform bacteria at the most upstream sites, 4 & 5, with r values of 0.55 ($p = 0.05$) and 0.76 ($p = 0.004$), respectively. There was a significant inverse correlation between DO and campylobacters downstream of the WWTP (site 2).

Relationship between campylobacters and precipitation

Basin wide campylobacter concentrations (all sites) showed a significant response to several rainfall measures; the strongest response was to maximum daily rainfall in the month preceding the sampling date ($r = 0.41$; Fig. 3). Analyzed by site, campylobacter concentrations at site 3 showed the strongest response to rainfall, particularly 7-day maximum daily rainfall in the seven days preceding the sampling date ($r = 0.84$; Fig. 4). Other sites showed weaker associations. There was no significant correlation with any rainfall variables at site 2.

Influence of wastewater treatment and discharge on campylobacter levels

Over the period of study, the mean influent flow at the wastewater treatment plant was 5.12 ± 1.4 million gallons per day (MGD). While not related to any measure of rainfall, campylobacter levels detected at site 2 were significantly correlated with WWTP flow ($r = 0.62$,

p=0.02). During the summer, influent flow at the treatment plant was at its highest level with a mean flow of 6.43 MGD. Mean concentration was also at its highest during the summer (214 CFU ml^{-1}). When campylobacters reached their highest concentration (595 CFU ml^{-1}), influent flow was also at its peak (8.52 MGD; Fig. 5).

In vitro *survival studies*

There was no significant difference in *Campylobacter jejuni* survival between DI water and river water microcosms at either temperature (Fig. 6). However, temperature did have a significant effect on culture counts, regardless of medium, with loss of culturability occurring within 5 days at 25^{o}C and 10 days at 5^{o}C. Cells enumerated by direct counting were always higher than culturable counts and viable cells could be detected by direct counting through day 30 in all experiments. The decay functions were able to adequately model observations of direct viable counts in both media types and at both temperatures (R^2 = 0.87 to 0.96; Fig. 6); however, the exponential decay functions poorly fit the observations for culture counts (Fig. 6). There was a significant difference between culture survival curves at 5^{o} and 25^{o} C (p value <0.05; Tbl. 6). There was no significant difference between survival curves for direct viable counts at the two temperatures (Tbl. 6).

Temperature, time, and method of detection were all significant were all significant factors in the modeling of campylobacter decay ($p<0.05$). Medium (DI *versus* river water) did not have a significant effect on the survival of the bacteria. Pairwise interactions of all effects revealed that medium*time, medium*method, temperature*time, temperature*method, and time*method were significant at the 5% level; the interaction of medium*temperature was not significant.

DISCUSSION

Our objective in this study was to determine the prevalence of campylobacters in a mixed-use watershed and relate that to land-use, season and other environmental parameters. We used traditional culture-based detection methods to identify campylobacters directly from environmental waters (37). The bacteria were detected at all sites during this study and confirmed with simple biochemical tests (catalase and oxidase test) and motility testing (39, 32). This method was more recently used by Diergaardt et al. (12) with comparable results of presumptive *Campylobacter* spp. identification with phenotypic and biochemical identification. However, after 16S rRNA sequence analysis, the majority of the isolates identified biochemically as *Campylobacter* strains actually belonged to the genus *Arcobacter* (12). *A. butzleri* has been reported in drinking water reservoirs, in water treatment plants, rivers and well water (19, 35, 2). In humans *A. butzleri* causes enteritis and occasionally septicemia (48) and risk factors for human infection are similar to those for *Campylobacter* including consumption of contaminated poultry and contaminated water (12).

In the present study campylobacters were found at highest concentrations immediately downstream of a municipal wastewater treatment plant. Sewage and sewage sludge have been shown to contain campylobacters in concentrations of 10^2 to 10^5 CFU 100 ml^{-1} and 10^1 to 10^3 CFU 100 ml^{-1}, respectively (44, 17). Furthermore, studies have shown that *Campylobacter* spp. can survive typical wastewater treatment and can persist within sewage effluent (5, 44). Both *Campylobacter* and *Arcobacter* have been detected in contaminated river water and wastewater samples by culture and molecular direct detection methods (28). Although the numbers detected in the present study are higher than those reported in other surveys, the high loading of anthropogenic and agricultural inputs to this site maybe a factor driving for these high numbers.

The influent received by the WWTP in this study is split evenly between human waste (50%) and poultry processing waste (50%); of the poultry waste approximately 33% is slaughterhouse waste (plant operator, personal communication). The treatment plant's capacity is 6.00 million gallons per day (MGD). Increase in flow is related to changes in the volume received, which is often driven by increased rain (plant operator, personal communication).

The lack of rainfall through the spring of 2004 may be an important factor in the low levels of campylobacter noted during this time, especially by May 2004 when numbers were expected to increase based on the previous year's survey (high levels in early June 2003). In predominately agricultural reaches of the watershed, rainfall was an important environmental driver for campylobacter loading.

In this study campylobacters were more frequently isolated and present in larger numbers during the summer months, which is consistent with trends in clinical cases but was contradicted by low survival rates determined for summer temperatures *in vitro*. In particular the seasonal variation at the WWTP paralleled reported clinical cases of *Campylobacter* infections in Georgia Public Health District 9-2 (which contains the study area) (Fig. 5). During the summer months when case rates reached their maximum, precipitation was high and the WWTP was operating at or above permitted capacity. These results differ from other environmental studies in which detection of campylobacter peaked in late autumn-winter months (5, 21). Given the lower survival rate of *Campylobacter* at warm temperatures *in vitro*, we hypothesize that environmental loading is higher in the summer months. Carriage rates for animals are known to increase in the summer months (29) and therefore loading may be attributed to run-off containing contaminated animal feces. Our results are similar to the seasonal pattern found in chickens and

sewage sludge (20, 51, 33). However, clinical cases show similar summer peaks (31, 25) and human sewage may also contribute to the high numbers of campylobacters noted here.

Testing of waters for fecal indicator bacteria is commonly used as a proxy for the presence of enteric pathogens; however, many studies have demonstrated that indicators are poorly correlated with many pathogens including *Campylobacter* (50, 42). The Georgia standard for fecal coliform bacteria requires that in-stream samples not exceed a geometric mean of 200 CFU 100 ml^{-1} for four samples collected within 30 days (GA DNR EPD Chapter 391-3-6 Water Quality Control revised November 2004). In the present study, *Campylobacter* spp. were significantly ($r = 0.57$, $p<0.05$) correlated with fecal coliform bacteria suggesting that fecal coliforms may be a useful tool in predicting *Campylobacter* presence in environmental waters. However, campylobacters were found in waters below the State standard 56% of the time.

The results of our microcosm study indicate that over time temperature significantly influences the culturability and persistence of *C. jejuni*, regardless of water type. Survival in stationary river water and deionized water microcosms was demonstrated at both 5°C and 25°C. *C. jejuni* remained culturable for up to 10 days at 5°C, whereas bacteria held at 25°C could not be cultured after 5 days. Previous studies have shown that campylobacters survive longer at lower temperatures (23, 13, 45). In this study, viable cells detected by microscopy were noted through day 30 at both temperatures, 6-fold and 12-fold longer than culture counts at 5°C and 25°C, respectively. In all cases total viable counts were higher than culturable counts. This supports the notion that *C. jejuni* cells enter a VBNC state, which may have bearing on environmental surveillance.

CONCLUSION

Campylobacters were frequently present in surface waters receiving human and animal waste, and agriculture runoff; control area with little agricultural inputs showed very few detects. In the present study, campylobacters were detected at the highest concentrations, 595 CFU ml^{-1}, directly downstream from a wastewater treatment plant that processes both human and poultry slaughterhouse waste. While wastewater is driving a significant portion of the campylobacter loading in this watershed, upstream impacts are more likely related to the percentage of land in the drainage used for agriculture. Traditional detection methods may underestimate *C. jejuni* numbers as a method of detection and enumeration and future studies should evaluate *Campylobacter* spp. VBNC state for its potential environmental transmission of disease. Additional work is needed in evaluating factors that influence campylobacter loading in the environment.

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TABLES

Table 1. Description of the sample sites within the Satilla watershed.

	Drainage Area	Landuse (%)			
Site	(km^2)	Row-Crop	Pasture	Forest[a]	Other[b]
1	462	26	6	51	17
2	14	17	5	47	32
3	434	27	6	50	17
4	28	34	3	50	14
5	45	51	1	31	18
6[c]	21	9	1	74	16

[a] Includes deciduous forest, evergreen forest, mixed forest, and forested wetland

[b] Includes open water, transportation, utility swaths, urban, clearcut/sparse vegetation, and golf courses

[c] Control Site, located in the Lower Ocmulgee watershed

Table 2. Mean (minimum, maximum) of fecal coliform CFU 100 ml^{-1}, water quality and nutrient parameters at each site in the Satilla watershed.

Site	Fecal coliform (CFU $100ml^{-1}$)	Temperature (^oC)	Conductivity (mS/cm)[a]	DO (mg/L)	pH [a]	ORP (mV)[a]
1	173	17.6	0.177	7.2	7.21	147
	(15, 570)	(6.2, 25.1)	(0.048, 0.647)[c]	(4.8, 12.2)	(6.64, 7.6)	(63, 356)
2	698	20.7	0.612	7.8	7.21	131
	(0, 5000)	(10.0, 27.9)	(0.279, 0.895)[b]	(3.7, 12.1)	(6.92, 7.47)	(54, 310)
3	121	17.7	0.071	6.8	7.01	164
	(0, 315)	(5.8, 24.5)	(0.045, 0.098)	(3.2, 12.3)	(6.23, 7.8)	(81, 296)
4	186	17.1	0.073	5.2	7.09	217
	(20, 680)	(4.8, 24.9)	(0.05, 0.096)	(0.3, 12.5)	(6.45, 7.75)	(97, 459)
5	248	17.5	0.088	5.7	7.15	226
	(120, 490)	(5.7, 25.1)	(0.07, 0.106)	(2.2, 12.3)	(6.61, 7.52)	(80, 471)
6	55	16.3	0.041	8.2	6.49	227
	(0, 250)	(5.0, 25.3)	(0.037, 0.048)[c]	(2.8, 13.2)	(5.67, 7.39)[b]	(102, 423)
All Sites	249	17.8	0.182	6.9	7.03	183
	(0, 5000)	(4.8, 27.9)	(0.037, 0.895)	(0.3, 13.2)	(5.67, 7.8)	(54, 471)

[a] The P value for the differences between values is <0.05

[b] Value is significantly different at the 0.05 (95%) level from other values for individual site in the same column

[c] Site Comparison is significantly different at the 0.05 (95%) level in the same column

Table 2. Continued…Mean (minimum, maximum) of fecal coliform CFU 100 ml^{-1}, water quality and nutrient parameters at each site in the Satilla watershed continued.

Site	Turbidity (NTU)	NO3-N (mg/L)[a]	Cl (mg/L)[a]	PO4 (mg/L)[a]	NH3-N (mg/L)[a]	Potassium [a]
1	3.5	0.21	18.88	0.17	0.03	6.7
	(1.1, 10.0)	(0.00, 0.84)	(5.83, 52.15)	(0.01, 1.30)	(0.00, 0.06)	(1.4, 23.8)
2	2.0	8.06	2.63	56.75	2.78	0.3
	(0.0, 8.0)	(0.90, 7.14)[c]	(19.80, 88.00)[c]	(0.23, 6.61)[c]	(0.02, 1.48)[c]	(4.4, 33.8)[c]
3	3.8	0.16	9.83	0.10	0.04	3.2
	(1.0, 9.3)	(0.01, 0.86)	(3.77, 13.40)	(0.00, 0.99)	(0.01, 0.20)	(2.2, 4.4)
4	2.9	0.2	9.69	0.02	0.07	2.3
	(0.7, 6.3)	(0.00, 1.00)	(3.63, 13.30)	(0.00, 0.06)	(0.01, 0.21)	(1.0, 3.0)
5	3.2	0.19	13.28	0.01	0.07	2.8
	(1.0, 6.5)	(0.00, 0.87)	(7.91, 21.55)	(0.00, 0.06)	(0.01, 0.33)	(0.4, 3.6)
6	3.5	0.11	6.72	0.03	0.02	1.4
	(0.0, 10.0)	(0.00, 0.90)	(2.77, 8.95)	(0.00, 0.98)	(0.01, 0.04)	(0.8, 1.8)
All Sites	3.1	0.6	19.49	0.54	0.09	6.2
	(0.0, 10.0)	(0.00, 7.14)	(2.77, 88.00)	(0.00, 6.61)	(0.00, 1.48)	(0.4, 33.8)

[b] Value is significantly different at the 0.05 (95%) level from other values for individual site in the same column

[c] Site Comparison is significantly different at the 0.05 (95%) level in the same column

Table 3. Mean (± standard deviation) of water quality parameters at each season. Only parameters having significant ($p < 0.05$) differences are shown.

Season	Temperature ^{o}C	DO mg/L	pH	ORP	Turbidity NTU
Summer	23.8 (± 2.6)^{a}	4.85 (± 1.94)^{c}	6.77 (± 0.36)^{b}	211 (± 55)^{b}	4.4 (± 2.0)^{a}
Fall	13.0 (± 2.9)^{c}	7.51 (± 1.43)^{ab}	7.20 (± 0.42)^{ab}	194 (± 53)^{ab}	1.3 (± 0.7)^{ba}
Winter	10.3 (± 3.0)^{c}	9.68 (± 2.69)^{a}	7.42 (± 3.04)^{a}	104 (± 88)^{a}	2.6 (± 0.1)^{b}
Spring	19.4 (± 6.3)^{b}	6.32 (± 2.39)^{bc}	6.89 (± 0.36)^{ab}	209 (± 47)^{ab}	3.4 (± 1.0)^{a}

$^{a\,b\,c}$ Values with same letter are NOT significantly different at the 0.05 (95%) level in the same column

Table 4. Summary statistics of campylobacter by site, limit of detection = 10 CFU ml^{-1}

Site	Mean (minimum, maximum) (CFU ml^{-1})	% + for *Campylobacter*	N
1	65 (0, 325)[ab]	64%	14
2	158 (0, 595)[a]	93%	14
3	9 (0, 60)[b]	29%	14
4	30 (0, 115)[b]	50%	14
5	10 (0, 50)[b]	62%	13
6	2 (0, 10)[b]	15%	13

[a b] Values with same letter are NOT significantly different at the 0.05 (95%) level

Table 5. Correlations between water quality variables and campylobacter (significant values only)

WQ parameter	Spearman r	p
Fecal coliform (CFU $100ml^{-1}$)	0.36	0.0016
Temp (^{o}C)	0.45	< 0.0001
Conductivity (mS/cm)	0.42	0.0002
Chlorophyll *a* (mg/L)	0.24	0.0433
Fluorescence (%)	0.23	0.0479
NO_3^--N (mg/L)	0.34	0.0034
Cl (mg/L)	0.34	0.0033
PO_4^{3-} (mg/L)	0.40	0.0004
Potassium (mg/L)	0.34	0.0033
DIC (mg/L)	0.25	0.0344

Table 6. *Campylobacter jejuni in vitro* survival study direct counts one Log exponential decay.

Temperature (^{o}C)	Medium	Method	Decay formula	R square value
5	DI	Culture[a]	$y = 7.87\ e^{(-0.04663x)} - 2.74$	0.57
		Direct[b]	$y = 14.19\ e^{(-0.9847x)} + 7.30$	0.87
5	River	Culture[a]	$y = 7.40\ e^{(-0.06076x)} - 1.96$	0.59
		Direct[b]	$y = 16.18\ e^{(-1.157x)} + 7.64$	0.90
25	DI	Culture[a]	$y = 2.58\ e^{(-0.1713x)} - 0.20$	0.42
		Direct[b]	$y = 12.23\ e^{(-0.8569x)} + 7.19$	0.94
25	River	Culture[a]	$y = 7.80\ e^{(-0.5892x)} + 0.02$	0.82
		Direct[b]	$y = 14.42\ e^{(-1.136x)} + 7.62$	0.96

[a] Culture, significant difference between the curves ($p < 0.05$)

[b] Direct, no significant difference between the curves

FIGURE LEGENDS

Figure 1: Sampling site locations within the Satilla watershed in southeastern Georgia (USA).

Figure 2: Seasonal variation of Campylobacter.

Figure 3: Maximum daily rainfall in the month preceding sample date and Campylobacter concentration at all sites. Spearman r 0.41, p 0.0003

Figure 4: Seven day maximum rainfall preceding sample date and Campylobacter concentration at site 3. Spearman r 0.84, p 0.0006

Figure 5: Mean Campylobacter CFU ml^{-1} detected at Site 2 and WWTP flow (MGD).

Figure 6: *Campylobacter jejuni* survival in deionized (DI) and River water microcosms at $5^{o}C$ and $25^{o}C$ detected with culture and direct detection methods.

Figure 7: Campylobacter detected at Site 2 (WWTP) and Campylobacter cases in Georgia Public Health (PH) District 9-2 (Southeast).

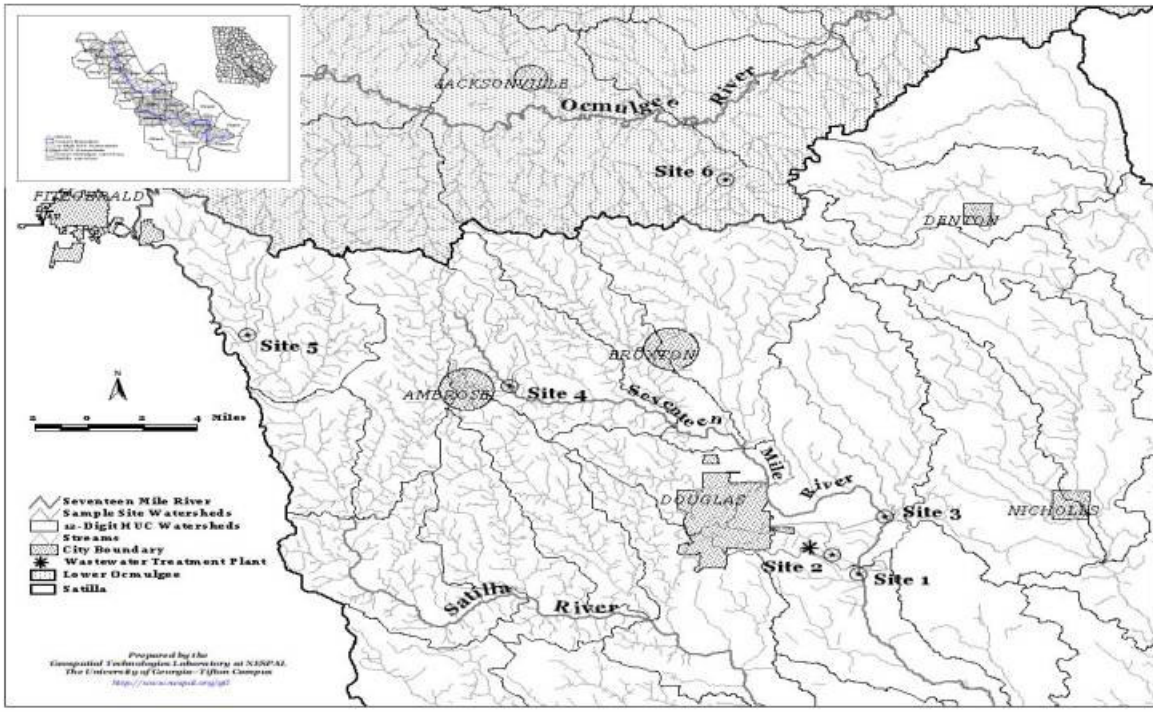
JACKSONVILLE
Ocmulgee River
Site 6
FITZGERALD
DENTON
Site 5
BROXTON
AMBROSE
Site 4
Seventeen Mile River
DOUGLAS
Site 3
NICHOLLS
Site 2
Site 1
Satilla River
Seventeen Mile River
Sample Site Watersheds
12-Digit HUC Watersheds
Streams
City Boundary
Wastewater Treatment Plant
Lower Ocmulgee
Satilla
Miles

Fig. 1.

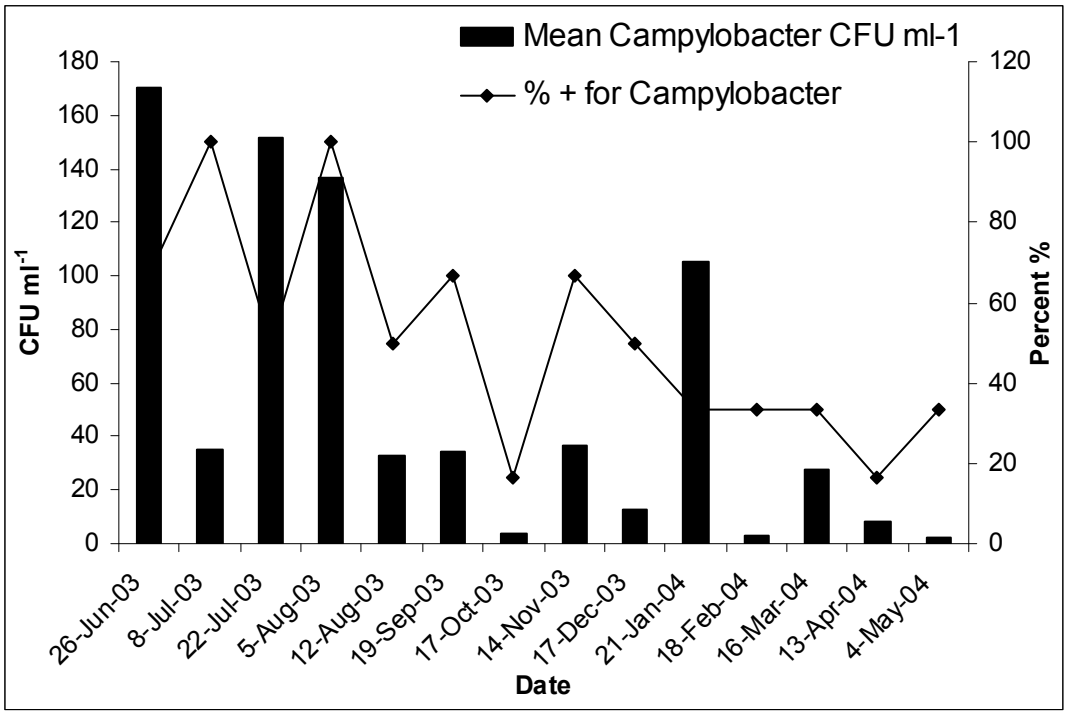
Mean Campylobacter CFU ml-1
% + for Campylobacter
CFU ml-1
Percent %
Date
180
160
140
120
100
80
60
40
20
0
120
100
80
60
40
20
0
26-Jun-03
8-Jul-03
22-Jul-03
5-Aug-03
12-Aug-03
19-Sep-03
17-Oct-03
14-Nov-03
17-Dec-03
21-Jan-04
18-Feb-04
16-Mar-04
13-Apr-04
4-May-04

Fig. 2.

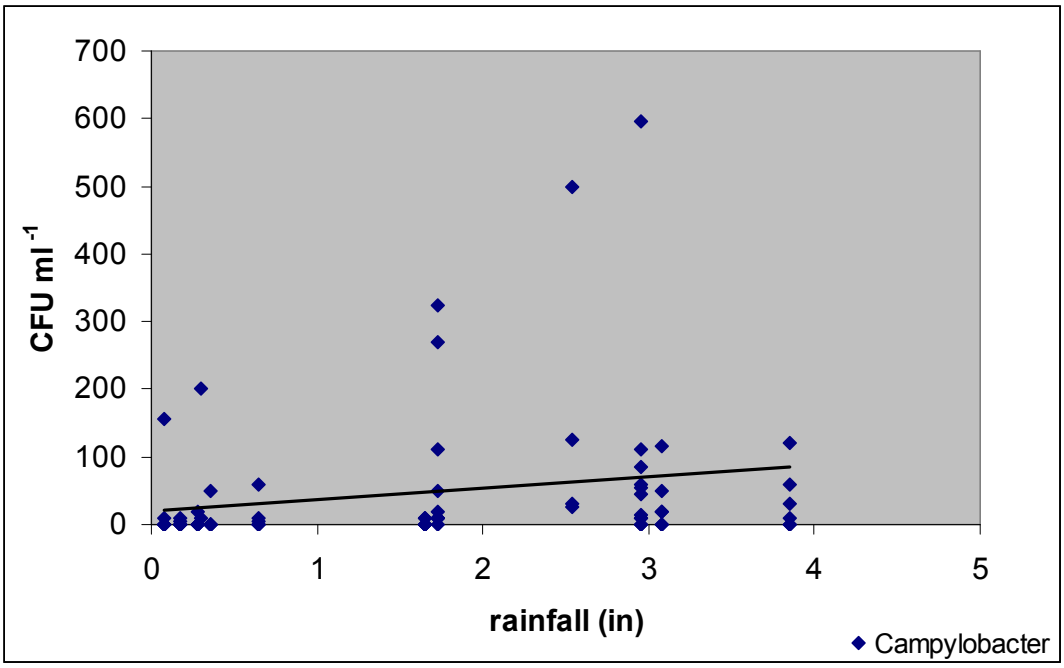
700
600
500
400
300
200
100
0
CFU ml -1
0
1
2
3
4
5
rainfall (in)
Campylobacter

Fig. 3.

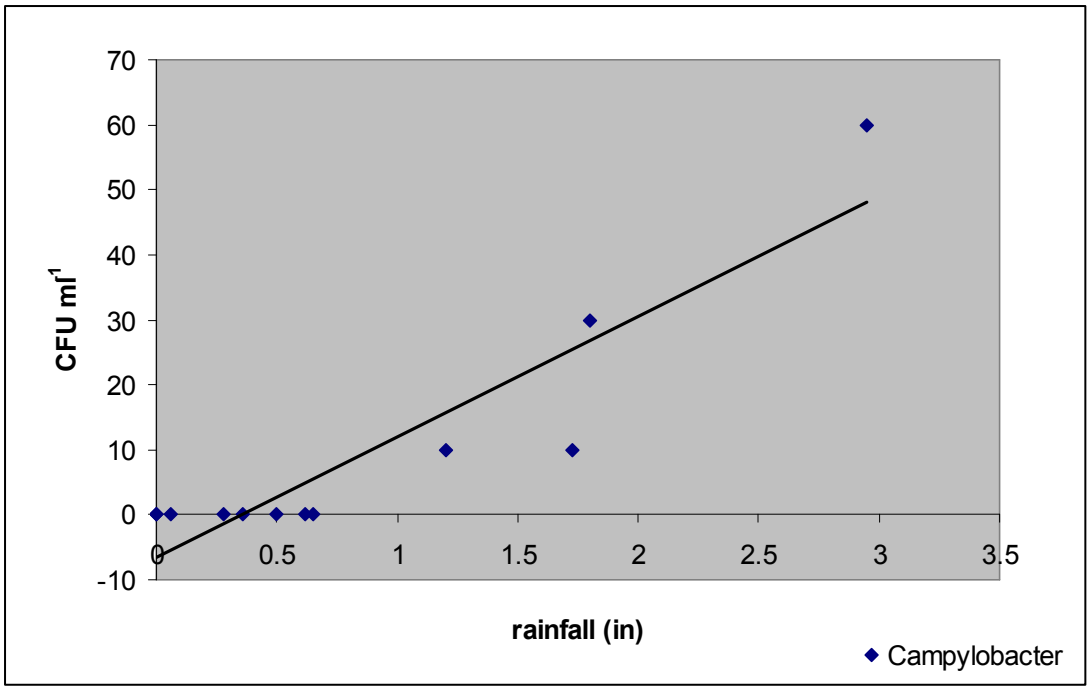
70
60
50
40
30
20
10
0
-10
CFU ml-1
0
0.5
1
1.5
2
2.5
3
3.5
rainfall (in)
Campylobacter

Fig. 4.

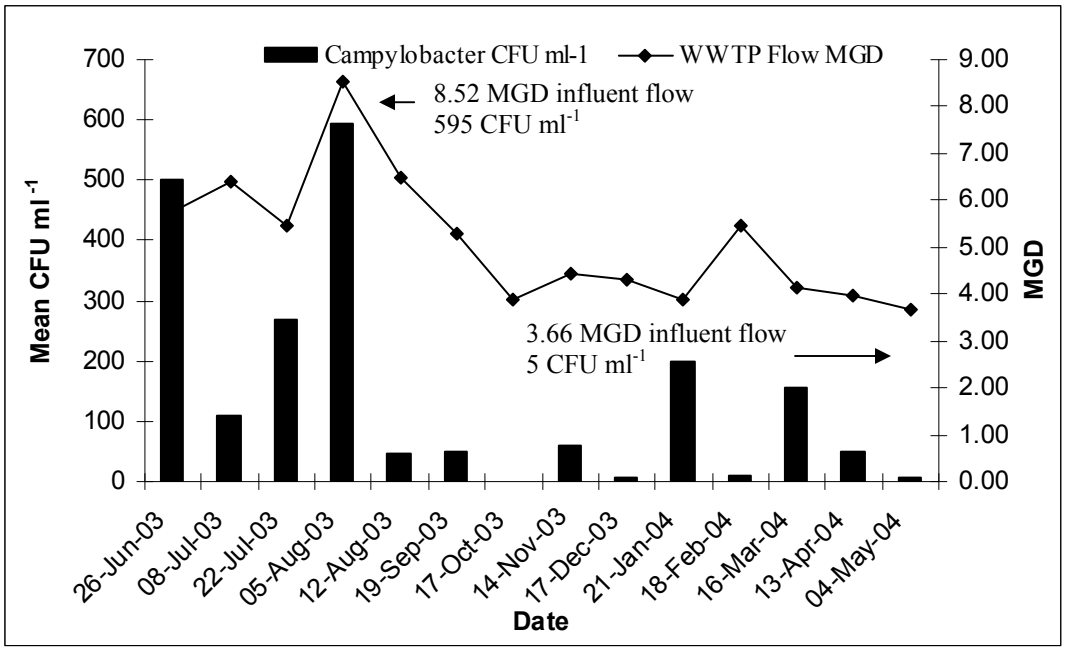
Campylobacter CFU ml-1
WWTP Flow MGD
8.52 MGD influent flow
595 CFU ml-1
3.66 MGD influent flow
5 CFU ml-1
Mean CFU ml-1
MGD
Date
700
600
500
400
300
200
100
0
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
26-Jun-03
08-Jul-03
22-Jul-03
05-Aug-03
12-Aug-03
19-Sep-03
17-Oct-03
14-Nov-03
17-Dec-03
21-Jan-04
18-Feb-04
16-Mar-04
13-Apr-04
04-May-04

Fig. 5.

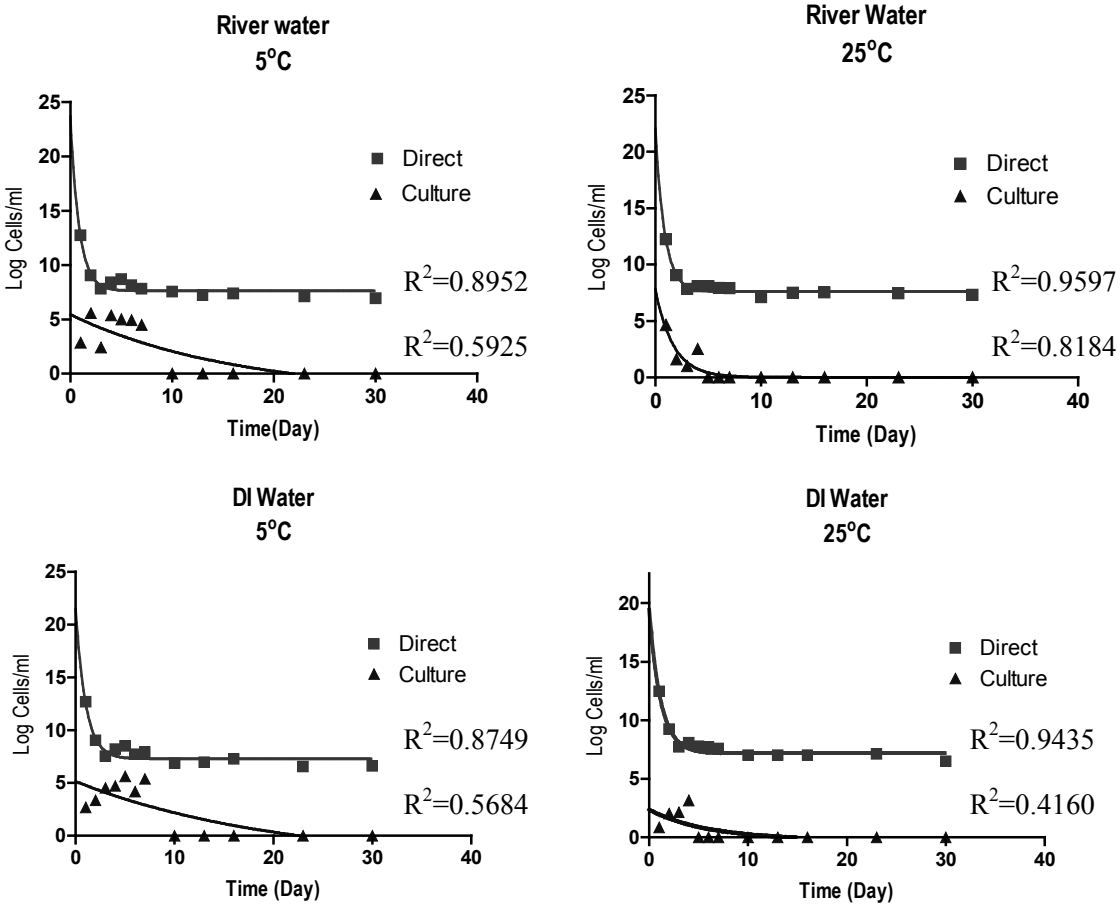
River water
5°C
Log Cells/ml
Time(Day)
Direct
Culture
R2=0.8952
R2=0.5925
River Water
25°C
Log Cells/ml
Time (Day)
Direct
Culture
R2=0.9597
R2=0.8184
DI Water
5°C
Log Cells/ml
Time (Day)
Direct
Culture
R2=0.8749
R2=0.5684
DI Water
25°C
Log Cells/ml
Time (Day)
Direct
Culture
R2=0.9435
R2=0.4160

Fig. 6.

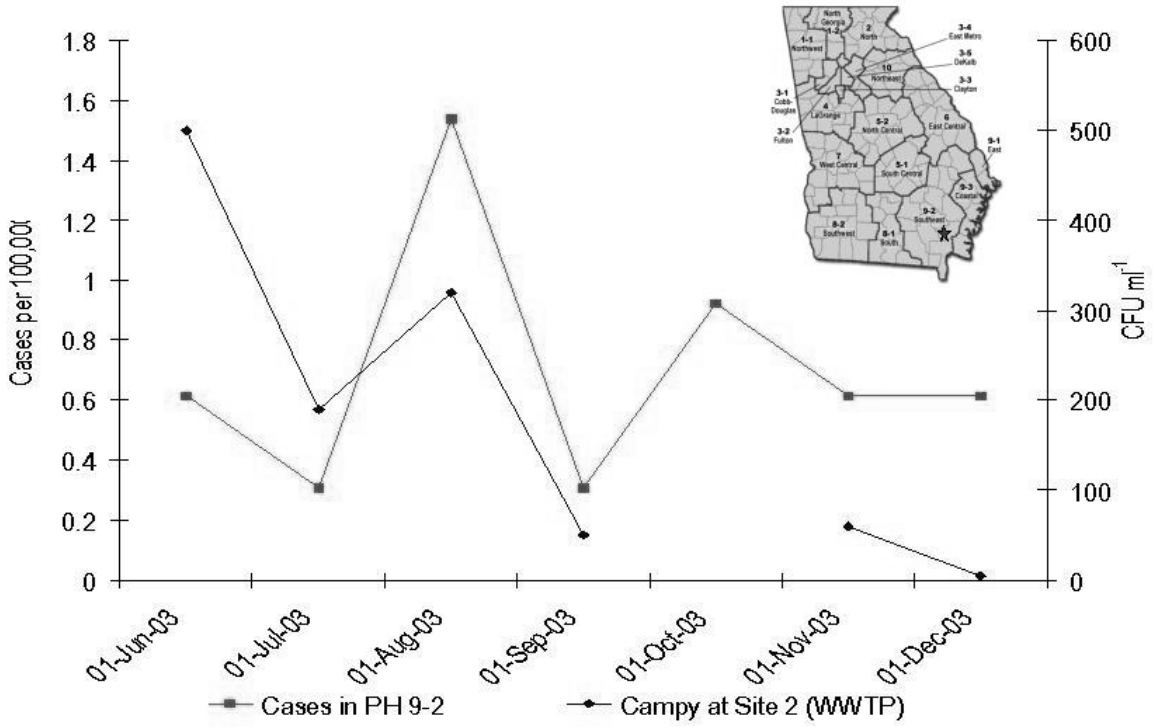
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Cases per 100,00(
600
500
400
300
200
100
0
CFU ml-1
01-Jun-03
01-Jul-03
01-Aug-03
01-Sep-03
01-Oct-03
01-Nov-03
01-Dec-03
Cases in PH 9-2
Campy at Site 2 (WWTP)

Fig. 7.

CHAPTER 4

SUMMARY AND CONCLUSIONS

The genus *Campylobacter* comprises a group of closely related gram-negative bacteria that primarily colonize the gastrointestinal tracts of a wide variety of host species. Some of these bacteria are commensals in birds (6), but many, particularly *Campylobacter jejuni*, and its close relative *Campylobacter coli*, are enteric pathogens of humans and wild, domestic, and domesticated animals (6, 7). Although it is widely assumed that campylobacteriosis is primarily a food-borne disease, the majority of infections are sporadic, and the sources of infection are rarely determined (8). Additionally, in the developed world, *Campylobacter* isolations typically increase during the summer and fall months (1). Water also is potentially an important reservoir of the thermophilic campylobacters and is an established vehicle for the transmission of these organisms to man and domestic animals (2, 4, 3). Although, studies have shown campylobacters to be common in natural waters such as streams, rivers, and lakes the seasonality exhibited in these environmental detections primarily occur during the fall and winter months (3, 5) in contrast to the seasonality of campylobacter enteritis.

The objectives of this study were to assess potential drivers for environmental loading, seasonality, and persistence of thermotolerant campylobacters in streams of southeast Georgia (USA) and begin to relate these to clinical burden in the region.

The results of this study indicate that campylobacters were detected frequently throughout the watershed. Campylobacters were more frequently isolated and present in larger numbers during the summer months, which is consistent with trends in clinical cases but was

contradicted by low survival rates determined for summer temperatures *in vitro*. Campylobacters were found at highest concentrations immediately downstream of a municipal wastewater treatment plant and clinical cases of *Campylobacter* within the Southeastern Public Health District in the study area showed similar seasonal trends to culturable counts at this site**;** suggesting a tentative association between human illness and loading in the watershed. The apparent discrepancy between field data and controlled survival microcosms indicates that loading of campylobacters in the watershed is much higher in the summer months and may be related to shedding rates in agricultural animals or a higher likelihood of camylobacters in human sewage. Finally, data from our *in vitro* survival study suggest that over time temperature significantly influences the culturability and persistence of *C. jejuni*, regardless of water type. Traditional detection methods may underestimate *C. jejuni* numbers as a method of detection and enumeration and future studies should evaluate *Campylobacter* spp. VBNC state for its potential environmental transmission of disease. Additional work is needed in evaluating factors that influence campylobacter loading in the environment.

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