ABSTRACT

BOLINGER, HANNAH K. Emerging Trends of Antibiotic Resistance and Risk Factors for *Campylobacter* spp. in Commercially Produced Turkey Flocks (Under the direction of Dr. Sophia Kathariou).

Campylobacter is a common foodborne pathogen often linked to poultry. Infections are generally self-limiting, however some may require treatment with antibiotics, like macrolides. Resistance to macrolides is commonly conferred by substitutions in the 23S rRNA gene and frequently encountered in *C. coli.* In 2014 a macrolide resistance determinant new to *Campylobacter, erm*(B), was reported. To date, *erm*(B) has been largely confined to China, though the true prevalence remains poorly characterized. This study sought to determine the source of macrolide resistance in *Campylobacter* from turkeys and flies. *erm*(B) was absent in178 erythromycin-resistant *Campylobacter* and sequence analysis of the 23S rRNA gene of 48 isolates indicated that all harbored the A2075G substitution, including the resistance noted in *C. jejuni.* Gentamicin is another viable antibiotic therapy and resistance in the US has been uncommon. However, resistance rose sharply between 2007 and 2011. In this study, 361 *Campylobacter* isolates were evaluated for their ability to grow in 50 μg/ml gentamicin and for the presence of *aph(2")-If* or *aph(2")-Ig*, two gentamicin-resistance determinants. Of 362 gentamicin-resistant isolates, 352 carried *aph(2")-If* while only eight carried *aph(2")-Ig.* All resistant isolates from North Carolina carried *aph(2")-If* while all isolates harboring *aph(2")-Ig* were from Arkansas. No isolates from a smaller panel of gentamicin-susceptible isolates carried either of these resistance genes.

Risk factor analyses have identified actions on the farm that increase the risk of *Campylobacter* carriage in broiler flocks with fewer reports in turkey flocks. Thus, routes of transmission to turkeys is still largely undetermined. This study aimed to characterize *Campylobacter* from flies, one identified risk factor, and feces to determine similarities between

the two samples. Flies and turkey feces were sampled for *Campylobacter* and subsequent isolates were characterized by species and antimicrobial resistances; representative isolates were typed via multilocus sequence typing. On all farms where feces were *Campylobacter*-positive the flies were as well, and vice versa. Flies were more likely to carry *C. coli* and turkeys *C. jejuni*. Multidrug-resistant strains were the most commonly isolated strains from both sample types, and flies were found to carry a more diverse population of *Campylobacter*. Finally, the presence of certain strains in either sample type was predictive of its presence in the other sample type at the same farm. Fly populations, *Campylobacter*-positive broiler flocks, and the incidence of campylobacteriosis follow seasonal trends, so an analysis was performed to evaluate the relationship between weather and *Campylobacter* in turkey flocks. Weather variables were recorded for five weeks prior to flock sampling resulting in a final model containing an indication of cloud cover and dew temperature with a random farm effect. Surveys were sent to farm managers requesting details of the farm to assess which actions were risk factors for *Campylobacter* in the flocks. Audits were performed to verify information and collect more data. These audits and surveys resulted in a final model with fixed effects of season and barrier and a random farm effect.

Finally, public knowledge of *Campylobacter* is far lower than for pathogens like *Salmonella* and *Escherichia coli*. A survey was distributed to students enrolled in Food Microbiology to assess their knowledge of *Campylobacter.* More than half of students responded positively to having heard of *Campylobacter,* but there were major knowledge gaps regarding routes of transmission and risky foods. A Twitter campaign was started in 2015 to disseminate information on *Campylobacter* and a website, campylobacter.fbns.ncsu.edu, was created where articles regarding *Campylobacter* were written and posted. In addition, several articles were

published in trade magazines focused on food safety and poultry production in an attempt to increase knowledge of *Campylobacter* in the general public as well as in poultry production circles.

Emerging trends of antibiotic resistance and risk factors for *Campylobacter* spp. in commercially produced turkey flocks.

by
Hannah K Bolinger

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Food Science

Raleigh, North Carolina

2017

APPROVED BY:

_____	_____
Sophia Kathariou	Donna Carver
Committee Co-Chair	

_____	_____	_____
Benjamin Chapman	Andrew Binder	Jonathan Allen

DEDICATION

To my husband, Pierce. Thank you for seeing me through this, being eternally patient with me, and always telling me that I can do it. I love you.

BIOGRAPHY

Hannah Bolinger was born and raised in Bartlesville, Oklahoma, a fact which she is very proud of. She graduated with her bachelor's degree in Chemistry from the University of Oklahoma where she was able to spend her senior year performing research with the Natural Products Discovery Group. Always a lover of food, health, and nutrition, an undergraduate Biochemistry course sparked her interest in pursuing a master's degree in Nutritional Biochemistry and Physiology from Rutgers University. At Rutgers and under the mentorship of Dr. Don Schaffner, she studied the survival of bacterial species on surfaces for the purpose of developing an alternative diagnostic for Norovirus and was awarded a NoroCORE fellowship. Hannah fell in love with North Carolina during a summer internship at NCSU and decided to apply there for her PhD. She was thrilled to be accepted into the lab of Dr. Sophia Kathariou in 2014. Her doctoral work focused on emerging antibiotic resistance trends in *Campylobacter* sourced from commercial turkey farms as well as identifying risk factors for *Campylobacter* colonization of commercial turkey flocks.

ACKNOWLEDGMENTS

I will be forever grateful for the opportunity to pursue my doctoral work at North Carolina State University. Dr. Sophia Kathariou has taught me to look at the world through the eyes of an ever-curious scientist and offered me encouragement along the way. Dr. Donna Carver has taught me the value of approaching risk assessments in a pragmatic, thoughtful way and the importance of understanding your data.

I would like to offer a very special thank you to Jeffrey Niedermeyer who, in addition to becoming my close friend, has worked without tire to support not only my experiments, but those of all the students in the lab. He really needs to take the GRE.

To my incredible labmates, Cameron, Victor, Midya, Fan, Meg, Robert, and Kartheek. I want you to know that you have become my second family, and I can only thank you all for your encouragement, patience, and brilliance. I am lucky to know you.

Finally, I need to acknowledge the support that my canine companion Bianca has provided. As the only other Okie I knew on the East Coast, she has always provided me with a sense of home. She has seen me through my bachelor's, master's, and now doctoral degrees, moved with me on an almost yearly basis for the past decade, and I can't image how I would have done it without her.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1 Literature Review - *Campylobacter* as a Public-Health Threat

The genus

Campylobacter spp. were first reported in the early 19th century by Theodore Escherich, who observed a non-cultivable spiral shaped organism in the intestinal tracts of children suffering from "cholera infantum" (Epps et al., 2013). Other accounts came from veterinarians who observed it in animals and described it as a "vibrio-like" organism. In fact *C. jejuni,* the species responsible for the majority of human cases, has gone by the names *V. fetus, C. fetus* subsp. *jejuni*, and others until 1963 when Seabald and Vernon proposed changing the name from *Vibrio* to *Campylobacter,* from the Greek word for curved rod (Epps et al., 2013; Ketley, 1997; Park, 2002).

Campylobacter are Gram-negative bacteria, 0.5 to 8 μm long with a characteristic curved, spiral, or S-shaped cell, sometimes referred to as "gull-shaped" due to the likeness of children's drawings of birds (Perez-Perez, Medical Microbiolgoy 4th edition). Characteristically rapid, this bacterium is motile due to a single polar unsheathed flagellum at one or both ends. Additionally, the guanine-plus-cytosine content of the genome is rather low ranging from 28% to 38% (Park, 2002; Penner, 1988; Veron & Chatelain, 1973). Historically, it was rather difficult to distinguish between the two thermophilic *Campylobacter* spp. responsible for the majority of human cases, *C. coli* and *C. jejuni*. Both species are unable to utilize carbohydrates and use amino acids and Krebs cycle intermediates in their respiration (Guccione et al., 2008; Hoffman & Goodman, 1982; SMIBERT, 1978; Westfall, Rollins, & Weiss, 1986). However, the majority of *C. jejuni* strains are able to hydrolyze hippurate which has become the main distinction between these two species (Epps et al., 2013; Harvey, 1980; Penner, 1988). Hippuricase is the enzyme responsible for the hydrolysis of hippurate and PCR can be used to target the gene that encodes it to identify *C. jejuni* (Harvey, 1980; Houng et al., 2001; Penner, 1988).

Campylobacter spp. are known as fastidious organisms requiring a very specific environment for growth. *C. coli* and *C. jejuni* grow best at 42°C and will not grow below 30°C. They also require microaerobic conditions of less than 15% oxygen (Ketley, 1997; Park, 2002). Additionally, they are quite susceptible to many environmental conditions such as high salt and low pH. They are sensitive to drying and subsequently do not survive for long periods of time on dry surfaces. Just 2% sodium chloride is enough to inhibit their growth as they are quite sensitive to osmotic stress, and their growth is inhibited at a pH below 4.9 (Park, 2002).

Total human illnesses

Despite *Campylobacter's* fastidious nature, they are one of the most common causes of bacterial foodborne illness. Of the 37.2 million foodborne illnesses every year in the US, *Campylobacter* spp. are responsible for 9%, or 800,000 cases (Elaine Scallan et al., 2011). However, illnesses with *Campylobacter* spp. are thought to be under-reported and may be as high as 1.3 million cases annually in the US (CDC 2017). *Campylobacter* is responsible for 15% of hospitalizations due to foodborne disease, and 90% of campylobacteriosis cases are caused by *C. jejuni* with close to 10% being caused by *C. coli* (*NARMS Integrated Report : 2012-2013*, 2013). Campylobacteriosis is normally self-limiting and characterized by watery diarrhea which may or may not be bloody, severe abdominal cramps, nausea, and fever lasting from three to seven days (Centers for Disease Control and Prevention, 2014b).

While the majority of people recover from a *Campylobacter* infection without the need for antibiotics, certain populations are at a higher risk for severe infection, and immune-compromised populations are more likely to require treatment with antibiotics such as macrolides (i.e. erythromycin and azithromycin) (Centers for Disease Control and Prevention,

2013a). Additionally, *C. jejuni* is recognized to be the most common trigger for Guillain-Barre Syndrome (GBS), the most common cause of acute, flaccid paralysis since the near-eradication of Polio (Nachamkin, Allos, & Ho, 1998; Yang, Lian, Liu, Wu, & Duan, 2016; Yuki & Hartung, 2012). GBS is characterized by a loss of sensation, weakness, pain, paralysis, or a combination thereof in the limbs over a period of 12 hours to 28 days (Sejvar et al., 2011). Estimations of the occurrence of GBS following a *Campylobacter* infection range from as low as 1 to as high as 100 per 100,000 cases (*Centers for Disease Control and Prevention 2017*; E. Scallan, Hoekstra, Mahon, Jones, & Griffin, 2015).

Routes of transmission

The most common vehicles for campylobacteriosis are poultry, untreated water, and unpasteurized milk are (Gillespie et al., 2002; Hopkins & Scott, 1983; T. Humphrey, O'Brien, & Madsen, 2007; Pires, Vigre, Makela, & Hald, 2010; E. V. Taylor et al., 2012). Multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) have been used to verify that isolates from poultry, sheep, and cattle are identical to those that have caused illness in humans (Kärenlampi, Rautelin, Hakkinen, & Hänninen, 2003; Kramer, Frost, Bolton, & Wareing, 2000; Tyson et al., 2016). *C. jejuni* and *C. coli* are thermophilic and grow best at 42°C, the body temperature of poultry. Both the European Authority on Food Safety and the CDC agree that eating undercooked poultry, or cooked poultry that has been exposed to raw product is the most common source of infection (Centers for Disease Control and Prevention, 2014a; European Food Safety Authority, 2014). Infections are usually sporadic and large outbreaks are rare (CDC, 2015). However, when campylobacteriosis outbreaks do occur, raw milk or untreated water are the most common vehicles of transmission (Lahti et al., 2017).

Campylobacter prevalence in poultry

As previously mentioned, the handling or consumption of poultry has been found to be associated with *Campylobacter* infections (Hopkins & Scott, 1983). Numerous studies worldwide report that *Campylobacter* is extremely common in conventional poultry flocks (Lawes, J.R., Vidal, A., Clifton-Hadley, F.A., Sayers, R., Rodgers, J., Snow, L., Evans, S.J., Powell, 2012; Torralbo et al., 2014; Wallace, Stanley, & Jones, 1998; Wright et al., 2008), and an analysis of organic turkey production in Germany found that 147 (98%) of the 150 birds tested were positive for *Campylobacter* via cloacal swabbing (Fawzy, Metwaly, Adawy, Hotzel, & Tomaso, 2016). Recent reports in the U.S. indicate isolation of *Campylobacter* from retail chicken meat between 33-52% of retail chicken and 6.1% of turkey ceca (US Food and Drug Administration, 2014). However, as most studies focus on *Campylobacter* in broilers, there is a knowledge gap regarding the prevalence of *Campylobacter* in turkeys.

Review of quantitative risk assessments of *Campylobacter* in poultry

Numerous quantitative microbial risk assessments (QMRA) have identified risk factors for *Campylobacter* colonization of commercial poultry flocks. However, the majority of these have only examined broiler flocks, with far fewer studies looking at commercial turkey production (Cardinale, Tall, Guèye, Cisse, & Salvat, 2004; Kapperud et al., 1993; Newell et al., 2011; Sommer et al., 2016; a W. van de Giessen, Bloemberg, Ritmeester, & Tilburg, 1996). Additionally, there are differences in production based on the specific company, country, or geographic location within a country that adds to the difficulty in interpreting QMRAs. However, the literature does indicate a consensus for some factors. Manure management,

livestock or other animals, water chlorination, and management practices have all been cited as important variables in keeping flocks *Campylobacter* free (Guerin et al., 2007).

A number of studies have identified the age of the broiler flock to be positively associated with the risk of being positive for *Campylobacter* (Berndtson, Emanuelson, Engvall, & Danielsson-Tham, 1996; Bouwknegt et al., 2004). One explanation might be that the birds will have more opportunity for exposure the longer they are alive and may be especially important in turkey production due to their longer life spans. Another risk factor would be hiring external employees as has been found in previous studies (Agunos, Waddell, Léger, & Taboada, 2014; Newell & Fearnley, 2003a). Properly cleaning and disinfecting poultry houses, especially between flocks, is another commonly cited protective action that poultry growers can take against *Campylobacter* (Berndtson et al., 1996; Evans & Sayers, 2000; a W. van de Giessen et al., 1996). However, the ability to properly clean a poultry-house may be related to the age and maintenance of that house, as buildings that are newer or in good repair can have fewer flocks become colonized, however not all studies found this association (Berndtson et al., 1996; Cardinale et al., 2004; Evans & Sayers, 2000; Sommer et al., 2016).

Changing separate boots, clean coveralls, and using hand sanitizer between each house has a protective effect as *Campylobacter* can be tracked between houses on these items (Agunos et al., 2014; A. van de Giessen et al., 1992; a W. van de Giessen et al., 1996). In fact, *Campylobacter* isolated from broilers has been found to be genetically identical *Campylobacter* recovered on the boots of farm workers (Agunos et al., 2014). Additionally, birds are at a higher risk of becoming positive if adjacent houses already are (Agunos et al., 2014; a W. van de Giessen et al., 1996).

The presence of other animals on the farms is also a risk factor as *Campylobacter* thrives in the intestinal tracts of many mammals as well as poultry (Agunos et al., 2014; A. van de Giessen et al., 1992; a W. van de Giessen et al., 1996). Controlling rodents on the farm is sometimes cited as an important measure to reduce the risk of *Campylobacter,* but there are multiple ways to measure this variable and not all studies find significance (Berndtson et al., 1996; Evans & Sayers, 2000; Kapperud et al., 1993; Sommer et al., 2016; Torralbo et al., 2014). In fact, at least one study found a negative association between controlling for rodents and the risk of *Campylobacter* (Arsenault, Letellier, Quessy, Normand, & Boulianne, 2007). Additionally, rodents trapped on farms are not always positive for *Campylobacter* thus making it unlikely they would be spreading this bacterium (Gregory, Barnhart, Dreesen, Stern, & Corn, 1997).

While insects like flies and darkling beetles are not necessarily always carriers of *Campylobacter* (Gregory et al., 1997)*,* the literature strongly suggests that they are an important vector of transmission (Ekdahl, Normann, & Andersson, 2005; Birt Hald, Sommer, & Skovgard, 2007; Nichols, 2005). For example, the use of fly screens to decrease fly populations in the houses has been identified to have a protective effect (Birt Hald et al., 2007; Sommer et al., 2016). Several studies have isolated and investigated *Campylobacter* from flies, and *C. jejuni* has been shown to be transferrable from a *Campylobacter*-positive flock to flies and then onward to a *Campylobacter*-negative flock(Birthe Hald et al., 2004; Rosef & Kapperud, 1983; Shane, Montrose, & Harrington, 1985). Additionally, MLST analysis has identified strains of *Campylobacter* from flies can match the strains from broiler-derived isolates (Royden et al., 2016). Several studies have investigated the relationship between fly populations, season, and

incidence of campylobacteriosis (Ekdahl et al., 2005; Nichols, 2005; Wagenaar, J.A. Newell, D.G., Kalupahana, R.S., Mughini-Gras, 2015).

Season and climatic events have been identified in several studies to affect the prevalence of *Campylobacter* in poultry flocks, although to the best of the author's knowledge no studies have specifically investigated climate's effect on turkey carriage of *Campylobacter.* Heavy rainfall has been identified (Jonsson, Chriél, Norström, & Hofshagen, 2012) as increasing the risk of *Campylobacter* in broiler flocks*,* possibly through tracking of mud into poultry houses. Sky conditions appear to strongly affect the survival of *Campylobacter* as sunny days appear to inhibit the recovery of *Campylobacter* from environmental samples compared to samples taken on rainy or cloudy days (Hansson, Vågsholm, Svensson, & Olsson Engvall, 2007). Additionally, warmer weather appears to be favorable to the survival of *Campylobacter* (Jonsson et al., 2012) as more campylobacteriosis is reported during warmer weeks (Tam, Rodrigues, O'Brien, & Hajat, 2006).

Antimicrobial resistance in the food chain

Increases in antimicrobial resistance in *Campylobacter* is a rising threat and the continued use of whole-genome sequencing (WGS) has allowed the identification of several new determinants of resistance in *Campylobacter* (Y. Chen et al., 2013; Miller, Huynh, Parker, & Niedermeyer, 2016; S. Qin et al., 2014). In 2013, the CDC produced a report in which they named *Campylobacter* as a "microorganism with a threat level serious", the same threat level given to organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Centers for Disease Control and Prevention, 2013a). Specifically, concerns regarding ciprofloxacin, tetracycline, and/or

azithromycin-resistant *Campylobacter* have been raised (Centers for Disease Control and Prevention, 2013a; Organization, 2012).

The use of antimicrobials in animal-production industry is one venue by which *Campylobacter* may be experiencing a selective pressure. One recent example of this is the use of enrofloxacin and subsequent rise of fluoroquinolone resistance in *Campylobacter* (Endtz et al., 1991). Until FDA banned enrofloxacin in 2005, it was approved for use in broilers and turkeys to control mortality associated with *E. coli* and *Pasterella multocida (US Food and Drug Adminimstration, 2005). According to FDA's own website, the Center for Veterinary Medicine (CVM) determined that "the use of fluoroquinolones in poultry caused the development of fluoroquinolone-resistant Campylobacter species in poultry* that these fluoroquinolone-resistant organisms are transferred to humans and cause the development of fluoroquinolone-resistant *Campylobacter* in humans and fluoroquinolone-resistant *Campylobacter* infections in humans are a health hazard." (US Food and Drug Adminimstration, 2005)

As previously mentioned, macrolides, like erythromycin and azithromycin, are the drug of choice for treating severe campylobacteriosis infections. Macrolides function in the same manner as lincosamides, which are approved for treating illness in food-production animals (*NARMS Integrated Report : 2012-2013*, 2013). Because of their shared mechanism of action, resistance to one class confers resistance to the other. Macrolide-resistance is uncommon in *C. jejuni*, but is commonly encountered in *C. coli,* and between the years 2011 and 2012 the number of macrolide-resistant *C. coli* isolated doubled (US Food and Drug Administration, 2014).

Another example of an antibiotic's use that is possibly selecting toward antibiotic resistance may be gentamicin, an aminoglycoside. CFR Title 21 Sec. 529.1044b allows a gentamicin solution for dipping turkey eggs as long as the eggs do not go toward human

consumption (current as of April 1, 2017) (FDA CFR 2017). While previously uncommon, the prevalence of gentamicin-resistant *Campylobacter* has increased in the past few years.

Increasing resistance to gentamicin in *Campylobacter* spp.

Gentamicin resistance in *Campylobacter* has experienced a major increase in prevalence in the past few years. A 2012 report from China was the first to document the drastically increasing prevalence of gentamicin resistance (X. Chen et al., 2010). Gentamicin-resistance in *Campylobacter* isolated from Chinese broilers was as high as 92.3% in *C. coli* and 27.2% in *C. jejuni* in the year 2010. Prior to this time the prevalence of gentamicin-resistant *Campylobacter* worldwide was believed to be quite low, reported as less than 1% in studies from various countries around the globe (Shangshang Qin et al., 2012). NARMS surveillance data shows that gentamicin resistance was first documented in the United States in a *C. coli* isolate of human origin in 2000 and in an isolate of *C. jejuni* from retail chicken in 2003 (Y. Chen et al., 2013; Zhao et al., 2015). The 2013 Retail Meat NARMS report shows that as recently as 2002, there was no reported gentamicin resistance in either *C. jejuni* or *C. coli* isolated from retail chicken (Centers for Disease Control and Prevention, 2013b). The year 2012 showed a prevalence of 4.1% in *C. coli* and 0.2% in *C. jejuni*. However, 2011 was the year with the highest reported prevalence with *C. coli* showing resistance in 18.1% of isolates.

Numerous gentamicin resistance genes have been identified in *Campylobacter* including *aacA4*, *aac(6')-Ie/aph(2")-Ia*, *aph(2")-If*, and *aph(2")-Ig*, and in July 2015 *aac(6')-Ie/aph(2")-If2, aph(2")-If3, aph(2")-Ih, aph(2")-Ib,* and *aph(2")-Ic.*(Lee et al., 2002; Zhao et al., 2015). In 2013, a plasmid (pN29710-1) carrying multiple antibiotic resistance genes, including a novel gentamicin-resistance gene, *aph(2")-Ig,* was identified in an isolate of *C. coli* sourced from U.S.

(Y. Chen et al., 2013). pN29710-1 is 95% homologous with a pTet plasmid carrying *tet(O)* except the additional antibiotic resistance genes (ARGs) carried by pN29710-1 are lacking in pTet. Similarly, a *tet(O)*-carrying, multidrug-resistant plasmid was identified from a clinical isolate of *C. jejuni* from Thailand, pCG8245. Both of these plasmids also carry the gene cluster *aadE, sat4,* and *aphA-3* (Y. Chen et al., 2013; Nirdnoy, Mason, & Guerry, 2005). This gene cluster was first identified in the chromosome of *C. coli* in 2012, but had been found on the plasmid, pCG8245, from *C. jejuni* in 2005 (Nirdnoy et al., 2005; Shangshang Qin et al., 2012). pCG8245 contains 29 open reading frames (ORFs), ten of which encode genes for aminoglycoside resistance including an additional gentamicin resistance gene, *aph(2")-If* (Toth, Frase, Antunes, & Vakulenko, 2013). Continued monitoring of clinical and foodborne *Campylobacter* isolates for their resistance profiles and determination of the genetic basis of gentamicin resistance are crucial for understanding the epidemiology of this threat to public health.

REFERENCES

Agunos, A., Waddell, L., Léger, D., & Taboada, E. (2014). A systematic review characterizing on-farm sources of *Campylobacter* spp. for broiler chickens. *PloS One*, *9*(8), e104905. http://doi.org/10.1371/journal.pone.0104905

Arsenault, J., Letellier, A., Quessy, S., Normand, V., & Boulianne, M. (2007). Prevalence and risk factors for *Salmonella* spp. and *Campylobacter* spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. *Preventive Veterinary Medicine*, *81*(4), 250–64. http://doi.org/10.1016/j.prevetmed.2007.04.016

Berndtson, E., Emanuelson, U., Engvall, A., & Danielsson-Tham, M. L. (1996). A 1-year epidemiological study of campylobacters in 18 Swedish chicken farms. *Preventive Veterinary Medicine*, *26*(3–4), 167–185. http://doi.org/10.1016/0167-5877(95)01008-4

Bouwknegt, M., van de Giessen, A. W., Dam-Deisz, W. D. C., Havelaar, A. H., Nagelkerke, N. J. D., & Henken, A. M. (2004). Risk factors for the presence of *Campylobacter* spp. in Dutch broiler flocks. *Preventive Veterinary Medicine*, *62*, 35–49. http://doi.org/10.1016/j.prevetmed.2003.09.003

Centers for Disease Control and Prevention. (2017). *Campylobacter* (Campylobacteriosis). Retrieved September 11, 2017, from https://www.cdc.gov/campylobacter/faq.html

Cardinale, E., Tall, F., Guèye, E. F., Cisse, M., & Salvat, G. (2004). Risk factors for *Campylobacter* spp. infection in Senegalese broiler-chicken flocks. *Preventive Veterinary Medicine*, *64*(1), 15–25. http://doi.org/10.1016/j.prevetmed.2004.03.006

Centers for Diseas Control and Prevention. (2015). FoodNet *2015 Surveillance Report*. Retrieved from: https://www.cdc.gov/foodnet/reports/annual-reports-2015.html

Centers for Disease Control and Prevention. (2013a). *Antibiotic resistance threats in the United*

States. Retrieved from www.cdc.gov/drugresistance/threat-report-2013/.

Centers for Disease Control and Prevention. (2013b). *National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Human Isolates Final Report, 2010* (Vol. 104). Retrieved from http://www.cdc.gov/narms

Centers for Disease Control and Prevention. (2014a). *Campylobacter*. Retrieved February 20, 2016, from http://www.cdc.gov/foodsafety/diseases/campylobacter/index.html

Centers for Disease Control and Prevention. (2014b). *Campylobacter*, General Information. Retrieved from http://www.cdc.gov/nczved/divisions/dfbmd/diseases/campylobacter/

US Food and Drug Administration, Code of Federal Regulations Title 21. (2017). Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=529.1044b

Chen, X., Naren, G. W., Wu, C. M., Wang, Y., Dai, L., Xia, L. N., … Shen, J. Z. (2010). Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China. *Veterinary Microbiology*, *144*(1–2), 133–139. http://doi.org/10.1016/j.vetmic.2009.12.035

Chen, Y., Mukherjee, S., Hoffmann, M., Kotewicz, M. L., Young, S., Abbott, J., … McDermott, P. F. (2013). Whole-genome sequencing of gentamicin-resistant *Campylobacter coli* isolated from U.S. retail meats reveals novel plasmid-mediated aminoglycoside resistance genes. *Antimicrobial Agents and Chemotherapy*, *57*(11), 5398–5405. http://doi.org/10.1128/AEM.02873-15

Ekdahl, K., Normann, B., & Andersson, Y. (2005). Could flies explain the elusive epidemiology of campylobacteriosis? *BMC Infectious Diseases*, *5*(1), 11. http://doi.org/10.1186/1471-2334-5-11

Endtz, H. P., Ruijs, G. J., van Klingeren, B., Jansen, W. H., van der Reyden, T., & Mouton, R. P. (1991). Quinolone resistance in *Campylobacter* isolated from man and poultry following the

introduction of fluoroquinolones in veterinary medicine. *Journal of Antimicrobial Chemotherapy*, *27*(2), 199–208. http://doi.org/10.1093/jac/27.2.199

Epps, S., Harvey, R., Hume, M., Phillips, T., Anderson, R., & Nisbet, D. (2013). Foodborne *Campylobacter*: Infections, metabolism, pathogenesis and reservoirs. *International Journal of Environmental Research and Public Health*, *10*(12), 6292–6304. http://doi.org/10.3390/ijerph10126292

European Food Safety Authority. (2014). EFSA explains zoonotic diseases. *Campylobacter*. Fact Sheet (2014) http://doi.org/10.2805/59450

Evans, S. J., & Sayers, A. R. (2000). A longitudinal study of *Campylobacter* infection of broiler flocks in Great Britain. *Preventive Veterinary Medicine*, *46*(3), 209–223. http://doi.org/10.1016/S0167-5877(00)00143-4

Fawzy, M., Metwaly, E., Adawy, H. El, Hotzel, H., & Tomaso, H. (2016). Prevalence , genotyping and risk factors of thermophilic *Campylobacter* spreading in organic turkey farms in Germany. *Gut Pathogens*, *8*(28), 1–9. http://doi.org/10.1186/s13099-016-0108-2

Gillespie, I. A., O'Brien, S. J., Frost, J. A., Adak, G. K., Horby, P., Swan, A. V, … Neal, K. R. (2002). A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: a tool for generating hypotheses. *Emerging Infectious Diseases*, *8*(9), 937–942. http://doi.org/10.3201/eid0809.010187

Gregory, E., Barnhart, H., Dreesen, D. W., Stern, N., & Corn, J. L. (1997). Epidemiological Study of *Campylobacter* spp. in broilers: Source , time of colonization, and prevalence. *Avian Diseases*, *41*(4), 890–898. Retrieved from http://www.jstor.org/stable/1592343?seq=1#page_scan_tab_contents

Guccione, E., Del Rocio Leon-Kempis, M., Pearson, B. M., Hitchin, E., Mulholland, F., Van

Diemen, P. M., … Kelly, D. J. (2008). Amino acid-dependent growth of *Campylobacter jejuni*: Key roles for aspartase (AspA) under microaerobic and oxygen-limited conditions and identification of AspB (Cj0762), essential for growth on glutamate. *Molecular Microbiology*, *69*(1), 77–93. http://doi.org/10.1111/j.1365-2958.2008.06263.x

Guerin, M. T., Martin, W., Reiersen, J., Berke, O., McEwen, S. A., Bisaillon, J.-R., & Lowman, R. (2007). A farm-level study of risk factors associated with the colonization of broiler flocks with *Campylobacter* spp. in Iceland, 2001-2004. *Acta Veterinaria Scandinavica*, *49*(1), 18. http://doi.org/10.1186/1751-0147-49-18

Hald, B., Skovgård, H., Bang, D. D., Pedersen, K., Dybdahl, J., Jespersen, J. B., & Madsen, M. (2004). Flies and *Campylobacter* infection of broiler flocks. *Emerging Infectious Diseases*, *10*(8), 1490–1492. http://doi.org/10.3201/eid1008.040129

Hald, B., Sommer, H. M., & Skovgard, H. (2007). Use of fly screens to reduce *Campylobacter* spp. introduction in broiler houses. *Emerging Infectious Diseases*, *13*(12), 1951–1953. http://doi.org/10.3201/eid1312.070488

Hansson, I., Vågsholm, I., Svensson, L., & Olsson Engvall, E. (2007). Correlations between *Campylobacter* spp. prevalence in the environment and broiler flocks. *Journal of Applied Microbiology*, *103*(3), 640–649. http://doi.org/10.1111/j.1365-2672.2007.03291.x

Harvey, S. M. (1980). Hippurate hydrolysis by *Campylobacter fetus*. *Journal of Clinical Microbiology*, *11*(4), 435–437.

Hoffman, P. S., & Goodman, T. G. (1982). Respiratory physiology and energy-conservation efficiency of *Campylobacter jejuni*. *Journal of Bacteriology*, *150*(1), 319–326.

Hopkins, R. S., & Scott, A. S. (1983). Handling raw chicken as a source for sporadic *Campylobacter jejuni* infections. *Jounral of Infectious Diseases*, *148*(4), 770.

Houng, H.-S. H. S. H. H., Sethabutr, O., Nirdnoy, W., Katz, D. E., Pang, L. W., Altekruse, S. F., … Dean, R. A. (2001). Development of a ceuE-based multiplex polymerase chain reaction (PCR) assay for direct detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in Thailand. *Diagnostic Microbiology and Infectious Disease*, *40*(1–2), 11–19. http://doi.org/10.1016/S0732-8893(01)00251-6

Humphrey, T., O'Brien, S., & Madsen, M. (2007). Campylobacters as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology*, *117*(3), 237–257. http://doi.org/10.1016/j.ijfoodmicro.2007.01.006

Jonsson, M. E., Chriél, M., Norström, M., & Hofshagen, M. (2012). Effect of climate and farm environment on *Campylobacter* spp. colonisation in Norwegian broiler flocks. *Preventive Veterinary Medicine*, *107*(1–2), 95–104. http://doi.org/10.1016/j.prevetmed.2012.05.002

Kapperud, G., Skjerve, E., Vik, L., Hauge, K., Lysaker, a, Aalmen, I., … Potter, M. (1993). Epidemiological investigation of risk factors for campylobacter colonization in Norwegian broiler flocks. *Epidemiology and Infection*, *111*(2), 245–255. http://doi.org/10.1017/S0950268800056958

Kärenlampi, R., Rautelin, H., Hakkinen, M., & Hänninen, M. L. (2003). Temporal and geographical distribution and overlap of Penner heat-stable serotypes and pulsed-field gel electrophoresis genotypes of *Campylobacter jejuni* isolates collected from humans and chickens in Finland during a seasonal peak. *Journal of Clinical Microbiology*, *41*(10), 4870–4872. http://doi.org/10.1128/JCM.41.10.4870-4872.2003

Ketley, J. M. (1997). Pathogenesis of enteric infection by *Campylobacter*. *Microbiology*, *143*(1 997), 5–21. http://doi.org/10.1099/00221287-143-1-5

Kramer, J. M., Frost, J. A., Bolton, F. J., & Wareing, D. R. A. (2000). *Campylobacter*

contamination of raw meat and poultry at retail sale: Identification of multiple types and comparison with isolates from human infection. *Journal of Food Protection*, *63*(12), 1654–1659. Retrieved from http://jfoodprotection.org.prox.lib.ncsu.edu/doi/pdf/10.4315/0362-028X-63.12.1654

Lahti, E., Rehn, M., Ockborn, G., Hansson, I., Ågren, J., Engvall, E. O., & Jernberg, C. (2017). Outbreak of campylobacteriosis following a dairy farm visit: Confirmation by genotyping. *Foodborne Pathogens and Disease*, *XX*(Xx), fpd.2016.2257. http://doi.org/10.1089/fpd.2016.2257

Lawes, J.R., Vidal, A., Clifton-Hadley, F.A., Sayers, R., Rodgers, J., Snow, L., Evans, S.J., Powell, L. F. (2012). Investigation of prevalence and risk factors for *Campylobacter* in broiler flocks at slaughter : results from a UK survey. *Epidemiol Infect*, *140*, 1725–1737. http://doi.org/10.1017/S0950268812000982

Lee, M. D., Sanchez, S., Zimmer, M., Idris, U., Berrang, M. E., & McDermott, P. F. (2002). Class 1 integron-associated tobramycin-gentamicin resistance in *Campylobacter jejuni* isolated from the broiler chicken house environment. *Antimicrobial Agents and Chemotherapy*, *46*(11), 3660–3664. http://doi.org/10.1128/AAC.46.11.3660-3664.2002

Miller, W. G., Huynh, S., Parker, C. T., & Niedermeyer, J. A. (2016). Complete genome sequences of multidrug-resistant *Campylobacter jejuni* strain 14980A (turkey feces) and *Campylobacter coli* strain 14983A (housefly from a turkey farm), harboring a novel gentamicin resistance mobile element. *Genome Announcement*, *4*(5), 16–17. http://doi.org/10.1128/genomeA.01175-16.Copyright

Nachamkin, I., Allos, B. M., & Ho, T. (1998). *Campylobacter* species and Guillain-Barre Syndrome. *Clin. Microbiol. Rev.*, *11*(3), 555–567. Retrieved from

http://cmr.asm.org/content/11/3/555.long

US Food and Drug Administration. (2013). *National Antimicrobial Resistance Monitoring System Integrated Report - Enteric Bacteria. NARMS Integrated Report: 2012-2013*.

Newell, D. G., Elvers, K. T., Dopfer, D., Hansson, I., Jones, P., James, S., … Allen, V. M. (2011). Biosecurity-based interventions and strategies to reduce *Campylobacter* spp. on poultry farms. *Applied and Environmental Microbiology*, *77*(24), 8605–8614. http://doi.org/10.1128/AEM.01090-10

Newell, D. G., & Fearnley, C. (2003). Sources of *Campylobacter* colonization in broiler chickens. *Applied and Environmental Microbiology*, *69*(8), 4343–4351. http://doi.org/10.1128/AEM.69.8.4343

Nichols, G. L. (2005). Fly transmission of *Campylobacter*. *Emerging Infectious Diseases*, *11*(3), 361–364. http://doi.org/10.3201/eid1103.040460

Nirdnoy, W., Mason, C. J., & Guerry, P. (2005). Mosaic structure of a multiple-drug-resistant, conjugative plasmid from *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy*, *49*(6), 2454–2459. http://doi.org/10.1128/AAC.49.6.2454-2459.2005

World Health Organization. (2012). The global view of campylobacteriosis: report of an expert consultation. World Health Organization, Utrecht, The Netherlands. *http://apps.who.int/iris/bitstream/10665/80751/1/9789241564601_eng.pdf*

Park, S. F. (2002). The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. *International Journal of Food Microbiology*, *74*(3), 177–188. http://doi.org/10.1016/S0168-1605(01)00678-X

Penner, J. L. (1988). The genus *Campylobacter*: A decade of progress. *Clinical Microbiology Reviews*, *1*(2), 157–172. http://doi.org/10.1128/CMR.1.2.157.Updated

Pires, S. M., Vigre, H., Makela, P., & Hald, T. (2010). Using outbreak data for source attribution of human salmonellosis and campylobacteriosis in Europe. *Foodborne Pathogens and Disease*, *7*(11), 1351–61. http://doi.org/10.1089/fpd.2010.0564

Qin, S., Wang, Y., Zhang, Q., Chen, X., Shen, Z., Deng, F., … Shen, J. (2012). Identification of a novel genomic island conferring resistance to multiple aminoglycoside antibiotics in *Campylobacter coli*. *Antimicrobial Agents and Chemotherapy*, *56*(10), 5332–5339. http://doi.org/10.1128/AAC.00809-12

Qin, S., Wang, Y., Zhang, Q., Zhang, M., Deng, F., Shen, Z., … Shen, J. (2014). Report of ribosomal RNA methylase gene *erm*(B) in multidrug-resistant *Campylobacter coli*. *Journal of Antimicrobial Chemotherapy*, *69*(4), 964–968. http://doi.org/10.1093/jac/dkt492

Rosef, O., & Kapperud, G. (1983). House flies (Musca domestica) as possible vectors of *Campylobacter fetus* subsp. *jejuni*. *Applied and Environmental Microbiology*, *45*(2), 381–383. Retrieved from http://aem.asm.org.prox.lib.ncsu.edu/content/45/2/381.short

Royden, a, Wedley, a, Merga, J. Y., Rushton, S., Hald, B., Humphrey, T., & Williams, N. J. (2016). A role for flies (Diptera) in the transmission of *Campylobacter* to broilers? *Epidemiology and Infection*, *144*(15), 3326–3334. http://doi.org/10.1017/S0950268816001539

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., … Griffin, P. M. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. http://doi.org/10.3201/eid1701.091101p1

Scallan, E., Hoekstra, R. M., Mahon, B. E., Jones, T. F., & Griffin, P. M. (2015). An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiology and Infection*, *143*(13), 2795–2804.

http://doi.org/10.1017/S0950268814003185

Sejvar, J. J., Kohl, K. S., Gidudu, J., Amato, A., Bakshi, N., Baxter, R., … Wiznitzer, M. (2011). Guillain-Barre syndrome and Fisher syndrome: Case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine*, *29*(3), 599–612. http://doi.org/10.1016/j.vaccine.2010.06.003

Shane, S. M., Montrose, M. S., & Harrington, K. S. (1985). Transmission of *Campylobacter jejuni* by the Housefly (Musca domestica). *Source: Avian Diseases*, *29*(2), 384–391. Retrieved from http://www.jstor.org/stable/1590499

SMIBERT, R. M. (1978). the Genus *Campylobacter*. *Ann. Rev. Microbiol.*, 673–709.

Sommer, H. M., Hog, B. B., Larsen, L. S., Sorensen, A. I. V, Williams, N., Merga, J. Y., … Rosenquist, H. (2016). Analysis of farm specific risk factors for *Campylobacter* colonization of broilers in six European countries. *Microbial Risk Analysis*, *2–3*, 16–26. http://doi.org/10.1016/j.mran.2016.06.002

Tam, C. C., Rodrigues, L. C., O'Brien, S. J., & Hajat, S. (2006). Temperature dependence of reported *Campylobacter* infection in England, 1989-1999. *Epidemiol Infect*, *134*(1), 119–125. http://doi.org/10.1017/S0950268805004899

Taylor, E. V., Herman, K. M., Ailes, E. C., Fitzgerald, C., Yoder, J. S., Mahon, B. E., & Tauxe, R. V. (2012). Common source outbreaks of *Campylobacter* infection in the USA, 1997–2008. *Epidemiology and Infection*, *141*(2013), 1–10. http://doi.org/10.1017/S0950268812001744

Torralbo, A., Borge, C., Allepuz, A., García-Bocanegra, I., Sheppard, S. K., Perea, A., & Carbonero, A. (2014). Prevalence and risk factors of *Campylobacter* infection in broiler flocks from southern Spain. *Preventive Veterinary Medicine*, *114*(2), 106–113.

http://doi.org/10.1016/j.prevetmed.2014.01.019

Toth, M., Frase, H., Antunes, N. T., & Vakulenko, S. B. (2013). Novel aminoglycoside 2???-phosphotransferase identified in a gram- negative pathogen. *Antimicrobial Agents and Chemotherapy*, *57*(1), 452–457. http://doi.org/10.1128/AAC.02049-12

Tyson, G. H., Tate, H. P., Abbott, J., Tran, T.-T., Kabera, C., Crarey, E., … Zhao, S. (2016). Molecular subtyping and source attribution of *Campylobacter* isolated from food animals. *Journal of Food Protection*, *79*(11), 1891–1897. http://doi.org/10.4315/0362-028X.JFP-16-195

US Food and Drug Adminimstration. (2005). Withdrawal of Enrofloxacin for Poultry. Retrieved March 28, 2017, from https://www.fda.gov/AnimalVeterinary/SafetyHealth/RecallsWithdrawals/ucm042004.htm

US Food and Drug Administration. (2014). *NARMS Integrated Report: 2014*. Retrieved from https://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm059103.htm

van de Giessen, A., Mazurier, S. I., Jacobs-Reitsma, W., Jansen, W., Berkers, P., Ritmeester, W., & Wernars, K. (1992). Study on the epidemiology and control of *Campylobacter jejuni* in poultry broiler flocks. *Appl. Environ. Microbiol.*, *58*(6), 1913–1917. Retrieved from http://aem.asm.org/cgi/content/abstract/58/6/1913

van de Giessen, a W., Bloemberg, B. P., Ritmeester, W. S., & Tilburg, J. J. (1996). Epidemiological study on risk factors and risk reducing measures for *Campylobacter* infections in Dutch broiler flocks. *Epidemiology and Infection*, *117*(2), 245–250. http://doi.org/10.1017/S0950268800001412

Veron, M., & Chatelain, R. (1973). Taxonomic study of the genus *Campylobacter* Sebald and

Veron and designation of the neotype strain for the type species, *Campylobacter* fetus (Smith and Taylor) Sebald and Veron. *International Journal of Systematic Bacteriology*, *23*(2), 122–134. http://doi.org/10.1099/00207713-23-2-122

Wagenaar, J.A. Newell, D.G., Kalupahana, R.S., Mughini-Gras, L. (2015). *Zoonoses-Infections Affecting Humans and Animals: Focus on Public Health Aspects*. Retrieved from: http://doi.org/10.1007/978-94-017-9457-2

Wallace, J. S., Stanley, K. N., & Jones, K. (1998). The colonization of turkeys by thermophilic campylobacters. *Journal of Applied Microbiology*, *85*(2), 224–230. http://doi.org/10.1046/j.1365-2672.1998.00470.x

Westfall, H. N., Rollins, D. M., & Weiss, E. (1986). Substrate utilization by *Campylobacter jejuni* and *Campylobacter coli*. *Appl.Environ.Microbiol.*, *52*(4), 700–705. Retrieved from pm:2877615

Wright, S. L., Carver, D. K., Siletzky, R. M., Romine, S., Morrow, W. E. M., & Kathariou, S. (2008). Longitudinal study of prevalence of *Campylobacter jejuni* and *Campylobacter coli* from turkeys and swine grown in close proximity. *Journal of Food Protection*, *71*(9), 1791–1796.

Yang, B., Lian, Y., Liu, Y., Wu, B.-Y., & Duan, R.-S. (2016). A retrospective analysis of possible triggers of Guillain–Barre syndrome. *Journal of Neuroimmunology*, *293*, 17–21. http://doi.org/10.1016/j.jneuroim.2016.02.003

Yuki, N., & Hartung, H.-P. (2012). Guillain-Barré syndrome. *The New England Journal of Medicine*, *366*(24), 2294–2304. http://doi.org/10.1056/NEJMra1114525

Zhao, S., Mukherjee, S., Chen, Y., Li, C., Young, S., Warren, M., … McDermott, P. F. (2015). Novel gentamicin resistance genes in *Campylobacter* isolated from humans and retail meats

in the USA. *Journal of Antimicrobial Chemotherapy*, *70*(5), 1314–1321. http://doi.org/10.1093/jac/dkv001

Chapter 2 The Current State of Macrolide Resistance in *Campylobacter*: A Review of Trends and Impacts of Resistance Mechanisms

SUMMARY

Campylobacter spp., especially *Campylobacter jejuni* and *C. coli*, are leading bacterial foodborne pathogens worldwide. In the United States, an estimated 0.8 million cases of campylobacteriosis occur annually, mostly involving *C. jejuni.* Campylobacteriosis is generally self-limiting but in severe cases treatment with antibiotics may be mandated. Increasing incidence of fluoroquinolone resistance in *Campylobacter* has rendered macrolides like erythromycin and azithromycin the drugs of choice for human campylobacteriosis. Prevalence of macrolide resistance in *C. jejuni* remains low, but can be common in *C. coli*. Substitutions in the 23S rRNA gene, specifically A2075G, and less frequently A2074C/G, remain the most common mechanism for high-level resistance to macrolides. In *C. jejuni*, resistance mediated by such substitutions is accompanied by reduced ability to colonize chickens and other fitness costs, potentially contributing to the low incidence of macrolide resistance. Interestingly, similar fitness impacts have not been noted in *C. coli*. Also noteworthy is a novel mechanism first reported in 2014 in *C. coli* from China and mediated by *erm*(B) harbored on multidrug-resistance genomic islands. Incidence of *erm*(B) appears to reflect clonal expansion of certain strains and whole-genome sequencing has been critical to the elucidation of *erm*(B)-associated macrolide resistance in *Campylobacter* spp. Except for one report from Spain, *erm*(B)-mediated macrolide resistance has been restricted to *Campylobacter,* mostly *C. coli*, of animal and human origin from China. If *erm*(B)-mediated macrolide resistance does not confer fitness costs in *C. jejuni* the range of this gene may expand in *C. jejuni*, threatening to compromise treatment effectiveness of severe campylobacteriosis cases.

INTRODUCTION

In 2013 the Centers for Disease Control and Prevention listed fluoroquinolone and macrolide-resistant *Campylobacter* among serious antibiotic resistance threats to public health (Centers for Disease Control and Prevention, 2013a). *Campylobacter* spp. are major etiologic agents for human foodborne illness (campylobacteriosis), resulting in an estimated 0.8 million annual cases of disease in the United States alone (Centers for Disease Control and Prevention, 2014b; E. Scallan et al., 2015; Elaine Scallan et al., 2011). *Campylobacter jejuni* is responsible for about 90% of campylobacteriosis cases, with *C. coli* accounting for the majority of the remainder (E. Scallan et al., 2015; Elaine Scallan et al., 2011). Poultry, contaminated water and raw milk are the most frequently identified vehicles for illness, with most outbreaks attributed to the latter two (Gillespie et al., 2002; Hermans et al., 2012; T. Humphrey et al., 2007; E. V. Taylor et al., 2012). In addition to acute gastroenteritis, campylobacteriosis may result in severe autoimmune sequelae including reactive arthritis and, in about one of every 1,000 cases, Guillain-Barré syndrome (GBS). Campylobacteriosis is considered to be the most frequent antecedent for GBS (Yang et al., 2016; Yuki & Hartung, 2012).

Antimicrobial treatment is not routinely recommended as most human cases resolve on their own within 3-5 days, but may be recommended for patients with unusually severe and prolonged symptoms, especially those with AIDS, the elderly or other vulnerable categories (Centers for Disease Control and Prevention, 2014b; Ruiz-palacios, 2007). Treatment with fluoroquinolones such as ciprofloxacin has been challenged by high incidence of fluoroquinolone resistance among human isolates, which led to the 2005 ban of the fluoroquinolone enrofloxacin for use in poultry (US Food and Drug Adminimstration, 2005). Resistance to the fluoroquinolone ciprofloxacin among human clinical isolates continues to be

frequently encountered, e.g. 21.6% of *C. jejuni* and 24.5% *C. coli* at the time of the 2005 ban vs. 26.7% and 35.6% of *C. jejuni* and *C. coli,* respectively, in 2014 (US Food and Drug Administration, 2014). In view of the continuing relatively high incidence of fluoroquinolone resistance in *Campylobacter* spp. from human cases, macrolides such as erythromycin and azithromycin are considered the drugs of choice for treatment of human campylobacteriosis (Centers for Disease Control and Prevention, 2013a, 2014b; Luangtongkum et al., 2010; US Food and Drug Administration, 2013).

Historically, the incidence of resistance to erythromycin and other macrolides has been low, especially in *C. jejuni*, even though there are several mechanisms by which *Campylobacter* can acquire resistance to these antimicrobials. Data from the most recent National Antimicrobial Resistance Monitoring System (NARMS) report indicate that the prevalence of erythromycin resistance (MIC ≥ 8 µg/ml) in *C. jejuni* has remained below 4% in human and chicken isolates since NARMS testing began in 1997 and 2001, respectively (US Food and Drug Administration, 2014). Macrolide resistance was noted in 22% of *C. jejuni* from hogs, but this was based on only nine isolates, and *C. jejuni* is generally uncommon in swine (Tyson et al., 2016). In contrast to the stable, low prevalence of macrolide resistance in *C. jejuni*, an overall trend for increased prevalence of macrolide-resistance (MIC ≥ 16 µg/ml) was noted in in *C. coli*; erythromycin resistance in *C. coli* derived from humans more than tripled in 2014 compared to 2011 (10.3% vs. 2.7%), and more than doubled in retail chicken isolates in the same time frame (11.4% vs 5.2%) (US Food and Drug Administration, 2014). Analysis of *C. coli* from samples collected at slaughter revealed macrolide resistance in 21% of isolates from sows, 40% from market swine, 11% from chickens and 6.7% from turkeys (US Food and Drug Administration, 2014). Analysis of 678 *C. jejuni* and 119 *C. coli* from human cases in Spain indicated that prevalence of

erythromycin resistance (MIC ≥ 32 μg/ml) was at relatively low levels (3.8%) similar to those in the U.S NARMS report, and that most (28/30) of the resistant isolates were *C. coli* (Perez-Boto, Lopez-Portoles, Simon, Valdezate, & Echeita, 2010).

Macrolide resistance in *C. jejuni* and *C. coli* has been the focus of intense study due to its potential to compromise therapeutic effectiveness of macrolides, and several excellent reviews previously addressed resistance mechanisms and implications (Gibreel et al., 2005a; Gibreel & Taylor, 2006; Luangtongkum et al., 2010; Payot et al., 2006). Nonetheless, more recent investigations have expanded our understanding of the biological implications of macrolide resistance, especially in regard to associated fitness costs in *C. jejuni* (Han, Pu, Wang, Meng, & Ge, 2009; Hao et al., 2009; Luangtongkum et al., 2012; Zeitouni, Collin, Andraud, Ermel, & Kempf, 2012). In addition, employment of whole genome sequencing (WGS) has not only advanced understanding of the distribution of previously known resistance-associated mutations but, starting in 2014, also revealed the emergence of *erm*(B), a macrolide resistance determinant not previously detected in *Campylobacter* spp. (S. Qin et al., 2014). For these collective reasons we considered the timing appropriate for an updated review of mechanisms and implications of macrolide resistance in this pathogen.

MECHANISMS OF RESISTANCE

Target mutations in 23S rRNA genes

Campylobacter spp. may evade macrolide binding by alteration of the antimicrobial's 23S rRNA target at position 2074 or 2075. The substitutions A2075G, A2074G, A2074C, and the more rarely encountered A2074T have been shown to confer high-level (> 512 μg/ml) resistance to erythromycin when present in all three copies of the 23S rRNA gene in *C. jejuni* and *C. coli*

(Caldwell, Wang, & Lin, 2008; Lin et al., 2007; Payot et al., 2006; Perez-Boto et al., 2010). High-level resistance also requires an intact CmeABC efflux system as will be discussed in detail below (Caldwell et al., 2008; Lin et al., 2007). The ribosomal substitutions may not always occur in all three copies of the gene, resulting in a lower levels of resistance (Gibreel et al., 2005a; Luangtongkum et al., 2010; Vacher, Menard, Bernard, Santos, & Megraud, 2005). Additionally, isolates may have different substitutions on different copies of the 23S rRNA gene. For example, one *C. jejuni* strain with erythromycin MIC > 128 μg/ml harbored A2074C and A2075G substitutions in different copies of the 23S rRNA (Vacher et al., 2005).

WGS analysis of 114 *Campylobacter* spp. isolates identified 52 (46%), including 17 *C. jejuni* and 35 *C. coli,* that were resistant to erythromycin; all but one harbored the A2075G substitution in the 23S rRNA gene with the remaining, a *C. jejuni* isolate, harboring A2074T, suggesting that A2075G remains the most prevalent genetic event conferring high-level resistance to erythromycin (Zhao et al., 2016). All erythromycin-resistant isolates were co-resistant to azithromycin and vice versa, with the exception of one strain found to be azithromycin-resistant but erythromycin-susceptible and harboring an amino acid substitution (A86E) in ribosomal protein L22 (Zhao et al., 2016).

Target mutations in ribosomal proteins

In the absence of mutations in 23S rRNA genes, mutations in *rplD* and *rplV* (ribosomal proteins L4 and L22, respectively) are associated with a lower level of resistance to macrolides (erythromycin MIC, 32 μg/ml) in comparison to isolates with ribosomal substitutions that exhibit erythromycin MICs > 512 μg/ml (Gibreel et al., 2005a; Hao et al., 2013; Luangtongkum et al., 2012). Strains with L4 and L22 mutations developed high-level resistance (>256 μg/ml) upon

acquisition of mutations in 23S rRNA (Hao et al., 2013). Numerous macrolide resistance-conferring substitutions and insertions in these ribosomal proteins have been recorded. For instance, various substitutions (e.g. G74D, G67V, R72I, A71D), and a glycine insertion at position 60 were noted In L4 (C. Cagliero, Mouline, Cloeckaert, & Payot, 2006; Caldwell et al., 2008; Hao et al., 2013; Luangtongkum et al., 2010). For L22, A88E and G86E have been noted as well as a nine-base duplication at position 292 of *rplV* and insertions at position 86 or 98 (Caldwell et al., 2008; Hao et al., 2013; Luangtongkum et al., 2010).

Ribosomal methylation-*erm*(B)

For many years, base substitutions in the 23S rRNA sequence were the only mechanism known to specifically mediate high-level macrolide resistance in *Campylobacter* spp. (Luangtongkum et al., 2010). However, in 2014 a ribosomal methylase encoded by *erm*(B) was reported for the first time in *C. coli* strain ZC113 of swine origin in China (S. Qin et al., 2014). *Erm*(B) dimethylates a single adenine in the 23S rRNA leading to decreased binding of macrolides (Leclercq, 2002). *C. coli* ZC113 and the majority of other *erm*(B)-harboring strains are constitutively resistant to macrolides and also express *erm*(B) constitutively (Deng, Shen, Zhang, Wu, & Zhang, 2015). However, a small number of *erm*(B)-harboring strains were found to be susceptible to erythromycin; such strains expressed *erm*(B) and became macrolide-resistant, with MICs as high as strains constitutively expressing *erm*(B), only upon pre-incubation with erythromycin or clindamycin (Deng, Shen, et al., 2015). Interestingly, the majority of *erm*(B)-harboring strains appear to harbor deletions in the *erm*(B) regulatory region which may account for their constitutive resistance to macrolides (Deng, Shen, et al., 2015). It is tempting to speculate that

such deletion derivatives have been selected upon exposure to macrolides, e.g. in animal production.

In *C. coli* ZC113 *erm*(B) was harbored by a chromosomal multidrug-resistance genomic island (MDRGI) composed of 17 open reading frames (ORFs), eight of which encoded antimicrobial resistance determinants (S. Qin et al., 2014). Besides *erm*(B) the MDRGI included a truncated *tet*(O) and the aminoglycoside resistance cassette *aadE-sat4-aphA3,* previously found in conjunction with *erm*(B) in *Enterococcus* (S. Qin et al., 2014; Werner, Hildebrandt, & Witte, 2003). The MDRGI-associated *erm*(B) showed 100% identity with *erm*(B) of Gram-positive bacteria such as *Streptococcus suis, Enterococcus faecium* and *Lactobacillus plantarum* and most other MDRGI ORFs exhibited high homology with counterparts in Gram-positive bacteria, suggesting a Gram-positive origin for the *erm*(B)-harboring island in *Campylobacter* (S. Qin et al., 2014; Shangshang Qin et al., 2012; Wang et al., 2014).

Subsequent reports have identified *erm*(B) in several isolates, almost always in *C. coli* from China (Deng, Wang, Zhang, & Shen, 2015; Florez-Cuadrado et al., 2016; S. Qin et al., 2014; Zhang et al., 2016). Multilocus sequence typing (MLST) analysis of 58 *erm*(B)-harboring *C. coli* isolates from China identified 30 different sequence types, many of which clustered into one clonal complex (CC 828) (Wang et al., 2014). *Erm*(B)-positive isolates from another study also mostly clustered within CC828 (41). *Erm*(B)-harboring isolates exhibited high levels of erythromycin resistance (MIC, 512 μg/ml) with the exception of two of the five *erm*(B)-positive *C. jejuni* isolates identified to date (MIC, 16 μg/ml) (Florez-Cuadrado et al., 2016; S. Qin et al., 2014; Zhou et al., 2016). Interestingly, three *erm*(B)-positive *C. coli* isolates also harbored the A2075G substitution in 23S rRNA (Zhang et al., 2016).

In all investigated cases *erm*(B) was harbored by a MDRGI, frequently (57%) on the chromosome or on plasmids of varying size (Wang et al., 2014; Zhang et al., 2016). Sequence analysis has revealed at least eight different *erm*(B) genomic organizations, with the arrangement in the first reported *erm*(B)-harboring strain, *C. coli* ZC113, being designated as type I (Fig. 1) (Florez-Cuadrado et al., 2016; Wang et al., 2014). In the figure, shaded regions indicate areas with greater than 98% identity. The *erm*(B) gene is shown in red, aminoglycoside resistance genes are in yellow, the tetracycline resistance gene *tet(O)* is in purple, genes with predicted functions are in green, and genes encoding hypothetical proteins are in white with bordering regions shown in black in accordance with previously published MDRGIs (20, 32, 40). The comparisons were performed using Geneious (version 9.1.4 http://www.geneious.com, Kearse et al., 2012). Varying insertion sites were identified for different MDRGIs, but *erm*(B) was highly conserved (>98% identity at the nucleotide sequence level) among all eight MDRGIs (Fig.1) (Deng, Wang, et al., 2015; Florez-Cuadrado et al., 2016). The most divergent erm(B) (type III MDRGI) only had four amino acid substitutions in comparison to *erm*(B) in the type I MDRGI. The lone *erm*(B)-positive strain reported outside of China (MDRGI type VIII) only had one amino acid substitution in erm(B), while *erm*(B) in the type VII MDRGI of *C. jejuni* C179b (accession no. KF864551) had one nucleotide substitution in comparison to its type I homolog, which did not result in an amino acid change (Deng, Wang, et al., 2015; Florez-Cuadrado et al., 2016).

Detection of *erm*(B) remains uncommon in *Campylobacter* and as of this writing *erm*(B) seems to be largely confined to China, with only one *erm*(B)-positive strain reported elsewhere (Spain) (Florez-Cuadrado et al., 2016). WGS analysis of 114 *C. jejuni* and *C. coli* from the NARMS collection failed to identify *erm*(B) among any of the 52 macrolide-resistant isolates

(Zhao et al., 2016). It is not clear why reports for *erm*(B)-harboring strains have been largely absent from nations other than China.. China's antimicrobial use may have conferred unique selective pressures on *Campylobacter* spp. there. While antimicrobial use is difficult to measure, China has been estimated to be responsible for 23% of the global antimicrobial use, with the United States estimated at 13% (Van Boeckel et al., 2015). It is also conceivable that *erm*(B)-harboring *Campylobacter* spp. strains arose in China via horizontal gene transfer from Gram-positive microbes co-occurring with *Campylobacter,* e.g. in the gut of swine,, in response to animal husbandry attributes that might be more common in animal production in China than elsewhere.

Even in China, however, incidence of *erm*(B)-harboring strains appears to be low. For instance, only 58 of 1,554 (3.7%) *C. jejuni* and *C. coli* isolates from animal and human cases were PCR-positive for *erm*(B) (Wang et al., 2014). It is noteworthy that most (53/58) of these *erm*(B)-harboring isolates were *C. coli* obtained in 2011 and 2012, with only five from previous years (2007-2009) suggesting that *erm*(B) may be expanding (Wang et al., 2014). The earliest *erm*(B)-positive *Campylobacter* isolate was *C. jejuni* cj94473, isolated from human diarrheal disease in China in 1994, and exhibiting intermediate (MIC, 16μg/ml) resistance to erythromycin (Zhou et al., 2016). Another PCR analysis of 858 *C. jejuni* and *C. coli* isolates from human diarrheal cases, chicken, and swine from China identified only 30 (3.5%) that were *erm*(B)-positive; all were *C. coli*, with a substantial fraction (13/30, 43%) originating from human diarrheal cases (Zhang et al., 2016). To the best of the authors' knowledge, fitness impacts of these MDRGIs in *Campylobacter* have not yet been described.

The sole *erm*(B)-positive strain reported outside of China may warrant special attention. This strain, *C. coli* ZTA09/02204, harbored *erm*(B) on a MDRGI (type VIII) that was genetically

different enough from that of *C. coli* ZC113 (Fig. 1) to prompt the speculation that it may have originated independently (Florez-Cuadrado et al., 2016). The type VIII MDRGI harbors 12 ORFs including 5 AMR genes with an intact *tet*(O), in contrast to the type I MDRGI from *C. coli* ZC113 which contained 17 ORFs with eight AMR genes and a truncated *tet*(O) (Fig. 1) (Florez-Cuadrado et al., 2016; S. Qin et al., 2014). Especially interesting is the high (99%) similarity of the type VIII MDRGI to a region on the previously characterized plasmid pN29710-1, identified in *C. coli* from retail chicken in the United States and harboring the recently identified aminoglycoside resistance gene *aph(2")-Ig* (Y. Chen et al., 2013). In the type VIII MDRGI a cluster of genes, including *erm*(B), an omega transcriptional repressor, a toxin-antitoxin system, and *aadE* (Fig. 1) may have been inserted between *aad9* and the truncated *tet*(O) of pN29710-1 (Florez-Cuadrado et al., 2016).

Multidrug efflux pumps

Macrolide resistance mediated by specific mutations or horizontally acquired determinants such as discussed above operates over and above baseline resistance levels conferred by innate efflux systems such as the CmeABC efflux pump. CmeABC is a member of the RND efflux transporter family; it harbors the typical structural motif of 12 transmembrane α-helices and mediates efflux of various compounds (Guo, Lin, Reynolds, & Zhang, 2010; Luangtongkum et al., 2010; Mamelli, Amoros, Pagès, & Bolla, 2003; Pumbwe & Piddock, 2002; Tseng et al., 1999). CmeABC inactivation resulted in up to a 64-fold decrease in the erythromycin MIC of *C. jejuni* harboring the A2075G substitution in the 23S rRNA (Gibreel, Wetsch, & Taylor, 2007; Guo et al., 2010; Pumbwe & Piddock, 2002). The extent to which CmeABC inactivation may decrease erythromycin MICs of *erm*(B)-harboring strains remains to be determined.

Mutations in the regulatory region of *cmeABC* may affect the MIC of macrolide-resistant isolates, and recently a *C. jejuni* variant with enhanced macrolide resistance and harboring a single A to G substitution in the CmeR-binding site of *cmeABC* (resistance enhancing CmeABC, RE-CmeABC) was identified (Yao et al., 2016). CmeR is located directly upstream of *cmeABC* and represses *cmeABC* transcription by binding to an inverted repeat in the CmeR-CmeA intergenic region (Guo et al., 2008; Lin, Akiba, Sahin, & Zhang, 2005). Mutations in the CmeR-binding site have been shown to result in overexpression of CmeABC (Cedric Cagliero, Maurel, Cloeckaert, & Payot, 2007; Grinnage-Pulley & Zhang, 2015), and indeed one RE-CmeABC variant, which was identified using WGS, exhibited a 5-fold increase in *cmeABC* transcript levels (Yao et al., 2016). Such enhanced-resistance variants may be expanding within *Campylobacter.* Prevalence of RE-CmeABC among chicken and swine-derived *C. jejuni* and *C. coli* increased from 7% to 20% between 2012 and 2014 (Yao et al., 2016). It is noteworthy that RE-CmeABC was noticeably more common in *C. jejuni* than *C. coli*, possibly suggesting a fitness advantage in *C. jejuni* (Yao et al., 2016).

Fitness costs

The finding that macrolide resistance is much more common in *C. coli* than *C. jejuni* prompts the hypothesis that fitness costs associated with substitutions in the 23S rRNA macrolide target keep prevalence low in *C. jejuni*. *In vitro* competitive fitness assays have in fact indicated markedly impaired fitness associated with erythromycin resistance for certain strains of *C. jejuni* during growth in laboratory media (Han et al., 2009; Luangtongkum et al., 2012; Ohno et al., 2016; Zeitouni et al., 2012). Erythromycin-susceptible strains of *C. jejuni* and their isogenic erythromycin-resistant mutants (harboring either A2074G or A2075G 23S rRNA substitutions)

had equal ability to colonize chickens when inoculated as monocultures, but in mixtures and in absence of erythromycin the erythromycin-resistant mutants were readily outcompeted by their parental strain counterparts (Luangtongkum et al., 2012; Zeitouni et al., 2012). Interestingly, such fitness costs in bird colonization were not noted with macrolide-resistant mutants of *C. coli* (Zeitouni et al., 2012).

The impaired ability of erythromycin-resistant *C. jejuni*, but not *C. coli*, to colonize chickens may account for the overall low prevalence of erythromycin resistance in *C. jejuni* from human disease, for which chicken is a major vehicle. It also raises questions not only about the mechanisms underlying the observed differences in fitness impacts between *C. jejuni* and *C. coli* but also about mechanisms that allow stable, high-level erythromycin resistance in certain *C. jejuni* strains (Hao et al., 2009). Compensatory mutations in such strains may counteract the fitness impacts. One study employed inoculation of chickens with mixtures of a *C. jejuni* strain and an isogenic erythromycin-resistant mutant harboring A2074G in the 23S rRNA. A spontaneous colonization-proficient derivative of the erythromycin-resistant mutant was recovered from the inoculated birds, harboring a potentially compensatory C2551G substitution in the 23S rRNA in addition to the original A2074G, and retaining resistance to erythromycin (Zeitouni et al., 2012). In another study, erythromycin-resistant mutants selected *in vitro* upon exposure to increasing levels of macrolides were found to harbor numerous mutations in addition to 23S rRNA substitutions (Hao et al., 2013). WGS analysis is expected to further elucidate possible compensatory mechanisms in *C. jejuni* which harbor specific substitutions in 23S rRNA and also exhibit stable, high-level resistance to macrolides.

C. jejuni strains with amino acid substitutions in L4 and L22 also exhibited down-regulation of motility and energy metabolism genes; in addition, they had slower growth kinetics

and became outcompeted in poultry hosts (Hao et al., 2013; Luangtongkum et al., 2012). It is possible that the physiological impacts of L4 and L22 mutations that may precede those in 23S rRNA may contribute to the observed fitness impacts of macrolide resistance in *C. jejuni* (Hao et al., 2013). However, the roles of ribosomal proteins in macrolide resistance and fitness costs remain to be further elucidated. While some studies identified substitutions in the ribosomal proteins in conjunction with those in the 23S rRNA gene, others did not find such associations (Gibreel et al., 2005a; Hao et al., 2013; Ladely, Meinersmann, Englen, Fedorka-Cray, & Harrison, 2009; Vacher et al., 2005; Zhao et al., 2016; Zhou et al., 2016).

The potential impacts of *erm*(B) on *Campylobacter*'s ability to colonize animals or other adaptations remain to be determined. Additionally, while the increasing prevalence of RE-CmeABC in *C. jejuni* may indicate a fitness advantage, experimental fitness assessments of these strains have not bene reported.

It should be kept in mind that mutations conferring antimicrobial resistance do not always affect *Campylobacter* fitness in a negative way. Consider fluoroquinolone resistance of *C. jejuni,* mediated by a C257T substitution in *gyrA* (Luo et al., 2005). Although growth kinetics, motility, and ability to colonize chickens were similar between the parental strain and isogenic resistant mutants in monoculture, when inoculated as mixed cultures the resistant mutants frequently outcompeted their susceptible parental counterparts, even in the absence of ciprofloxacin (Luangtongkum et al., 2010; Luo et al., 2005). This contrasts with macrolide resistance where, as discussed above, resistant *C. jejuni* mutants were outcompeted by their susceptible counterparts.

Dissemination of macrolide resistance

Mutations in the 23S rRNA gene as well as those in *rplV and rplD* can be transferred from erythromycin-resistant to erythromycin-susceptible *Campylobacter* strains by natural transformation (C. Cagliero et al., 2006; Gibreel et al., 2005a; Kim, Carver, & Kathariou, 2006). Interestingly, turkey-derived erythromycin-susceptible *C. coli* strains were more efficiently transformed to erythromycin resistance than *C. coli* from swine and transformation frequency was significantly higher at 42°C, the body temperature of poultry, than at 25°C (Kim et al., 2006; Kim, Kim, & Kathariou, 2008). Certain strains of *Campylobacter* have fragmented 23S rRNA stemming from post-transcriptional excision of intervening sequences (IVS) (Trust et al., 1994). Analysis of erythromycin-resistant *C. coli* from turkeys revealed that, in addition to harboring the A2075G substitution in the 23S rRNA gene, these strains also tended to harbor IVS in all three 23S rRNA genes (Chan, Miller, Mandrell, & Kathariou, 2007). Both the 23S rRNA A2075G mutation and IVS were transferrable to erythromycin-susceptible, IVS-free *C. coli* by natural transformation, rendering the latter resistant to erythromycin (Chan et al., 2007). It remains to be determined whether the higher prevalence of A2075G-mediated erythromycin resistance in *C. coli*, in comparison to *C. jejuni,* may reflect differences in frequency of IVS and 23S rRNA fragmentation between these two species.

Erm(B) is also transferable via natural transformation. Even though most of the reported *erm*(B)-harboring isolates have been *C. coli*, with only five *erm*(B)-positive *C. jejuni* strains reported to date (Deng, Wang, et al., 2015; Zhou et al., 2016), *erm*(B)-mediated resistance was transferable from *C. coli* to *C. jejuni* by natural transformation with total genomic DNA (S. Qin et al., 2014; Wang et al., 2014). The type VII *erm*(B)-carrying MDRGI in the chromosome of *C. jejuni* C179b (Fig. 1) was also transferable via natural transformation into erythromycin-

susceptible *C. jejuni* (Deng, Wang, et al., 2015). Along with macrolide resistance, *C. jejuni* transformants acquired additional AMR genes harbored in the MDRGI, gaining resistance to the corresponding antimicrobials including lincosamides, tetracycline, ciprofloxacin and gentamicin (S. Qin et al., 2014; Wang et al., 2014). Finally, The RE-CmeABC mutation was also transformable, with transformants exhibiting up to a 32-fold increase in erythromycin MIC (from 0.5 μg/ml to 16 μg/ml) (Yao et al., 2016).

Whole genome sequencing

The massive increase in WGS data for *Campylobacter* spp. (P. Chen et al., 2016; Y. Chen et al., 2013; Dutta et al., 2016; Miller et al., 2016; S. Qin et al., 2014; Weis, Clothier, Huang, Kong, & Weimer, 2016; Zhao et al., 2016) has yielded a rich resource that can be mined to identify determinants associated with resistance to macrolides and other antimicrobials. The earlier-discussed WGS analysis of 114 *C. jejuni* and *C. coli* isolates from humans, retail meats, and food animals in the United States revealed that detection of specific AMR genes could accurately predict the corresponding resistance phenotype. For example, resistance to tetracycline and fluoroquinolones perfectly correlated with presence of *tet*(O) or mutations in *gyrA*, respectively (Zhao et al., 2016). WGS was also critical in the identification of *erm*(B) in *Campylobacter* and in analysis of the contents and insertional sites of the *erm*(B)-harboring MDRGIs in *Campylobacter* (Deng, Wang, et al., 2015; Florez-Cuadrado et al., 2016; S. Qin et al., 2014; Zhang et al., 2016). However, differences in levels of macrolide resistance among isolates meeting the resistance threshold, e.g. ≥ 8 μg/ml and ≥ 16 μg/ml for *C. jejuni* and *C. coli*, respectively (Centers for Disease Control and Prevention, 2016) may not be readily predictable based on presence or absence of specific resistance mutations or determinants, as has also been recognized for other bacterial pathogens (Ellington et al., 2016). Further integration of WGS data

and phenotypic macrolide resistance assessments may yield WGS signatures with capacity to predict differences in levels of macrolide resistance in *Campylobacter* spp. beyond the resistance threshold.

CONCLUSIONS

Conserved (primarily A2075G) substitutions in domain V of the 23S rRNA gene still represent the most commonly encountered mechanism for macrolide resistance in *C. jejuni* and *C. coli*. The accompanying fitness costs in *C. jejuni*, especially in colonization of chickens, may be responsible for the relatively low prevalence of macrolide resistance in this species. For reasons that remain to be elucidated, similar fitness costs in colonization have not been observed with *C. coli*, which also exhibits markedly higher prevalence of macrolide resistance, especially among isolates from food and animal sources. The issue of erythromycin resistance in *C. jejuni* is intriguing; in spite of the documented fitness costs, stable erythromycin-resistant strains have been described and poultry flocks colonized by such strains have been reported (Bolinger, H., Kirchner, M., Chandrashekhar, K., Miller, W., Niedermeyer, J., Carver, D., Kathariou, 2016; X. Chen et al., 2010; Frediani-Wolf & Stephan, 2003; Gibreel et al., 2005b; Jacobs-Reitsma, Koenraad, Bolder, & Mulder, 1994; Luangtongkum et al., 2006). Fitness costs in *C. jejuni* may be strain-dependent or alleviated by compensatory mutations. Increasing availability and use of WGS data is expected to make major contributions in the further elucidation of these issues.

Even though to date *erm*(B) remains primarily confined to *C. coli* from China, it has been shown to be transferrable via natural transformation to *C. jejuni*, the species responsible for the majority of human campylobacteriosis, and was recently also identified outside of China (Spain). Further clonal expansion of *erm*(B)-harboring strains and infiltration of *erm*(B) into *C. jejuni* or

C. coli populations in other regions can compromise the clinical effectiveness of macrolides for severe campylobacteriosis. Dissemination of the newly described resistance- enhancing CmeABC mutations (RE-CmeABC) may also have public health implications, especially if they emerge in *C. jejuni* strains already harboring 23S rRNA point mutations or *erm*(B).

REFERENCES

Bolinger, H., Kirchner, M., Chandrashekhar, K., Miller, W., Niedermeyer, J., Carver, D., Kathariou, S. (2016). Investigation of erythromycin-resistant *Campylobacter jejuni* from turkey farms in North Carolina, P2-192, Annu Meet IAFP. International Association for Food Protection,St. Louis, MO.

Cagliero, C., Maurel, M.-C., Cloeckaert, A., & Payot, S. (2007). Regulation of the expression of the CmeABC efflux pump in *Campylobacter jejuni*: Identification of a point mutation abolishing the binding of the CmeR repressor in an *in vitro* -selected multidrug-resistant mutant. *FEMS Microbiology Letters*, *267*(1), 89–94. http://doi.org/10.1111/j.1574-6968.2006.00558.x

Cagliero, C., Mouline, C., Cloeckaert, A., & Payot, S. (2006). Synergy between efflux pump CmeABC and modifications in ribosomal proteins L4 and L22 in conferring macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Antimicrobial Agents and Chemotherapy*, *50*(11), 3893–3896. http://doi.org/10.1128/AAC.00616-06

Caldwell, D. B., Wang, Y., & Lin, J. (2008). Development, stability, and molecular mechanisms of macrolide resistance in *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy*, *52*(11), 3947–3954. http://doi.org/10.1128/AAC.00450-08

Centers for Disease Control and Prevention. (2013). *Antibiotic resistance threats in the United States*. Retrieved from www.cdc.gov/drugresistance/threat-report-2013/.

Centers for Disease Control and Prevention. (2014). *Campylobacter*, General Information. Retrieved from http://www.cdc.gov/nczved/divisions/dfbmd/diseases/campylobacter/

Centers for Disease Control and Prevention. (2016). National Antimicrobial Resistance

Monitoring System (NARMS): 2014 Human Isolates Surveillance Report, 1–83. http://doi.org/10.1002/ejoc.201200111

Chan, K., Miller, W. G., Mandrell, R. E., & Katharíou, S. (2007). The absence of intervening sequences in 23S rRNA genes of *Campylobacter coli* isolates from turkeys is a unique attribute of a cluster of related strains which also lack resistance to erythromycin. *Applied and Environmental Microbiology*, *73*(4), 1208–1214. http://doi.org/10.1128/AEM.01995-06

Chen, P., Arabyan, N., Gilpin, B., Mason, C., Ak, T., Wa, S., … Weimer, B. C. (2016). Large-scale release of *Campylobacter* draft genomes: Resources for food safety and public health from the 100K pathogen genome project. *Genome Announcements*, *5*(1), 1–6. http://doi.org/10.1128/genomeA.00925-16

Chen, X., Naren, G. W., Wu, C. M., Wang, Y., Dai, L., Xia, L. N., … Shen, J. Z. (2010). Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China. *Veterinary Microbiology*, *144*(1–2), 133–139. http://doi.org/10.1016/j.vetmic.2009.12.035

Chen, Y., Mukherjee, S., Hoffmann, M., Kotewicz, M. L., Young, S., Abbott, J., … McDermott, P. F. (2013). Whole-genome sequencing of gentamicin-resistant *Campylobacter coli* isolated from U.S. retail meats reveals novel plasmid-mediated aminoglycoside resistance genes. *Antimicrobial Agents and Chemotherapy*, *57*(11), 5398–5405. http://doi.org/10.1128/AEM.02873-15.

Deng, F., Shen, J., Zhang, M., Wu, C., & Zhang, Q. (2015). Constitutive and inducible expression of the rRNA methylase gene *erm*(B) in *Campylobacter*. *Antimicrobial Agents and Chemotherapy*, *59*(10), 6661–6664. http://doi.org/10.1128/AAC.01103-15

Deng, F., Wang, Y., Zhang, Y., & Shen, Z. (2015). Characterization of the genetic environment of the ribosomal rna methylase gene *erm*(B) in *Campylobacter jejuni*. *Journal of*

Antimicrobial Chemotherapy, *70*(2), 613–615. http://doi.org/10.1093/jac/dku418

Dutta, V., Altermann, E., Olson, J., Wray, G. A., Siletzky, R. M., & Kathariou, S. (2016). Whole-genome sequences of agricultural, host-associated *Campylobacter coli* and *Campylobacter jejuni* strains. *Genome Announcements*, *4*(4), e00833-16. http://doi.org/10.1128/genomeA.00833-16

Ellington, M. J., Ekelund, O., Aarestrup, F. M., Canton, R., Doumith, M., Giske, C., … Woodford, N. (2016). The role of whole genome sequencing (WGS) in antimicrobial susceptibility testing of bacteria: report from the EUCAST subcommittee. *Clinical Microbiology and Infection*, *23*, 2–22. http://doi.org/10.1016/j.cmi.2016.11.012

Florez-Cuadrado, D., Ugarte-Ruiz, M., Quesada, A., Palomo, G., Domínguez, L., & Porrero, M. C. (2016). Description of an *erm*(B)-carrying *Campylobacter coli* isolate in Europe. *Journal of Antimicrobial Chemotherapy*, *71*(3), 841–843. http://doi.org/10.1093/jac/dkv383

Frediani-Wolf, V., & Stephan, R. (2003). Resistance patterns of *Campylobacter* spp. strains isolated from poultry carcasses in a big Swiss poultry slaughterhouse. *International Journal of Food Microbiology*, *89*(2), 233–240. http://doi.org/10.1016/S0168-1605(03)00152-1

Gibreel, A., Kos, V. N., Keelan, M., Trieber, C. a, Levesque, S., Michaud, S., & Taylor, D. E. (2005). Molecular mechanism and stability of the resistance phenotype. *Antimicrobial Agents and Chemotherapy*, *49*(7), 2753–2759. http://doi.org/10.1128/AAC.49.7.2753

Gibreel, A., & Taylor, D. E. (2006). Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *The Journal of Antimicrobial Chemotherapy*, *58*(2), 243–255. http://doi.org/10.1093/jac/dkl210

Gibreel, A., Wetsch, N. M., & Taylor, D. E. (2007). Contribution of the CmeABC efflux pump to macrolide and tetracycline resistance in *Campylobacter jejuni*. *Antimicrobial Agents and*

Chemotherapy, *51*(9), 3212–3216. http://doi.org/10.1128/AAC.01592-06

Gillespie, I. A., O'Brien, S. J., Frost, J. A., Adak, G. K., Horby, P., Swan, A. V, … Neal, K. R. (2002). A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: a tool for generating hypotheses. *Emerging Infectious Diseases*, *8*(9), 937–942. http://doi.org/10.3201/eid0809.010187

Grinnage-Pulley, T., & Zhang, Q. (2015). Genetic basis and functional consequences of differential expression of the CmeABC efflux pump in *Campylobacter jejuni* isolates. *PLOS One*, *10*(7), e0131534. http://doi.org/10.1371/journal.pone.0131534

Guo, B., Lin, J., Reynolds, D. L., & Zhang, Q. (2010). Contribution of the multidrug efflux transporter CmeABC to antibiotic resistance in different *Campylobacter* species. *Foodborne Pathogens and Disease*, *7*(1), 77–83. http://doi.org/10.1089/fpd.2009.0354

Guo, B., Wang, Y., Shi, F., Barton, Y.-W., Plummer, P., Reynolds, D. L., … Zhang, Q. (2008). CmeR functions as a pleiotropic regulator and is required for optimal colonization of *Campylobacter jejuni* in vivo. *Journal of Bacteriology*, *190*(6), 1879–1890. http://doi.org/10.1128/JB.01796-07

Han, F., Pu, S., Wang, F., Meng, J., & Ge, B. (2009). Fitness cost of macrolide resistance in *Campylobacter jejuni*. *International Journal of Antimicrobial Agents*, *34*(5), 462–466. http://doi.org/10.1016/j.ijantimicag.2009.06.019

Hao, H., Dai, M., Wang, Y., Peng, D., Liu, Z., & Yuan, Z. (2009). 23S rRNA mutation A2074C conferring high-level macrolide resistance and fitness cost in *Campylobacter jejuni*. *Microbial Drug Resistance*, *15*(4), 239–244. http://doi.org/10.1089/mdr.2009.0008

Hao, H., Yuan, Z., Shen, Z., Han, J., Sahin, O., Liu, P., & Zhang, Q. (2013). Mutational and transcriptomic changes involved in the development of macrolide resistance in

Campylobacter jejuni. *Antimicrobial Agents and Chemotherapy*, *57*(3), 1369–1378. http://doi.org/10.1128/AAC.01927-12

Hermans, D., Pasmans, F., Messens, W., Martel, A., Van Immerseel, F., Rasschaert, G., … Haesebrouck, F. (2012). Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*. *Vector-Borne and Zoonotic Diseases*, *12*(2), 89–98. http://doi.org/10.1089/vbz.2011.0676

Humphrey, T., O'Brien, S., & Madsen, M. (2007). Campylobacters as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology*, *117*(3), 237–257. http://doi.org/10.1016/j.ijfoodmicro.2007.01.006

Jacobs-Reitsma, W. F., Koenraad, P., Bolder, N., & Mulder, R. (1994). In vitro susceptibility of *Campylobacter* and *Salmonella* isolates from broilers to quinolones, ampicillin, tetracycline, and erythromycin. *Veterinary Quarterly*, *16*(4), 206–208.

Kim, J.S., Carver, D. K., & Kathariou, S. (2006). natural transformation-mediated transfer of erythromycin resistance in *Campylobacter coli* strains from turkeys and swine. *Applied and Environmental Microbiology*, *72*(2), 1316–1321. http://doi.org/10.1128/AEM.72.2.1316–1321.2006

Kim, J.S., Kim, J.W., & Kathariou, S. (2008). Differential effects of temperature on natural transformation to erythromycin and nalidixic acid resistance in *Campylobacter coli*. *Applied and Environmental Microbiology, 74*(19), 6121–6125. http://doi.org/10.1128/AEM.01075-08

Ladely, S. R., Meinersmann, R. J., Englen, M. D., Fedorka-Cray, P. J., & Harrison, M. A. (2009). 23S rRNA gene mutations contributing to macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Foodborne Pathogens and Disease*, *6*(1), 91–98. Retrieved from http://online.liebertpub.com.prox.lib.ncsu.edu/doi/pdf/10.1089/fpd.2008.0098

Leclercq, R. (2002). Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *34*(4), 482–492. http://doi.org/10.1086/324626

Lin, J., Akiba, M., Sahin, O., & Zhang, Q. (2005). CmeR functions as a transcriptional repressor for the multidrug efflux pump CmeABC in *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy*, *49*(3), 1067–75. http://doi.org/10.1128/AAC.49.3.1067-1075.2005

Lin, J., Yan, M., Sahin, O., Pereira, S., Chang, Y.-J. Y.-J., & Zhang, Q. (2007). Effect of macrolide usage on emergence of erythromycin-resistant *Campylobacter* isolates in chickens. *Antimicrobial Agents and Chemotherapy*, *51*(5), 1678–86. http://doi.org/10.1128/AAC.01411-06

Luangtongkum, T., Jeon, B., Han, J., Plummer, P., Logue, C. M., & Zhang, Q. (2010). Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. *Future Microbiology*, *4*(2000), 189–200. http://doi.org/10.2217/17460913.4.2.189.Antibiotic

Luangtongkum, T., Morishita, T. Y. T., Ison, A. J., Huang, S., McDermott, P. F., & Zhang, Q. (2006). Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. *Applied and Environmental Microbiology…*, *72*(5), 3600–3607. http://doi.org/10.1128/AEM.72.5.3600

Luangtongkum, T., Shen, Z., Seng, V. W., Sahin, O., Jeon, B., Liu, P., & Zhang, Q. (2012). Impaired fitness and transmission of macrolide-resistant *Campylobacter jejuni* in its natural host. *Antimicrobial Agents and Chemotherapy*, *56*(3), 1300–1308. http://doi.org/10.1128/AAC.05516-11

Luo, N., Pereira, S., Sahin, O., Lin, J., Huang, S., Michel, L., & Zhang, Q. (2005). Enhanced in

vivo fitness of fluoroquinolone-resistant *Campylobacter jejuni* in the absence of antibiotic selection pressure. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(3), 541–546. http://doi.org/10.1073/pnas.0408966102

Mamelli, L., Amoros, J. P., Pagès, J. M., & Bolla, J. M. (2003). A phenylalanine-arginine β-naphthylamide sensitive multidrug efflux pump involved in intrinsic and acquired resistance of *Campylobacter* to macrolides. *International Journal of Antimicrobial Agents*, *22*(3), 237–241. http://doi.org/10.1016/S0924-8579(03)00199-7

Miller, W. G., Huynh, S., Parker, C. T., & Niedermeyer, J. A. (2016). Complete genome sequences of multidrug-resistant *Campylobacter jejuni* strain 14980A (turkey feces) and *Campylobacter coli* strain 14983A (housefly from a turkey farm), harboring a novel gentamicin resistance mobile element. *Genome Announcement*, *4*(5), 16–17. http://doi.org/10.1128/genomeA.01175-16.Copyright

Ohno, H., Wachino, J. I., Saito, R., Jin, W., Yamada, K., Kimura, K., & Arakawa, Y. (2016). A highly macrolide-resistant campylobacter *jejuni* strain with rare A2074T mutations in 23s rRNA genes. *Antimicrobial Agents and Chemotherapy*, *60*(4), 2580–2581. http://doi.org/10.1128/AAC.02822-15

Payot, S., Bolla, J.-M., Corcoran, D., Fanning, S., Mégraud, F., & Zhang, Q. (2006). Mechanisms of fluoroquinolone and macrolide resistance in *Campylobacter* spp. *Microbes and Infection*, *8*(7), 1967–1971. http://doi.org/10.1016/j.micinf.2005.12.032

Perez-Boto, D., Lopez-Portoles, J. A., Simon, C., Valdezate, S., & Echeita, M. A. (2010). Study of the molecular mechanisms involved in high-level macrolide resistance of Spanish *Campylobacter jejuni* and *Campylobacter coli* strains. *Journal of Antimicrobial Chemotherapy*, *65*(10), 2083–2088. http://doi.org/10.1093/jac/dkq268

Pumbwe, L., & Piddock, L. (2002). Identification and molecular characterisation of CmeB, a *Campylobacter jejuni* multidrug effux pump. *FEMS Microbiology Letters*, *206*, 185–189. http://doi.org/10.1111/j.1574-6968.2002.tb11007.x

Qin, S., Wang, Y., Zhang, Q., Chen, X., Shen, Z., Deng, F., … Shen, J. (2012). Identification of a novel genomic island conferring resistance to multiple aminoglycoside antibiotics in *Campylobacter coli*. *Antimicrobial Agents and Chemotherapy*, *56*(10), 5332–5339. http://doi.org/10.1128/AAC.00809-12

Qin, S., Wang, Y., Zhang, Q., Zhang, M., Deng, F., Shen, Z., … Shen, J. (2014). Report of ribosomal RNA methylase gene *erm*(B) in multidrug-resistant *Campylobacter coli*. *Journal of Antimicrobial Chemotherapy*, *69*(4), 964–968. http://doi.org/10.1093/jac/dkt492

Ruiz-palacios, G. M. (2007). The health burden of *Campylobacter* infection and the impact of antimicrobial resistance : playing chicken. *Clinical Infectious Diseases*, *44*, 701–703. http://doi.org/10.1086/509936

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., … Griffin, P. M. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. http://doi.org/10.3201/eid1701.091101p1

Scallan, E., Hoekstra, R. M., Mahon, B. E., Jones, T. F., & Griffin, P. M. (2015). An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiology and Infection*, *143*(13), 2795–2804. http://doi.org/10.1017/S0950268814003185

Taylor, E. V., Herman, K. M., Ailes, E. C., Fitzgerald, C., Yoder, J. S., Mahon, B. E., & Tauxe, R. V. (2012). Common source outbreaks of *Campylobacter* infection in the USA, 1997–2008. *Epidemiology and Infection*, *141*(2013), 1–10.

http://doi.org/10.1017/S0950268812001744

Trust, T. J., Logan, S. M., Gustafson, C. E., Romaniuk, P. J., Kim, N. W., Chan, V. L., … Gutell, R. R. (1994). Phylogenetic and molecular characterization of a 23S rRNA gene positions the genus *Campylobacter* in the epsilon subdivision of the Proteobacteria and shows that the presence of transcribed spacers is common in *Campylobacter* spp. *Journal of Bacteriology*, *176*(15), 4597–4609. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8045890

Tseng, T. T., Gratwick, K. S., Kollman, J., Park, D., Nies, D. H., Goffeau, a, & Saier, M. H. (1999). The RND permease superfamily: an ancient, ubiquitous and diverse family that includes human disease and development proteins. *Journal of Molecular Microbiology and Biotechnology*, *1*(1), 107–125.

Tyson, G. H., Tate, H. P., Abbott, J., Tran, T.-T., Kabera, C., Crarey, E., … Zhao, S. (2016). Molecular subtyping and source attribution of *Campylobacter* isolated from food animals. *Journal of Food Protection*, *79*(11), 1891–1897. http://doi.org/10.4315/0362-028X.JFP-16-195

US Food and Drug Adminimstration. (2017). Withdrawal of Enrofloxacin for Poultry. Retrieved March 28, 2017, from https://www.fda.gov/AnimalVeterinary/SafetyHealth/RecallsWithdrawals/ucm042004.htm

US Food and Drug Administration. (2013). *National Antimicrobial Resistance Monitoring System Integrated Report - Enteric Bacteria. NARMS Integrated Report: 2012-2013*.

US Food and Drug Administration. (2014). *NARMS Integrated Report: 2014*. Retrieved from https://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm059103.htm

Vacher, S., Menard, A., Bernard, E., Santos, A., & Megraud, F. (2005). Detection of mutations

associated with macrolide resistance in thermophilic *Campylobacter* spp. by real-time PCR. *Microbial Drug Resistance (Larchmont, N.Y.)*, *11*(1), 40–47. http://doi.org/10.1089/mdr.2005.11.40

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. a, Robinson, T. P., … Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences of the United States of America*, (16), 1–6. http://doi.org/10.1073/pnas.1503141112

Wang, Y., Zhang, M., Deng, F., Shen, Z., Wu, C., Zhang, J., … Shen, J. (2014). Emergence of multidrug-resistant *Campylobacter* species isolates with a horizontally acquired rRNA methylase. *Antimicrobial Agents and Chemotherapy*, *58*(9), 5405–5412. http://doi.org/10.1128/AAC.03039-14

Weis, A. M., Clothier, K. A., Huang, B. C., Kong, N., & Weimer, B. C. (2016). Draft genome sequences of *Campylobacter jejuni* strains that cause abortion in livestock. *Genome Announcements*, *4*(6), e01324-16. http://doi.org/10.1128/genomeA.01324-16

Werner, G., Hildebrandt, B., & Witte, W. (2003). Linkage of *erm*(B) and *aadE-sat4-aphA-3* in multiple-resistant *Enterococcus faecium* isolates of different ecological origins. *Microbial Drug Resistance. 1*(9 Supplement), S9-S16.

Yang, B., Lian, Y., Liu, Y., Wu, B.-Y., & Duan, R.-S. (2016). A retrospective analysis of possible triggers of Guillain–Barre syndrome. *Journal of Neuroimmunology*, *293*, 17–21. http://doi.org/10.1016/j.jneuroim.2016.02.003

Yao, H., Shen, Z., Wang, Y., Deng, F., Liu, D., Naren, G., … Shen, J. (2016). Emergence of a potent multidrug efflux pump variant that enhances *Campylobacter* resistance to multiple antibiotics. *mBio*, *7*(5), e01543-16. http://doi.org/10.1128/mBio.01543-16

Yuki, N., & Hartung, H.-P. (2012). Guillain-Barré syndrome. *The New England Journal of Medicine*, *366*(24), 2294–2304. http://doi.org/10.1056/NEJMra1114525

Zeitouni, S., Collin, O., Andraud, M., Ermel, G., & Kempf, I. (2012). Fitness of macrolide resistant *Campylobacter coli* and *Campylobacter jejuni*. *Microbial Drug Resistance*, *18*(2), 101–108. http://doi.org/10.1089/mdr.2011.0188

Zhang, A., Song, L., Liang, H., Gu, Y., Zhang, C., Liu, X., … Zhang, M. (2016). Molecular subtyping and erythromycin resistance of *Campylobacter* in China. *Journal of Applied Microbiology*, *121*(1), 287–293. http://doi.org/10.1111/jam.13135

Zhao, S., Tyson, G. H., Chen, Y., Li, C., Mukherjee, S., Young, S., … McDermott, P. F. (2016). Whole-genome sequencing analysis accurately predicts antimicrobial resistance phenotypes in *Campylobacter* spp. *Applied and Environmental Microbiology*, *82*(2), 459–466. http://doi.org/10.1128/AEM.02873-15

Zhou, J., Zhang, M., Yang, W., Fang, Y., Wang, G., & Hou, F. (2016). A seventeen-year observation of the antimicrobial susceptibility of clinical *Campylobacter jejuni* and the molecular mechanisms of erythromycin-resistant isolates in Beijing, China. *International Journal of Infectious Diseases*, *42*, 28–33. http://doi.org/10.1016/j.ijid.2015.11.005

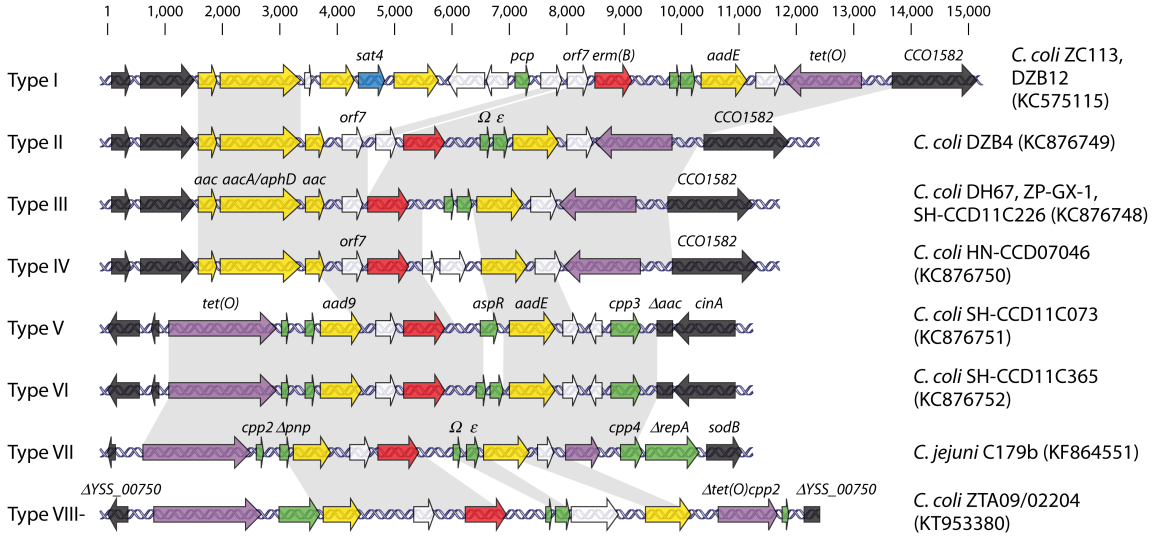


Figure 2-1 The genetic environment of *erm*(B) –harboring MDRGIs in *Campylobacter* spp.

Chapter 3 Emerging antimicrobial resistance determinants in *Campylobacter* spp. from conventionally grown turkeys: investigation of gentamicin resistance and *erm*(B)-mediated macrolide resistance

SUMMARY

Campylobacter generally causes an illness that subsides on its own, however vulnerable populations may require treatment with antibiotics. *Campylobacter* isolates are commonly resistant to fluoroquinolones and quickly becoming increasingly resistant to other antimicrobials such as macrolides and gentamicin. Despite the emerging nature of resistance to macrolides and gentamicin, the pre-harvest prevalence of resistance to gentamicin especially, but also macrolides, remains poorly characterized in poultry, especially so in turkeys. This study has found that of 2,564 C. *jejuni* and C. *coli* isolates sourced from turkeys between July 2012 and July 2016, 90.0% were resistant to gentamicin (50 μg/ml). Resistance was strongly associated ($p<0.001$) with the presence of either *aph(2")-If* or *aph(2")-Ig*. Additionally, there was a strong geographic trend in the distribution of these genes as *aph(2")-If* found only in isolates from North Carolina and *aph(2")-Ig* only in isolates from Arkansas. We also investigated the incidence of *erm*(B), which was recently described among macrolide resistant *Campylobacter*, mostly from China. However, *erm*(B) could not be detected among any of the 178 erythromycin-resistant isolates that we tested. The 23S rRNA gene of 52 erythromycin-resistant *Campylobacter* were sequenced to determine the cause of their resistance and all were found to harbor an A2075G substitution. This substitution is common in C. *coli*, but more rarely encountered in C. *jejuni*. These results underscore the need for stewardship in antibiotic use in animal agriculture to preserve the efficacy of available therapies.

INTRODUCTION

Campylobacter spp. are estimated to be responsible for approximately 850,000 illnesses in the U.S. every year (Elaine Scallan et al., 2011). Although most cases will resolve on their own, immunocompromised individuals are more susceptible to severe infections and may require antibiotic treatment. However, the high incidence of resistance to fluoroquinolones has brought about the need for alternative antibiotic therapies and resulted in macrolides becoming the drug of choice for treatment (Alfredson & Korolik, 2007; Lutgen et al., 2009; US Food and Drug Administration, 2014). Gentamicin has been considered as another viable option for treatment in cases of severe infection with *Campylobacter* such as severe *C. fetus* infections (Aarestrup & Engberg, 2001). *Campylobacter*, as a zoonotic pathogen, experiences selective pressures from antimicrobial use both in humans and livestock, and both gentamicin and macrolides are approved for use in animal agriculture (US Food and Drug Administration, 2013).

Until recently, gentamicin resistance was uncommonly encountered in *Campylobacter.* NARMS data beginning in 1998 indicates that historically there has been low prevalence of resistance in both human and animal isolates *of Campylobacter* (NARMS 2017). However, gentamicin-resistant *C. coli* from chickens has increased from 0% in 1998 to a high of 11.5% in 2011, and a WGS survey (published in 2016) of 114 *Campylobacter* isolates from humans, chicken, and beef found that 78 (68%) were gentamicin resistant (NARMS 2017; Zhao et al., 2016). Additionally, WGS of *Campylobacter* isolates from the recent puppy-associated outbreak found that 9 of the 13 analyzed isolates were resistant to gentamicin (CDC 2017). However, for all of the increase in resistance, very little is known about the determinants of resistance or the possibility and mechanisms of dissemination.

The pre-harvest incidence of gentamicin resistance is relatively unknown, however a 2006 study suggests that at the time the incidence was still quite low as analysis of turkey ceca from both conventional (n=360) and organic production (n=230) found no isolates with gentamicin resistance (Luangtongkum et al., 2006). There were no gentamicin-resistant *C. jejuni* isolated from retail ground turkey in 2013, and in 2014 there were no gentamicin-resistant *C. jejuni* from turkey ceca, however only one isolate was tested from this source (US Food and Drug Administration, 2014). In contrast, 13.3% of *C. coli* from turkey ceca was reported to be resistant to gentamicin in 2014 (US Food and Drug Administration, 2014). This has brought about the the need to clarify the true prevalence of gentamicin resistance in poultry and determine if resistance is indeed more common today than has previously been reported. Finally, due to the high levels of fluoroquinolone resistance in *Campylobacter,* the use of macrolides to treat confirmed cases of campylobacteriosis has increased. While *C. coli* isolates may commonly show resistance to macrolides, resistance is far less common in *C. jejuni*. Mechanisms of macrolide resistance in *Campylobacter* primarily include substitutions in the 23S rRNA genes. However, resistance can also involve mutations in active efflux determinants and the presence of a gene, *erm*(B), that was only reported in *Campylobacter* in 2014 and has remained limited mainly to China, although retrospective analyses placed the first *erm*(B)-positive *Campylobacter* isolates in 1994 (S. Qin et al., 2014; Zhou et al., 2016). Substitutions in 23S rRNA, specifically A2075G, A2074C, and less frequently A2074G, most commonly mediate macrolide resistance in *Campylobacter* spp. although there are associated fitness costs in *C. jejuni* (Zhao et al., 2016).

The goal of this study was to evaluate the current state (i.e. prevalence and genetic basis) of gentamicin resistance in *Campylobacter* isolates derived from turkeys and flies from

commercial turkey flocks. Additionally, the potential for dissemination of *aph(2")-If* via natural transformation was investigated. Finally, we screened for the presence of *erm(B)*.

MATERIALS and METHODS

Campylobacter strains and growth conditions

The isolates used in this study are listed in Appendix A. A total of 366 *Campylobacter* isolates (191 *C. coli,* 175 *C. jejuni*) were isolated from feces (n = 39), flies (n = 34), and intestinal samples (n = 283, 275 from ceca and 18 from jejunum) from 70 conventional turkey farms in North Carolina from July 2012 to July 2016. In addition, ten isolates from ceca provided by a commercial turkey company in Arkansas between December of 2014 and January of 2015 were also analyzed. Fly and fecal samples were only available through January, 2015 and jejunum samples were only available from May to June of 2016.

Fly and fecal samples were collected during on-farm evaluations from July, 2013 to October, 2015 and processed as described in Chapter 3. Intestinal samples were collected on a weekly basis by the turkey company for their own health surveillance program. One cecum per bird and six ceca per flock were made available to us. Individually packed ceca were shipped to the laboratory overnight on ice. Cecal composites were made by combining 0.1 g of cecal contents from the mid-section of each of the six ceca samples into 1 ml of sterile MHB broth in order to make a 1:10 dilution. Serial dilutions of these suspensions were plated onto modified *Campylobacter* blood-free selective agar (mCCDA; Oxoid, Hampshire, UK; SE 155, Oxoid), and incubated microaerobically at 42°C for 48 hours in anaerobic jars containing CampyPak Plus microaerobic sachets (Becton Dickinson, Sparks, MD). A total of six colonies per composite sample were purified on Mueller-Hinton agar (MHA; Difco, Becton Dickinson), and pure

cultures were preserved in brain heart infusion broth (Becton Dickinson) supplemented with 20% glycerol at -80°C.

Antibiotic susceptibility determinations

Each isolate was tested to determine its resistance (AMR) against 7 antimicrobial agents: 16 μg/ml tetracycline, 64 μg/ml streptomycin, 8 μg/ml erythromycin, 64 μg/ml kanamycin, 32 μg/ml nalidixic acid, 4 μg/ml ciprofloxacin, 50 μg/ml gentamicin. A few isolated colonies of each strain were suspended in 100 μl of MHB. Two 3.5 μl spots from each isolate were placed onto each of the seven antimicrobial plates and an MHA positive control plate. *C. jejuni* ATCC 33560 (purchased from the American Type Culture Collection, Manassas, VA) was used as for quality assurance on each antibiotic plate. Resistance was determined by noting confluent growth of the bacteria after 48 hours of incubation at 42°C. AMR phenotypes are denoted by the first letter of each antimicrobial with the exception of the quinolones nalidixic acid and ciprofloxacin which usually shows co-resistance, as was the case for all isolates in this study, and is denoted by Q.

DNA extractions, species confirmation, and MLST

Multiplex PCR using primers targeting *ceuE* and *hip* was used to determine the species (*C. coli* or *C. jejuni* respectively) of the isolates as described previously (Smith et al., 2004). Isolate designations consisted of the combination of antimicrobial resistance profile and species designation. For example, *C. jejuni* isolate resistant to tetracycline, kanamycin, and gentamicin, was designated TKGcj.

Multi-locus sequence typing (MLST) was performed on a subset of isolates from 33 flocks. DNA was extracted from isolates grown on MHA using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the procedures suggested by the vendor. MLST analysis was performed as described previously (Miller et al., 2006).

Detection of Resistance Determinants

PCR based detection of resistance genes

Testing for the presence of the aminoglycoside resistance genes *aph(2")-If* and *aph(2")-Ig* was performed by PCR using primers YC21F and YC21R and SMIgF and SMIgR, respectively, (Zhao et al., 2015). Testing for the presence of *erm(B)* utilized primers ermB-F: GGGCATTTAACGACGAAACTGG and ermB-R: CTGTGGTATGGCGGGTAAGT, based on *erm*(B) sequences conserved in *C. coli*, *C. jejuni* (Wang et al., 2014). They were additionally PCR-tested for the aminoglycoside-resistance cluster (ARC) from the type I MDRGI (Shangshang Qin et al., 2012; Wang et al., 2014) with primers cluster -F: GGATGGATTCCTATGAAAACAT and cluster-R: GGCTTTGTTCATCTTCATACTCT, to detect MDRGIs that may either have lost or not yet acquired *erm*(B). Genomic DNAs of *C. coli* ZC113 and *C. coli* NADC2A were included each time as positive control for *erm*(B) and the ARC, respectively (S. Qin et al., 2014; Wang et al., 2014).

23S rRNA sequencing

Sequencing of an internal fragment of the 23S rRNA genes was performed as previously described using primers F2-campy-23S (5'-AATTG ATGGGGTTAGCATTAGC-3') and R1-

campy-23S (5'-AA CGATTTCCAACCGTTCTG-3') (Kim et al., 2006) with a difference in that sequencing was performed at either a different company (Genewiz Inc., South Plainfield, NJ, USA) or at the USDA-ARS lab of W. G. Miller.

Transformations

Natural Transformation was performed as previously described for a panel of isolates (Kim et al., 2006). Briefly, the turkey feces-derived *C. jejuni* isolate 14980A (TSKQG) and the fly-derived *C. coli* isolate 14983A (Miller et al., 2016) which both carry *aph(2")-If* served as the donor of gentamicin resistance in transformation assays. Mean transformation frequencies for the recipient strains 14158-1 and 81-176 were determined by calculating the ratio of the total number of gentamicin-resistant transformants to the total number of recipient cells at the end of the transformation period. Frequency of transformation was determined in duplicate, and from at least two independent transformation experiments.

RESULTS AND DISCUSSION

Gentamicin resistance is common in turkey and fly-derived *Campylobacter* isolates

Of the 2,564 isolates obtained between July 2012 and October 2016, 90.0% were found to be resistant to gentamicin. The distribution of these isolates sorted by year and source can be seen in Figure 3-1. These results agree with the recent increase in gentamicin resistance in *Campylobacter* in the U.S. and high levels reported in China (US Food and Drug Administration, 2014; Yao, Liu, Wang, Zhang, & Shen, 2017).

Gentamicin resistance genes were strongly correlated with the resistance phenotype

Although a large number of gentamicin-resistance genes have been identified in *Campylobacter,* we chose to test for the presence of *aph(2")-If* and *aph(2")-Ig* in a panel of both gentamicin-susceptible (n=14) and gentamicin-resistant isolates (n=361) (Zhao et al., 2015). Past studies have identified *aph(2")-If* and *aph(2")-Ig* as the most common genes conferring resistance to gentamicin in *Campylobacter* from broilers and humans (Yao et al., 2017; Zhao et al., 2015). Additionally, WGS of 114 *Campylobacter* isolates found that *aph(2")-If* and *aph(2")-Ig* were the only gentamicin-resistance genes shared between humans, poultry, and poultry meat (Y. Chen et al., 2013). The results from our study identified *aph(2")-If* and *aph(2")-Ig* in both *C. jejuni* and *C. coli* and no isolate contained both resistance genes. Species designation (*C. jejuni* vs. *C. coli*) had no significant effect on which gene was present (Fisher's exact test, $p = 0.108$).

The presence of *aph(2")-If* (n=352) was vastly more common than that of *aph(2")-Ig* (n=8), and the presence of a resistance gene was highly correlated with the resistant phenotype as shown in Table 3-1 ($p<0.0001$). No gentamicin-susceptible isolates carried a resistance gene, however two of the resistant isolates lacked either *aph(2")-If* or *aph(2")-Ig.* Additionally, all eight isolates harboring *aph(2")-Ig* were found in isolates (7 *C. coli,* 1 *C jejuni)* from Arkansas, and no isolates from Arkansas carried *aph(2")-If*, which was found only in isolates from North Carolina. However, many more isolates from North Carolina were included in this panel and isolates from Arkansas from only from late 2014 to early 2015. All gentamicin-susceptible isolates were inhibited at 4 μg/ml.

Gentamicin resistance determinants *aph(2")-If* and *aph(2")-Ig* were detected in *C. jejuni* and *C. coli* isolates of diverse genotypes and AMR profiles

From the 361 gentamicin resistant isolates analyzed, the most common AMR profile in gentamicin-resistant *C. coli* was TSEKQG (n=125) while for *C. jejuni* it was TSKQG (n=109). For gentamicin-susceptible *C. coli,* the most common AMR profile was T (n=3) while for susceptible *C. jejuni* it was TSKQ (n=3) as shown in figure 3-2. MLST analysis was performed on 71 *Campylobacter* spp. isolates resulting in 29 STs. ST-1839 was the most common ST identified (n=29), and all but one of the 29 *C. jejuni* isolates with ST-1839 harbored *aph(2")-If* and exhibited resistance to gentamicin with the remaining isolate being gentamicin susceptible and lacking a resistance gene (appendix A).

***aph(2")-If* is transferable to both *C. jejuni* and *C. coli* by natural transformation**

Previous literature indicates that *aph(2")-If* may be either chromosomal or plasmid mediated while *aph(2")-Ig* has been identified only on plasmids (Nirdnoy et al., 2005; Yao et al., 2017; Zhao et al., 2015, 2016). Successful transformation of *aph(2")-If* into gentamicin-susceptible *C. jejuni* has been reported (Yao et al., 2017), however transfer to *C. coli* and the frequency of transformation remains unevaluated. WGS identified *aph(2")-If* in a *C. coli* isolate from a fly in a commercial turkey farm (Miller et al., 2016).

Six of seven gentamicin-susceptible and *aph(2")-If* negative recipients were transformable by total genomic DNA from fly-derived *C. coli* strain 14983A and turkey feces-derived *C. jejuni* isolate 14980A as shown in Table 3-2. Both 14980A and 14983A acted as suitable donors of gentamicin resistance. The transformation frequencies were calculated for the donor-recipient pairs of 81-176, 14158-1, and 14983A. Recipient 14158-1 experienced the

greatest mean transformation frequency of $1.43\times10^{-5} \pm 4.52\times10^{-6}$ while 81-176 had a lower mean frequency of transformation of $1.49\times10^{-7} \pm 9.73\times10^{-8}$. The MICs of both of these recipient strains increased from 4 μg/ml to >512 μg/ml after transformation which agrees with previous gentamicin transformation work (Yao et al., 2017). The *C. jejuni* strain 15626C was unable to be transformed by the *C. coli* strain 14983A. A panel of eight randomly-chosen transformants from several independent transformations were tested by PCR for *aph(2")-If*. In all cases, the transformants were positive for this gene, while all control isolates (recipients incubated without donor DNA) were negative.

A recent report by Zhou et al performed whole genome sequencing of 114 *Campylobacter* isolates (Zhao et al., 2016). Of these 114 isolates, four were turkey-derived and resistant to gentamicin; three carried *aph(2")-If* while one carried *aph(2")-Ig*. However, the vast majority of *Campylobacter* isolates from chickens in this study carried *aph2")-Ig,* which is in opposition to our findings. This may be due to the difference in source or perhaps unreported geographical differences. For example, all of the *aph2")-Ig*-carrying *Campylobacter* in our study were from Arkansas. It may be that the geographical source of the chickens in the Zhou et al. was quite distant from the source of the *aph(2")-If*-carrying *Campylobacter* sourced from turkeys in North Carolina. Another difference between the samples would be that the *Campylobacter* isolates in our study were from pre-harvest birds, while those of Zhou et al were from retail chicken. Whole-genome sequencing of two *Campylobacter* spp. strains (one *C. jejuni,* ST-1839, accession numbers for genome/plasmid CP017025 to CP017028 from turkey feces, one *C. coli,* ST-1067, CP017029 to CP017030) from a fly) has revealed the genetic basis for similar multidrug-resistant phenotypes in turkeys and flies (Miller et al., 2016). Both of these strains harbored plasmids with *tet(O)* which confers resistance to tetracycline, as well as

kanamycin-resistance genes (Crespo et al., 2016; Lambert, T., Gerbaud, G., Trieu-Cuot, P., Courvalin, 1985; Liebert, Kanamycin, Gibreel, Sköld, & Taylor, 2004; Diane E. Taylor & Chau, 1996; Tenover, Gilbert, & O'Hara, 1989). The *C. coli* strain harbored the A2075G substitution in all three copies of the 23S rRNA gene, associated with macrolide resistance (Bolinger & Kathariou, 2017) and The 86 →Ile in *gyrA*, which has been shown to confer resistance to ciprofloxacin and nalidixic acid (Engberg, Aarestrup, Taylor, Gerner-Smidt, & Nachamkin, 2001; Jesse, Englen, Pittenger-Alley, & Fedorka-Cray, 2006; D. E. Taylor & Courvalin, 1988; Zirnstein, Helsel, Li, Swaminathan, & Besser, 2000). Both strains also carried *aph(2")-If*, which confers resistance to gentamicin.

The information reported here is even more recent than that in the last NARMS report which included data from 2014. It also includes both *C. jejuni* and *C. coli* and sources (turkeys and flies) not typically discussed in the literature. We believe that this report represents one of the largest scale investigations of gentamicin-resistant *Campylobacter* to date with a major focus on a food production animal and insect vectors.

Findings from *erm(B)* investigations

PCR with *erm*(B)-specific primers was employed to investigate 178 erythromycin-resistant (MIC ≥ 8 μg/ ml) *Campylobacter* isolates (174 *C. coli*, 4 *C. jejuni*) obtained between July 2013 and June 2016 from conventional turkey production in the southeastern United States. These isolates were derived from turkey feces (n=5), ceca (n=159), and flies in turkey houses (n=14). Multi-locus sequence typing (MLST) revealed four sequence types among *C. coli* (ST-1101, ST-8086, ST-8212, ST-8551) and two (ST-1839, ST-7729) among the four erythromycin-resistant *C. jejuni*.

No evidence for *erm*(B) or the ARC was obtained for any of the *C. coli* or *C. jejuni* isolates. Instead, the four erythromycin-resistant isolates harbored the typical A2075G substitution in 23S rRNA similarly to erythromycin-resistant *C. coli* (data not shown). Additionally, 48 erythromycin-resistant turkey-derived *C. coli* were analyzed by sequencing of PCR products of a fragment of the 23S rRNA obtained with primers F2-campy-23S and R1-campy-23S as described previously (Kim et al., 2006). The 48 erythromycin-resistant isolates included representatives of diverse MLST-based sequence types and AMR profiles, specifically TSEKQG (n=31), TEKQG (n=13), and one each of TSEKQ, TEK, TSEQG and TEKG. Sequence analysis revealed that all harbored the A2075G substitution in the 23S rRNA, known to be associated with macrolide resistance (Bolinger & Kathariou, 2017).

These findings suggest that *erm*(B) has not yet infiltrated the genomes of macrolide-resistant *C. coli* or *C. jejuni* from commercial turkey production in the United States. The data will contribute to worldwide efforts to monitor macrolide resistance and the presence of *erm*(B) in *Campylobacter* from food animal production and other sources.

REFERENCES

Aarestrup, F. M., & Engberg, J. (2001). Antimicrobial resistance of thermophilic *Campylobacter*. *Veterinary Research*, *32*(3–4), 311–321. http://doi.org/10.1051/vetres:2001127

Alfredson, D. A., & Korolik, V. (2007). Antibiotic resistance and resistance mechanisms in *Campylobacter jejuni* and *Campylobacter coli*. *FEMS Microbiology Letters*, *277*(2), 123–132. http://doi.org/10.1111/j.1574-6968.2007.00935.x

Bolinger, H., & Kathariou, S. (2017). The current state of macrolide resistance in *Campylobacter*: A review of trends and impacts of resistance mechanisms. *Applied and Environmental Microbiology*, http://doi.org/10.1128/AEM.00416-17

Chen, Y., Mukherjee, S., Hoffmann, M., Kotewicz, M. L., Young, S., Abbott, J., … McDermott, P. F. (2013). Whole-genome sequencing of gentamicin-resistant *Campylobacter coli* isolated from U.S. retail meats reveals novel plasmid-mediated aminoglycoside resistance genes. *Antimicrobial Agents and Chemotherapy*, *57*(11), 5398–5405. http://doi.org/10.1128/AEM.02873-15.

Crespo, M. D., Altermann, E., Olson, J., Miller, W. G., Chandrashekhar, K., & Kathariou, S. (2016). Novel plasmid conferring kanamycin and tetracycline resistance in the turkey-derived *Campylobacter jejuni* strain 11601MD. *Plasmid*, *86*, 32–37. http://doi.org/10.1016/j.plasmid.2016.06.001

Engberg, J., Aarestrup, F. M., Taylor, D. E., Gerner-Smidt, P., & Nachamkin, I. (2001). Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: Resistance mechanisms and trends in human isolates. *Emerging Infectious Diseases*. http://doi.org/10.3201/eid0701.700024

Jesse, T. W., Englen, M. D., Pittenger-Alley, L. G., & Fedorka-Cray, P. J. (2006). Two distinct mutations in *gyrA* lead to ciprofloxacin and nalidixic acid resistance in *Campylobacter coli* and *Campylobacter jejuni* isolated from chickens and beef cattle. *Journal of Applied Microbiology*, *100*(4), 682–688. http://doi.org/10.1111/j.1365-2672.2005.02796.x

Kim, J.-S., Carver, D. K., & Kathariou, S. (2006). Natural transformation-mediated transfer of erythromycin resistance in *Campylobacter coli* strains from turkeys and swine. *Applied and Environmental Microbiology*, *72*(2), 1316–1321. http://doi.org/10.1128/AEM.72.2.1316–1321.2006

Lambert, T., Gerbaud, G., Trieu-Cuot, P., Courvalin, P. (1985). Structural relationship between the genes encoding 3'-aminoglycoside phosphotransferases in *Campylobacter* and in Gram-positive cocci. *Annales de l'Institut Pasteur / Microbiologie*, 135–150.

Liebert, M. A., Kanamycin, P., Gibreel, A., Sköld, O. L. A., & Taylor, D. E. (2004). Characterization of plasmid-mediated *aphA-3* kanamycin resistance in *Campylobacter jejuni*, *10*(2), 98–105.

Luangtongkum, T., Morishita, T. Y. T., Ison, A. J., Huang, S., McDermott, P. F., & Zhang, Q. (2006). Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. *Applied and Environmental Microbiology…*, *72*(5), 3600–3607. http://doi.org/10.1128/AEM.72.5.3600

Lutgen, E. M., McEvoy, J. M., Sherwood, J. S., Logue, C. M., Mead, P., Slutsker, L., … Gaston, M. (2009). Antimicrobial resistance profiling and molecular subtyping of *Campylobacter* spp. from processed turkey. *BMC Microbiology*, *9*(1), 203. http://doi.org/10.1186/1471-2180-9-203

Miller, W. G., Englen, M. D., Kathariou, S., Wesley, I. V., Wang, G., Pittenger-Alley, L., …

Mandrell, R. A. (2006). Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. *Microbiology*, *152*(1), 245–255. http://doi.org/10.1099/mic.0.28348-0

Miller, W. G., Huynh, S., Parker, C. T., & Niedermeyer, J. A. (2016). Complete genome sequences of multidrug-resistant *Campylobacter jejuni* strain 14980A (turkey feces) and *Campylobacter coli* strain 14983A (housefly from a turkey farm), harboring a novel gentamicin resistance mobile element. *Genome Announcement*, *4*(5), 16–17. http://doi.org/10.1128/genomeA.01175-16.Copyright

Centers for Disease Control and Prevention. (2017). Multistate outbreak of human *Campylobacter* infections linked to pet store puppies. Retrieved October 4, 2017, from https://www.cdc.gov/campylobacter/outbreaks/puppies-9-17/index.html

US Food and Drug Administration (2017). NARMS Now: Interactive Data Displays. Retrieved October 4, 2017, from https://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/ucm416741.htm

Nirdnoy, W., Mason, C. J., & Guerry, P. (2005). Mosaic structure of a multiple-drug-resistant, conjugative plasmid from *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy*, *49*(6), 2454–2459. http://doi.org/10.1128/AAC.49.6.2454-2459.2005

Qin, S., Wang, Y., Zhang, Q., Chen, X., Shen, Z., Deng, F., … Shen, J. (2012). Identification of a novel genomic island conferring resistance to multiple aminoglycoside antibiotics in *Campylobacter coli*. *Antimicrobial Agents and Chemotherapy*, *56*(10), 5332–5339. http://doi.org/10.1128/AAC.00809-12

Qin, S., Wang, Y., Zhang, Q., Zhang, M., Deng, F., Shen, Z., … Shen, J. (2014). Report of

ribosomal RNA methylase gene *erm*(B) in multidrug-resistant *Campylobacter coli*. *Journal of Antimicrobial Chemotherapy*, *69*(4), 964–968. http://doi.org/10.1093/jac/dkt492

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., … Griffin, P. M. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. http://doi.org/10.3201/eid1701.091101p1

Taylor, D. E., & Chau, A. (1996). Tetracycline resistance mediated by ribosomal protection. *Antimicrobial Agents and Chemotherapy*, *40*(1), 1–5.

Taylor, D. E., & Courvalin, P. (1988). Mechanisms of antibiotic resistance in *Campylobacter* species. *Antimicrobial Agents and Chemotherapy*, *32*(8), 1107–1112.

Tenover, F. C., Gilbert, T., & O'Hara, P. (1989). Nucleotide sequence of a novel kanamycin resistance gene, *aphA-7*, from *Campylobacter jejuni* and comparison to other kanamycin phosphotransferase genes. *Plasmid*, *22*(1), 52–58. http://doi.org/10.1016/0147-619X(89)90035-8

US Food and Drug Administration. (2013). *National Antimicrobial Resistance Monitoring System Integrated Report - Enteric Bacteria. NARMS Integrated Report: 2012-2013*. Retrieved from: *https://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/.../UCM453398.pdf*

US Food and Drug Administration. (2014). *NARMS Integrated Report: 2014*. Retrieved from https://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm059103.htm

Wang, Y., Zhang, M., Deng, F., Shen, Z., Wu, C., Zhang, J., … Shen, J. (2014). Emergence of multidrug-resistant *Campylobacter* species isolates with a horizontally acquired rRNA methylase. *Antimicrobial Agents and Chemotherapy*, *58*(9), 5405–5412.

http://doi.org/10.1128/AAC.03039-14

Yao, H., Liu, D., Wang, Y., Zhang, Q., & Shen, Z. (2017). High prevlence and predominance of the *aph(2")-If* gene conferring aminoglycoside resistance in *Campylobacter*. *Antimicrobial Agents and Chemotherapy*, *61*(5), 1–9.

Zhao, S., Mukherjee, S., Chen, Y., Li, C., Young, S., Warren, M., … McDermott, P. F. (2015). Novel gentamicin resistance genes in *Campylobacter* isolated from humans and retail meats in the USA. *Journal of Antimicrobial Chemotherapy*, *70*(5), 1314–1321. http://doi.org/10.1093/jac/dkv001

Zhao, S., Tyson, G. H., Chen, Y., Li, C., Mukherjee, S., Young, S., … McDermott, P. F. (2016). Whole-genome sequencing analysis accurately predicts antimicrobial resistance phenotypes in *Campylobacter* spp. *Applied and Environmental Microbiology*, *82*(2), 459–466. http://doi.org/10.1128/AEM.02873-15

Zhou, J., Zhang, M., Yang, W., Fang, Y., Wang, G., & Hou, F. (2016). A seventeen-year observation of the antimicrobial susceptibility of clinical *Campylobacter jejuni* and the molecular mechanisms of erythromycin-resistant isolates in Beijing, China. *International Journal of Infectious Diseases*, *42*, 28–33. http://doi.org/10.1016/j.ijid.2015.11.005

Zirnstein, G., Helsel, L., Li, Y., Swaminathan, B., & Besser, J. (2000). Characterization of *gyrA* mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. *FEMS Microbiology Letters*, *190*, 1–7. http://doi.org/Doi 10.1016/S0378-1097(00)00306-2

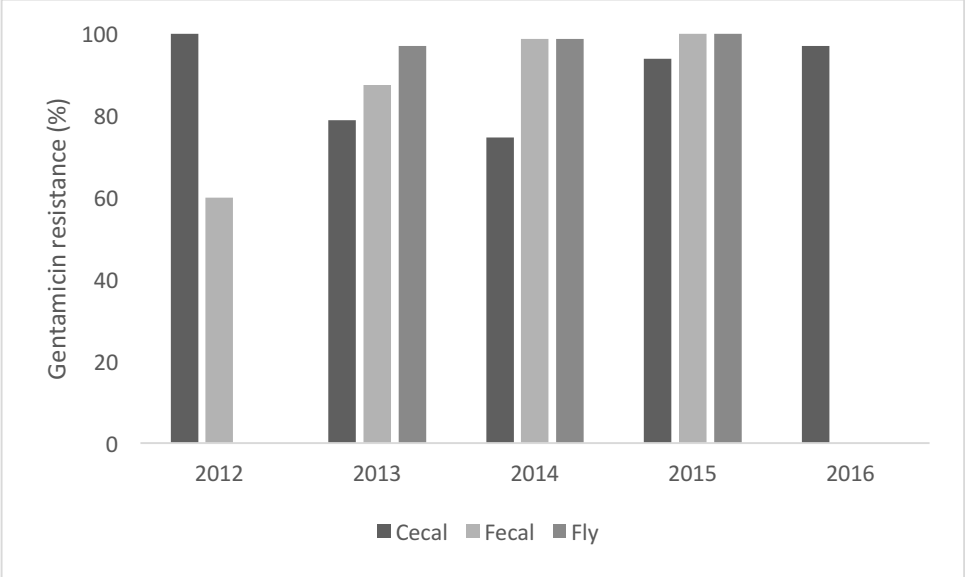


***Figure* 3-1** *The yearly percentages (resistant/number tested) of Campylobacter spp. isolates with resistance to gentamicin (50 mg/ml) by source. The number of isolates tested each year increased until 2015: 2012: n=11, 2013: n=252, 2014: n=634, 2015: n=913, 2016: n=754.*

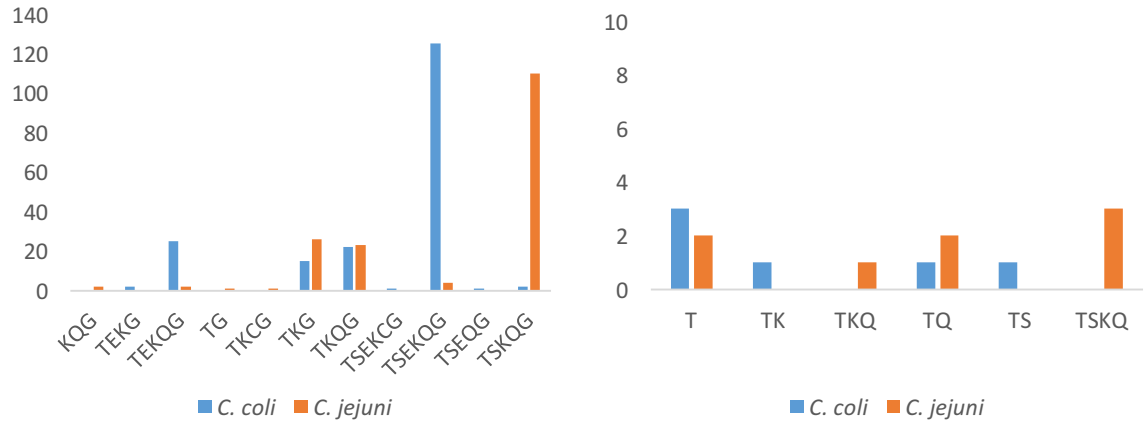


Figure 3-2. Distributions of antimicrobial resistance profiles of a) gentamicin-resistant *C. jejuni* and *C. coli* and b) gentamicin-susceptible *C. jejuni* and *C. coli*

Table 3-1 The correlation of the gentamicin-resistant phenotype with the presence of either *aph(2")-If* or *aph(2")-Ig*

	Resistant	Susceptible	Row Totals
Has Resistance Gene	360	0	360
Does Not Have Resistance Gene	2	14	16
Column Total	362	14	376

Table 3-2 Pairs of isolates used in natural transformation assays with positive/negative results

Recipient	Recipient Species	Recipient Source	Recipient AMR	Donor	Transformation Results
14240-6	*C. coli*	Turkey Feces	TS	14980A	+
14240-6	*C. coli*	Turkey Feces	TS	14983A	+
15626C	*C. jejuni*	Turkey Feces	TK	14980A	-
15626C	*C. jejuni*	Turkey Feces	TK	14983A	+
15147-A	*C. jejuni*	Turkey Cecum	PS	14980A	-
15147-A	*C. jejuni*	Turkey Cecum	PS	14983A	-
11168	*C. jejuni*	Reference	PS	14980A	+
11168	*C. jejuni*	Reference	PS	14983A	+
81-176	*C. jejuni*	Reference	S	14980A	+
81-176	*C. jejuni*	Reference	S	14983A	+
166 0hr BB-A	*C. jejuni*	Bovine Feces	TK	14980A	+

Chapter 4 Flies in commercial turkey farms harbor multidrug-resistant *Campylobacter jejuni* and *C. coli* with pronounced genotypic diversity

SUMMARY

Campylobacter is a leading cause of foodborne illness in the United States, and poultry are a major vehicle for infection. Houseflies play important roles in colonization of broiler flocks with *Campylobacter* but fly-mediated transmission of different strains remains poorly understood. Furthermore, little is known about roles of flies in colonization of turkey flocks with *Campylobacter*, as previous reports focused on broilers. Here we investigated flies as potential vectors for *Campylobacter* in commercial turkey flocks. We characterized antimicrobial susceptibility profiles and genotypes of *Campylobacter* from turkey feces and flies in the same turkey house of 28 different flocks. In all, 25 flocks were *Campylobacter*-positive in the feces and flies from the same turkey house. On average, feces carried a log CFU/g of 6.25 and flies had an average log CFU/ positive fly of 3.11. Both C. *coli* and C. *jejuni* were detected in turkey feces and flies, but C. *coli* was more likely to be recovered from flies than from feces. Up to six different strains could be isolated from flies of a single house, including multidrug resistant strains of C. *jejuni* and C. *coli*. Analysis of antimicrobial resistance profiles and multilocus sequence typing suggested greater diversity of *Campylobacter* among fly-derived isolates than those from feces. For the most common strain types, presence in flies was predictive of presence in feces, and vice versa. Findings suggest that flies may serve as vehicles for dissemination of *Campylobacter*, including multidrug resistant strains, among different houses at the same turkey farm, or different farms.

INTRODUCTION

Campylobacter jejuni and C. coli are major contributors to human disease worldwide. It ranks among the top five etiologic agents responsible for human foodborne illness in the United States and is the most prevalent in the European Union (European Food Safety Authority, 2014; Havelaar et al., 2015; Ruiz-palacios, 2007; E. Scallan et al., 2015; Elaine Scallan et al., 2011). Leading vehicles for human campylobacteriosis include poultry, raw milk and untreated water (Gillespie et al., 2002; Hermans et al., 2012; T. Humphrey et al., 2007; E. V. Taylor et al., 2012). *Campylobacter* is often recovered from broiler and turkeys flocks, and colonization can reach up to 10^9 CFU/g cecal content (Luangtongkum et al., 2006; Sahin, Morishita, & Zhang, 2002; K. Smith et al., 2004; US Food and Drug Administration, 2014; Wallace et al., 1998; Wright et al., 2008). While colonization of broiler chickens has been extensively investigated, less is known about the risk factors for *Campylobacter* colonization of turkey flocks.

Reducing the number of *Campylobacter*-positive poultry flocks may significantly reduce human illness, and there is therefore significant interest in producing *Campylobacter*-free poultry flocks (Efsa, 2011; Nauta, Johannessen, Laureano Adame, Williams, & Rosenquist, 2016; Rosenquist, Sommer, Nielsen, & Christensen, 2006). However, producing *Campylobacter*-free flocks can be extremely difficult and the routes of introduction into a flock are poorly understood. Insects such as flies and darkling beetles have been identified as possible vectors for the introduction of *Campylobacter* into a flock with most work focusing on flies (Berndtson et al., 1996; Fawzy et al., 2016; Jacobs-Reitsma, van de Giessen, Bolder, & Mulder, 1995; Newell & Fearnley, 2003b). Several studies have investigated fly-mediated transmission of *Campylobacter,* with successful isolation of *Campylobacter* spp. from flies found on commercial broiler farms (Berndtson, 1996; Birthe Hald et al., 2004; Rosef & Kapperud, 1983).

Additionally, *C. jejuni* was shown to be transferrable to flies from *Campylobacter*-positive broilers, and from these flies to previously *Campylobacter*-negative chickens (Shane et al., 1985).

Flies are known to travel several miles and may serve as vectors of transmission between farms (Greenberg & Bornstein, 1964; Pava-Ripoll et al., 2015). Studies in Denmark found that at least 900 flies enter the poultry house in their study every day (Birthe Hald et al., 2004), and a UK study was able to collect approximately 31 flies every two hours for a total of 1,644 flies (Royden et al., 2016). Thus, the use of fly screens greatly reduced the number of flies entering the houses and also significantly reduces the prevalence of *Campylobacter*-positive flocks at slaughter (Birt Hald et al., 2007).

However, limited information is available on the genotypes, and no information on the antimicrobial resistances, of fly-derived strains can be found (B. Hald et al., 2008; Birthe Hald et al., 2004; Rosef & Kapperud, 1983; Royden et al., 2016). For example, studies by Hald et al report only on the prevalence and species of *Campylobacter*-positive flies sourced from brooder farms in Denmark, and due to enrichment the results were not quantitative (B. Hald et al., 2008; Birthe Hald et al., 2004). A study from 1984 did speciate fly-derived strains from various animal farms, but was actually unable to isolate any *Campylobacter* from the flies collected from turkey farms (n=103) or cattle (n=100) (Rosef & Kapperud, 1983). However, successful isolation was accomplished from flies sourced from broiler (n=146) and hog farms (n=169). *C. coli* was drastically more common than *C. jejuni* (80.5-98.8% of isolates) in the fly-derived isolates from this study. Finally, just one study used MLST for characterizing their fly-derived strains (Royden et al., 2016). However, this study found a very low prevalence of flies (0.22-3.15%) carrying

Campylobacter spp. and no correlation between the STs isolated from flies and those from the broiler flocks.

All of the studies just mentioned collected their flies mainly from broiler farms, mostly in Europe and the UK, leaving turkey farms, of which the United States is the largest producer, almost completely unstudied ("Food and Agriculture Organization," 2017). In general, reports on the role of flies in colonization of turkey flocks with *Campylobacter* have been lacking. In spite of the high prevalence of *Campylobacter*, including multidrug resistant (MDR) strains, in turkey flocks and the strong suggestive evidence of the role of flies in *Campylobacter* transmission to broiler flocks, relevant studies with turkeys have been noticeably lacking (Luangtongkum et al., 2006). The goal of the current study was to investigate *Campylobacter* isolated from flies and feces sourced from commercial turkey farms in the southeastern United States. To expand upon this, we specifically aimed to determine prevalence of *Campylobacter* in flies on turkey farms, assess antimicrobial resistance in fly-derived isolates, and compare antimicrobial resistance profiles and genotypes of *Campylobacter* isolates from flies and turkey feces in the same house.

MATERIALS AND METHODS

Sample Collection and *Campylobacter* isolation

A total of 27 flocks from 28 distinct houses on 23 farms (n=15 brooder, n=8 growout) in eastern North Carolina were investigated in this study over three years (2013 - 2015). As is common practice for intensive, conventional turkey production in the United States, turkeys were kept on brooder farms from day-of-hatch to 4-5 weeks. At that point, brooder flocks are transported to multiple growout farms, where they remained until marketing. Both brooder and growout farms were visited in this study and the age of the flocks ranged from 11 days to 17 weeks of age.

Turkey fecal droppings and flies were collected from the turkey house at each visit. To collect the fecal samples, the poultry house was divided into quadrants and fecal droppings were collected from each quadrant, yielding a total of 12 fecal samples. Freshly voided fecal droppings were collected using sterile swabs, placed into sterile 15-mL polypropylene Corning tubes (Fisher Scientific, Pittsburgh, PA) and transported to the laboratory on ice. Up to 10 live flies were also collected from the same turkey house by trapping in Ziploc bags and transported to the laboratory on ice. Fecal samples and flies were typically processed *for Campylobacter* on the same day.

To process the fecal samples, the 6 fecal samples (0.1 g of each) from two of the quandrants were combined in 6 ml of sterile Mueller-Hinton broth (MHB; Difco, Becton Dickinson, Franklin Lakes, NJ) to make a fecal suspension, yielding two composite samples /flock. Serial dilutions of these suspensions were plated onto modified Charcoal-cefoperazone-deoxycholate agar (mCCDA; Oxoid, Hampshire, UK; SE 155, Oxoid) and incubated microaerobically at 42°C for 48 hours in anaerobic jars containing CampyPak Plus microaerobic sachets (Becton Dickinson, Franklin Lakes, NJ)). A total of six colonies per composite sample were purified on Mueller-Hinton agar (MHA; Difco, Becton Dickinson, Franklin Lakes, NJ), and pure cultures were preserved in brain heart infusion broth (Becton Dickinson) supplemented with 20% glycerol at -80°C. To isolate and enumerate *Campylobacter* spp. from the flies, each fly was homogenized in 1 ml of sterile Phosphate Buffered Saline and 100 μl of the suspension was plated on mCCDA. Subsequent incubations and purifications were as described for the fecal samples.

Antimicrobial susceptibility testing

Each isolate was tested against a panel of antibiotics to determine the antimicrobial resistances in the *Campylobacter* isolates. A few isolated colonies of each strain were suspended in 100 μl of MHB and 3.5 μl was spotted in duplicate onto each of the seven antimicrobial plates and an MHA positive control plate. *C. jejuni* ATCC 33560 (purchased from the American Type Culture Collection, Manassas, VA) was used as quality control each time. Resistance was based on confluent growth after 48 h of incubation at 42°C at the following antibiotic concentrations: 16 μg/ml tetracycline, 64 μg/ml streptomycin, 8 μg/ml erythromycin, 64 μg/ml kanamycin, 32 μg/ml nalidixic acid, 4 μg/ml ciprofloxacin, 50 μg/ml gentamicin.

Species determination, DNA preparation, and MLST

Multiplex PCR using primers targeting *ceuE* and *hip* was used to determine the species (*C. coli* or *C. jejuni* respectively) of the isolates as described previously (Smith et al., 2004). Isolate designations consisted of the combination of antimicrobial resistance profile and species designation. For example, a *C. jejuni* isolate resistant to tetracycline, kanamycin, and gentamicin, would be designated TKGcj.

Multi-locus sequence typing (MLST) was performed with a subset of isolates. DNA was extracted from isolates grown on MHA using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the procedures suggested by the vendor and DNA was eluted with 100-200 μl of AE buffer provided with the kit. MLST analysis was performed as described previously (Miller et al., 2006).

Statistical analysis

Analysis of data was carried out using various procedures in the SAS statistical software package (SAS Institute, Inc., Cary, NC). Relative abundance of *C. jejuni* and *C. coli* was compared across sources (fecal/fly) using chi-square statistics from two-way contingency tables. Additionally, a generalized linear mixed model for the probability that an observed isolate is *C. coli* was fit using the GLIMMIX procedure of SAS. This model included fixed source effects and random farm effects to account for the fact that

the counts populating the contingency table arose from repeated measurements on some of the replicate farms. To quantify diversity, the ratio of the number of different strains found to the total number of isolates collected was calculated for each farm and source. These ratios were modeled non-parametrically with additive sample type and farm effects and Friedman's test was used to test for sample type effects.

A manual search of the absolute frequencies also identified strains found in only one sample type. Fisher's Exact test was used to investigate the source effect on genotype (ST). To assess similarity between strains from flies and feces, a two-by-two table of frequencies was constructed for fly-and-fecal-positivity. For example, of the 25 flocks, 14 were found to be positive for strain TSKQGCj in both feces and flies, while five flocks were negative for this strain in both the flies and feces. One flock was positive for TSKQGCj in flies but not feces, while the reverse was found for five flocks (TSKQGCj-positive in feces, but not flies). Fischer's Exact Test was used to test the hypothesis that presence of a strain in one sample type is not associated with its presence in the other sample type from that same flock.

RESULTS

Campylobacter co-occurs in feces and flies from the same commercial turkey flock

Three flocks, all from different production cycles (August 2013, November 2013, and December 2014) at the same farm, were culture-negative for *Campylobacter* in both the turkey feces and flies. In the 25 remaining flocks both the flies and turkey droppings were culture-positive for *Campylobacter* with an average log CFU 6.25 ± 0.74 per g feces. In no case was a flock positive in just one source, i.e. only feces or only flies. A total of 232 flies were captured from the turkey houses with *Campylobacter*-positive flocks (average, 9.3 flies/turkey house). Of the captured flies, 66.2% +/- 25.4% were positive for *Campylobacter* with an average *Campylobacter* log CFU/positive fly of 3.11 ± 0.98. In all, 379 *Campylobacter* isolates (164 from flies and 215 from feces) were characterized from the 25 positive flocks. The isolates were nearly evenly split between *C. coli* (n= 192) and *C. jejuni* (n=187) (table 4-1).

Flies tended to harbor more *C. coli* than *C. jejuni* and more diverse *Campylobacter* strains than feces

C. coli and *C. jejuni* were isolated with similar frequencies from turkey droppings (48.8% and 51.2%, respectively). In contrast, *C. coli* was isolated more frequently from fly samples (53.1%) compared to *C. jejuni* (47.0%) (table 4-1). A generalized linear mixed model indicated that *Campylobacter* isolates from flies were more likely to be *C. coli* than isolates from feces ($p<0.0001$) with a mean probability of a feces or fly-derived isolate being *C. coli* of 0.3776 and 0.6967, respectively.

Seven distinct AMR profiles were detected among fly-derived *C. jejuni*, while five were detected from the feces (Fig. 1). Among *C. coli* isolates there was an equal number (nine) of

AMR profiles in both sample sources (Fig. 1). Diversity was greater ($p < 0.01$) among fly-derived isolates (0.5002) than among those from feces (0.2773). A panel of 51 *C. jejuni* and *C. coli* isolates representing different sources (flies vs. feces) and AMR profiles was genotyped by MLST, yielding four different STs for *C. jejuni* and 10 for *C. coli* (Fig.2) Six novel STs were identified, including two among *C. jejuni* (8227, 7730) and four among *C. coli* (8213, 8218, 8224, 8551) were novel (appendix A). In *C. jejuni* the most predominant ST was ST-1839, detected among *C. jejuni* isolates with AMR profiles TSKQG, TSKQ, TKQ, TKG, and TKQG (Fig. 1B). ST-1839 has previously only been reported from poultry from the southeastern United States (Gu, Siletzky, Wright, Islam, & Kathariou, 2009) as well as in yet unpublished data from the *Campylobacter* MLST database (https://pubmlst.org/bigsdb?db=pubmlst_campylobacter_isolates&page=profiles).

While there was not a definitive association between specific STs and AMR profiles, there does appear to be a relationship between related STs and AMRs. *C. coli* isolates with the same AMR profiles tended to exhibit the same or related STs. For example, TSEKQGCc, the second most common AMR profile for *C. coli* (Fig. 1), was associated with the most diverse set of STs (ST-889, 1101, 1017, 1067, and 8551). However these STs are highly related with ST-1101, 1017, 1067, and 8551 differing in only one allele, and 889 differing in only two (see appendix A). The most common ST in *C. coli* was ST-1101 (n=6). One exception was *C. coli* with AMR profile TEKQG, which was detected among isolates with the distantly related STs 1101 and 1192 (Fig. 2B). With the exception of the two closely related STs, 8213 and 8224, which were only detected from one farm, most STs were identified among isolates from different farms (Fig. 2).

C. jejuni isolates, with the exception of AMR profile TKG, with the same AMR profiles tended to exhibit identical or highly related (1-2 allele differences) STs (Fig. 2B). Most STs that were encountered more than once were detected among isolates from different flocks and farms (Fig. 2C), and STs detected more than once were found among both fly-and feces-derived isolates (Fig. 2A). However, of the 5 *C. coli* STs detected in multiple isolates two, ST-1067 and ST-1192, were from only one source, specifically flies (Fig. 2A), and from multiple farms (Fig. 2C). ST-1192, however, was closely related (one-allele difference) to the commonly-encountered ST-1833, detected among both fly and feces-derived *C. coli* isolates (Fig. 2A).

Multidrug Resistance was common in both sample types and shared between feces and fly-derived isolates from the same flock

Multidrug resistant (MDR) isolates (resistant to three or more antibiotic classes) represented the majority of isolates recovered either from feces (97.2%) or from flies (96.3%). Of the seven distinct AMR profiles seen in *C. jejuni,* by far the most common was TSKQG (152/187, 81.3%) followed by TSKQ (11/187, 5.9%), detected among isolates both from flies and from feces (Fig. 1). MLST-analyzed isolates with either of these predominant AMR profiles had the commonly-encountered ST-1839 (Fig. 2B). *C. coli* was found to exhibit several distinct AMR profiles and TSEKQG was the most prevalent (99/181, 54.7%), followed by TEKQG (38/181 21.0%) (table 4-2). These two AMR profiles were predominant among *C. coli* from flies as well as from feces (Fig. 1). When analyzed by MLST, *C. coli* with AMR profile TSEKQG had ST1101 and closely related STs, while those with TEKQG had ST-1101 and ST-1192 (Fig. 2B).

Strains that were not multidrug resistant were far less common, and of all strains detected more than once, only two were not shared between feces and flies: specifically, *C. coli* resistant

to only to tetracycline was recovered (n=5) only from feces of one flock, while *C. coli* resistant just to tetracycline and streptomycin (n=3) were only recovered from flies, again from a single flock (appendix B). MLST analysis of selected *C. coli* isolates with AMR profiles T and TS indicated that they had ST-1833 and 8218, respectively (Fig. 2B).

Detection of a specific species-AMR profile combination one sample predicts its presence in the other sample type in the same turkey house

There were some occurrences of shared STs and AMR profiles being found in the flies and feces of the same farm (Fig. 2). The results of the Fisher's Exact test indicate that the presence of five AMR profiles (TSKQGCj, p=0.0225, TSEKQGCc, p=0.0010, TKQGCc, p=0.0235, TKCc, p=0.0400, TEKQGCc, p=0.0333) in fecal droppings from a turkey house could be used to predict co-occurrence of the corresponding strain in flies from the same turkey house.

DISCUSSION

In this study, *Campylobacter* isolates sourced from flies and feces were typed based on the combination of their antimicrobial resistance profiles, species and MLST. Few studies have characterized fly-derived *Campylobacter* strains from poultry environments and none have characterized such a large number of both *C. jejuni and C. coli* or gone to the extent of MLST and antimicrobial resistance (B. Hald et al., 2008; Birthe Hald et al., 2004; Rosef & Kapperud, 1983; Royden et al., 2016; Szalanski, Owens, McKay, & Steelman, 2004). Only one study was able to recover and investigate *Campylobacter* species from flies sourced from turkey farms, but did not look at antimicrobial resistance or genotypic data (Szalanski et al., 2004). This study was able to isolate *Campylobacter* from 8.4-12.7% of houseflies (*Musca domestica)* from turkey

farms, although other types of flies were also tested and sometimes resulted in higher prevalence of *Campylobacter* such as *Hydrotaea aenescens* in which 25.5% of flies were *Campylobacter* positive. Feces from the turkeys on these farms were also analyzed and found to carry *Campylobacter.*

To the best of our knowledge only one study has examined fly-derived isolates by PFGE, however these were sourced from broiler production, (Birthe Hald et al., 2004) and only one study besides ours looked at MLST data although those fly-derived strains were also from broiler production (Royden et al., 2016). MLST analysis identified two STs in the *Campylobacter* isolated from the flock (*C. coli* ST 828, *C. jejuni* ST 257) and three from the fly isolates (*C. jejuni* STs 1701, 137, and 25). There was no overlap between these STs and the STs identified in either the turkey or fly-derived isolates from our study.

Other major differences include geographical differences in the studies – Royden et al occurred in the UK and the studies by Hald et al occurred in Denmark, while our study provides the first glimpse into the major AMRs and STs circulating within turkey production in the United States (B. Hald et al., 2008; B Hald, Wedderkopp, & Madsen, 2000; Royden et al., 2016). Finally, the results of this study indicate that *Campylobacter* may be more prevalent in flies than previously reported. Specifically, a previous study was able to detect *Campylobacter* spp. from only 10.9% of flies sourced from American turkey farms, whereas we found that 66.2% of flies from turkey production carried cultivable *Campylobacter* (Szalanski et al., 2004). Other studies have also tended to report low prevalence of *Campylobacter*-positive flies, and although this may reflect regional differences as most of these studies took place in Europe (B. Hald et al., 2008; Birthe Hald et al., 2004; Royden et al., 2016), even American-based studies (Gregory et al., 1997; Szalanski et al., 2004)report lower prevalence than what was found in this study. In

addition to regional differences, variables such as temperature may affect the survival of *Campylobacter* in flies as has been shown previously (Skovgård & Kristensen, 2017). Previous studies indicate that higher temperatures negatively affect the survival of *Campylobacter* in the fly, however this may be negated as higher temperatures allow increased propagation of house flies (B. Hald et al., 2008; Skovgård & Kristensen, 2017).

It is also of note that the literature indicates that *Campylobacter* does not persist in flies, and may be eliminated from the fly body within 24 hours (Gill et al., 2016; Skovgård & Kristensen, 2017). However, both of these studies only utilized *C. jejuni* strains, and there may be important strain to strain variation or other differences imparted by the flies' natural microbiota when found in the poultry houses. Finally unlike our study, none of these studies were able to quantify the *Campylobacter* found in or on the flies. The higher prevalence of *Campylobacter*-positive flies found in this study may be due to the geographical or seasonal differences in this study compared to those in the past, or may indicate further differences in the risks of *Campylobacter* in turkey production as opposed to the more commonly studied broiler production.

The results of this study also suggest that flies carry a greater diversity of *Campylobacter* strains than do turkeys from the same house. This is possibly due to the ability of the fly to sample a greater area of the poultry house or to bring in *Campylobacter* from outside of the poultry farm (Birthe Hald et al., 2004). While the prevalence of the two species was nearly evenly split between *C. coli* and *C. jejuni,* our results show that *C. coli* was statistically more likely to be found in flies and *C. jejuni* isolates were more likely to be found in fecal samples. *C. jejuni* can survive at least as well as *C. coli* in environmental samples (Buswell et al., 1998; Korhonen & Martikainen, 1991; Peyrat, Soumet, Maris, & Sanders, 2008; Solow, Cloak, &

Fratamico, 2003). Past studies had indicated that *C. jejuni* was more common than *C. coli* in flies (Berndtson, 1996; B. Hald et al., 2008; Szalanski et al., 2004) so it is surprising to find such a high prevalence of *C. coli* in the flies in this study. However, it may be that *C. jejuni* strains predominating in the turkeys, and excreted in the feces may have lower relative survival outside of the GI tract than similarly-excreted *C. coli*.

It is also of note that the most commonly isolated *Campylobacter* strains in the turkey feces were also those most commonly isolated from flies, and in agreement with past studies there was no occurrence of fly-positive flocks without the feces yielding positive results as well (Berndtson, 1996). The most common strains were all multidrug resistant and showed resistance to every antimicrobial they were challenged with (with the exception of erythromycin in *C. jejuni* which has associated fitness costs) (Bolinger & Kathariou, 2017). Recent whole-genome sequencing of two *Campylobacter* spp. strains (one *C. jejuni,* ST-1839, accession numbers for genome/plasmid CP017025 to CP017028 from turkey feces, one *C. coli,* ST-1067, CP017029 to CP017030) from a fly) has revealed the genetic basis for similar multidrug-resistant phenotypes in turkeys and flies (Miller et al., 2016). Both of these strains harbored plasmids containing *tet(O)* which confers resistance to tetracycline, as well as kanamycin-resistance genes (Lambert, T., Gerbaud, G., Trieu-Cuot, P., Courvalin, 1985; Liebert et al., 2004; Diane E. Taylor & Chau, 1996; Tenover et al., 1989). Resistance to erythromycin in the *C. coli* strain was conferred by an A2075G substitution in all three copies of the 23S rRNA gene, and The 86 →Ile in gyrA confer resistance to ciprofloxacin and nalidixic acid (Engberg et al., 2001; Jesse et al., 2006; D. E. Taylor & Courvalin, 1988; Zirnstein et al., 2000).

It is of note that that multidrug resistant *C. jejuni* of ST-1839, which has previously only been isolated from turkeys and broilers, (Gu et al., 2009; Miller et al., 2016) has now been

reported not only among additional turkey fecal isolates but also among multiple fly-derived isolates from the turkey farms. It is also interesting to note the shared STs between fecal and fly-derived isolates as well as farms harboring multiple STs. Indeed, the results of this study may indicate that flies from commercial turkey farms are often positive for *Campylobacter* and may be playing a role in the dissemination of this pathogen as well as associated antimicrobial resistance genes into the food supply.

REFERENCES

Berndtson, E. (1996). *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. *International Journal of Food Microbiology*, *32*, 35–47.

Berndtson, E., Emanuelson, U., Engvall, A., & Danielsson-Tham, M. L. (1996). A 1-year epidemiological study of campylobacters in 18 Swedish chicken farms. *Preventive Veterinary Medicine*, *26*(3–4), 167–185. http://doi.org/10.1016/0167-5877(95)01008-4

Bolinger, H., & Kathariou, S. (2017). The Current State of Macrolide Resistance in *Campylobacter* : A Review of Trends and Impacts of Resistance Mechanisms. *Applied and Environmental Microbiology*. http://doi.org/10.1128/AEM.00416-17

Buswell, C. M., Herlihy, Y. M., Lawrence, L. M., McGuiggan, J. T., Marsh, P. D., Keevil, C. W., & Leach, S. A. (1998). Extended survival and persistence of *Campylobacter* spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining. *Applied and Environmental Microbiology*, *64*(2), 733–41. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9464415

Efsa. (2011). Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal*, *9*(4), 1–141. http://doi.org/10.2903/j.efsa.2011.2105.

Engberg, J., Aarestrup, F. M., Taylor, D. E., Gerner-Smidt, P., & Nachamkin, I. (2001). Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: Resistance mechanisms and trends in human isolates. *Emerging Infectious Diseases*. http://doi.org/10.3201/eid0701.700024

European Food Safety Authority. (2014). EFSA explains zoonotic diseases. *Campylobacter*, 1–2.

http://doi.org/10.2805/59450

Fawzy, M., Metwaly, E., Adawy, H. El, Hotzel, H., & Tomaso, H. (2016). Prevalence , genotyping and risk factors of thermophilic *Campylobacter* spreading in organic turkey farms in Germany. *Gut Pathogens*, *8*(28), 1–9. http://doi.org/10.1186/s13099-016-0108-2

Food and Agriculture Organization. (2017). Data on live animals. Retrieved June 9, 2017, from http://www.fao.org/faostat/en/#data/QA

Gill, C. ;, Bahrndorff, S. ;, Lowenberger, C., Version, D., Gill, C. ;, Bahrndorff, S. ;, & Lowenberger, C. (2016). *Campylobacter jejuni* in Musca domestica : An examination of survival and transmission potential in light of the innate immune responses of the house flies. *Insect Science*, *24*(4), 584–598. http://doi.org/10.1111/1744-7917.12353

Gillespie, I. A., O'Brien, S. J., Frost, J. A., Adak, G. K., Horby, P., Swan, A. V, … Neal, K. R. (2002). A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: a tool for generating hypotheses. *Emerging Infectious Diseases*, *8*(9), 937–942. http://doi.org/10.3201/eid0809.010187

Greenberg, B., & Bornstein, A. A. (1964). FLY DISPERSION FROM A RURAL MEXICAN SLAUGHTERHOUSE*. *American Journal of Tropical Medicine and Hygiene*, *13*, 881–886. Retrieved from http://www.ajtmh.org.prox.lib.ncsu.edu/docserver/fulltext/14761645/13/6/TM0130060881.pdf?expires=1489174472&id=id&accname=12094&checksum=BFBDB8F99059604CCFDBF0E19EA15913

Gregory, E., Barnhart, H., Dreesen, D. W., Stern, N., & Corn, J. L. (1997). Epidemiological Study of *Campylobacter* spp . in Broilers : Source , Time of Colonization , and Prevalence. *Avian Diseases*, *41*(4), 890–898. Retrieved from

http://www.jstor.org/stable/1592343?seq=1#page_scan_tab_contents

Gu, W., Siletzky, R. M., Wright, S., Islam, M., & Kathariou, S. (2009). Antimicrobial susceptibility profiles and strain type diversity of *Campylobacter jejuni* isolates from turkeys in eastern North Carolina. *Applied and Environmental Microbiology*, *75*(2), 474–482. http://doi.org/10.1128/AEM.02012-08

Hald, B., Skovgård, H., Bang, D. D., Pedersen, K., Dybdahl, J., Jespersen, J. B., & Madsen, M. (2004). Flies and *Campylobacter* infection of broiler flocks. *Emerging Infectious Diseases*, *10*(8), 1490–1492. http://doi.org/10.3201/eid1008.040129

Hald, B., Skovgård, H., Pedersen, K., Bunkenborg, H., Skovgard, H., Pedersen, K., & Bunkenborg, H. (2008). Influxed insects as vectors for *Campylobacter jejuni* and *Campylobacter coli* in Danish broiler houses. *Poultry Science*, *87*(7), 1428–1434. http://doi.org/10.3382/ps.2007-00301

Hald, B., Sommer, H. M., & Skovgard, H. (2007). Use of fly screens to reduce *Campylobacter* spp. introduction in broiler houses. *Emerging Infectious Diseases*, *13*(12), 1951–1953. http://doi.org/10.3201/eid1312.070488

Hald, B., Wedderkopp, A., & Madsen, M. (2000). Thermophilic *Campylobacter* spp. in Danish broiler production: a cross-sectional survey and a retrospective analysis of risk factors for occurrence in broiler flocks. *Avian Pathology*, *29*(2), 123–31. http://doi.org/10.1080/03079450094153

Havelaar, A. H., Kirk, M. D., Torgerson, P. R., Gibb, H. J., Hald, T., Lake, R. J., … Group, on behalf of W. H. O. F. D. B. E. R. (2015). World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. *PLOS Medicine*, *12*(12), e1001923. http://doi.org/10.1371/journal.pmed.1001923

Hermans, D., Pasmans, F., Messens, W., Martel, A., Van Immerseel, F., Rasschaert, G., … Haesebrouck, F. (2012). Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*. *Vector-Borne and Zoonotic Diseases*, *12*(2), 89–98. http://doi.org/10.1089/vbz.2011.0676

Humphrey, T., O'Brien, S., & Madsen, M. (2007). Campylobacters as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology*, *117*(3), 237–257. http://doi.org/10.1016/j.ijfoodmicro.2007.01.006

Jacobs-Reitsma, W. F., van de Giessen, A. W., Bolder, N. M., & Mulder, R. W. (1995). Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. *Epidemiology and Infection*, *114*(3), 413–21. http://doi.org/10.1017/S0950268800052122

Jesse, T. W., Englen, M. D., Pittenger-Alley, L. G., & Fedorka-Cray, P. J. (2006). Two distinct mutations in gyrA lead to ciprofloxacin and nalidixic acid resistance in *Campylobacter coli* and *Campylobacter jejuni* isolated from chickens and beef cattle. *Journal of Applied Microbiology*, *100*(4), 682–688. http://doi.org/10.1111/j.1365-2672.2005.02796.x

Korhonen, L. K., & Martikainen, P. J. (1991). Comparison of the survival of *Campylobacter jejuni* and *Campylobacter coli* in culturable form in surface water. *Canadian Journal of Microbiology*, *37*(7), 530–533. http://doi.org/10.1139/m91-089

Lambert, T., Gerbaud, G., Trieu-Cuot, P., Courvalin, P. (1985). Structural relationship between the genes encoding 3'-aminoglycoside phosphotransferases in *Campylobacter* and in Gram-positive cocci. *Annales de l'Institut Pasteur / Microbiologie*, 135–150.

Liebert, M. A., Kanamycin, P., Gibreel, A., Sköld, O. L. A., & Taylor, D. E. (2004). Characterization of plasmid-mediated aphA-3 kanamycin resistance in *Campylobacter jejuni*, *10*(2), 98–105.

Luangtongkum, T., Morishita, T. Y. T., Ison, A. J., Huang, S., McDermott, P. F., & Zhang, Q.

(2006). Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. *Applied and Environmental Microbiology…*, *72*(5), 3600–3607. http://doi.org/10.1128/AEM.72.5.3600

Miller, W. G., Englen, M. D., Kathariou, S., Wesley, I. V., Wang, G., Pittenger-Alley, L., … Mandrell, R. A. (2006). Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. *Microbiology*, *152*(1), 245–255. http://doi.org/10.1099/mic.0.28348-0

Miller, W. G., Huynh, S., Parker, C. T., & Niedermeyer, J. A. (2016). Complete genome sequences of multidrug-resistant *Campylobacter jejuni* strain 14980A (turkey feces) and *Campylobacter coli* strain 14983A (housefly from a turkey farm), harboring a novel gentamicin resistance mobile element. *Genome Announcement*, *4*(5), 16–17. http://doi.org/10.1128/genomeA.01175-16.Copyright

Nauta, M., Johannessen, G., Laureano Adame, L., Williams, N., & Rosenquist, H. (2016). The effect of reducing numbers of *Campylobacter* in broiler intestines on human health risk. *Microbial Risk Analysis*. http://doi.org/10.1016/j.mran.2016.02.001

Newell, D. G., & Fearnley, C. (2003). Sources of *Campylobacter* Colonization in Broiler Chickens. *Applied and Environmental Microbiology*, *69*(8), 4343–4351. http://doi.org/10.1128/AEM.69.8.4343-4351.2003

Pava-Ripoll, M., Pearson, R. E. G., Miller, A. K., Tall, B. D., Keys, C. E., & Ziobro, G. C. (2015). Ingested Salmonella enterica, Cronobacter sakazakii, Escherichia *coli* O157:H7, and Listeria monocytogenes: transmission dynamics from adult house flies to their eggs and first filial (F1) generation adults. *BMC Microbiology*, *15*(1), 150. http://doi.org/10.1186/s12866-015-0478-5

Peyrat, M. B., Soumet, C., Maris, P., & Sanders, P. (2008). Recovery of *Campylobacter jejuni* from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: Analysis of a potential source of carcass contamination. *International Journal of Food Microbiology*, *124*(2), 188–194. http://doi.org/10.1016/j.ijfoodmicro.2008.03.030

Rosef, O., & Kapperud, G. (1983). House flies (Musca domestica) as possible vectors of *Campylobacter fetus* subsp. *jejuni*. *Applied and Environmental Microbiology*, *45*(2), 381–383. Retrieved from http://aem.asm.org.prox.lib.ncsu.edu/content/45/2/381.short

Rosenquist, H., Sommer, H. M., Nielsen, N. L., & Christensen, B. B. (2006). The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*. *International Journal of Food Microbiology*, *108*(2), 226–232. http://doi.org/10.1016/j.ijfoodmicro.2005.12.007

Royden, a, Wedley, a, Merga, J. Y., Rushton, S., Hald, B., Humphrey, T., & Williams, N. J. (2016). A role for flies (Diptera) in the transmission of *Campylobacter* to broilers? *Epidemiology and Infection*, *144*(15), 3326–3334. http://doi.org/10.1017/S0950268816001539

Ruiz-palacios, G. M. (2007). The health burden of *Campylobacter* infection and the impact of antimicrobial resistance : playing chicken. *Clinical Infectious Diseases*, *44*, 701–703. http://doi.org/10.1086/509936

Sahin, O., Morishita, T. Y., & Zhang, Q. (2002). *Campylobacter* colonization in poultry: sources of infection and modes of transmission. *Animal Health Research Reviews*, *3*(2), 95–105. http://doi.org/10.1079/AHRR200244

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., … Griffin, P. M. (2011). Foodborne illness acquired in the United States--major pathogens.

Emerging Infectious Diseases, *17*(1), 7–15. http://doi.org/10.3201/eid1701.091101p1

Scallan, E., Hoekstra, R. M., Mahon, B. E., Jones, T. F., & Griffin, P. M. (2015). An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiology and Infection*, *143*(13), 2795–2804. http://doi.org/10.1017/S0950268814003185

Shane, S. M., Montrose, M. S., & Harrington, K. S. (1985). Transmission of *Campylobacter jejuni* by the Housefly (Musca domestica). *Source: Avian Diseases*, *29*(2), 384–391. Retrieved from http://www.jstor.org/stable/1590499

Skovgård, H., & Kristensen, K. (2017). Retention of *Campylobacter* (Campylobacterales : Campylobacteraceae) in the House Fly (Diptera : Muscidae), (September), 1202–1209.

Smith, K., Reimers, N., Barnes, H. J., Lee, B. C., Siletzky, R., & Kathariou, S. (2004). *Campylobacter* colonization of sibling turkey flocks reared under different management conditions. *Journal of Food Protection*, *67*(7), 1463–1468.

Solow, B. T., Cloak, O. M., & Fratamico, P. M. (2003). Effect of Temperature on Viability of *Campylobacter jejuni* and *Campylobacter coli* on Raw Chicken or Pork Skin. *Journal of Food Protection*, *66*(11), 2023–2031. Retrieved from http://jfoodprotection.org/doi/pdf/10.4315/0362-028X-66.11.2023

Szalanski, A. L., Owens, C. B., McKay, T., & Steelman, C. D. (2004). Detection of *Campylobacter* and Escherichia *coli* O157:H7 from filth flies by polymerase chain reaction. *Med Vet Entomol*, *18*(3), 241–246. http://doi.org/10.1111/j.0269-283X.2004.00502.x

Taylor, D. E., & Chau, A. (1996). Tetracycline resistance mediated by ribosomal protection. *Antimicrobial Agents and Chemotherapy*, *40*(1), 1–5.

Taylor, D. E., & Courvalin, P. (1988). Mechanisms of antibiotic resistance in *Campylobacter*

species. *Antimicrobial Agents and Chemotherapy*, *32*(8), 1107–1112.

Taylor, E. V., Herman, K. M., Ailes, E. C., Fitzgerald, C., Yoder, J. S., Mahon, B. E., & Tauxe, R. V. (2012). Common source outbreaks of *Campylobacter* infection in the USA, 1997–2008. *Epidemiology and Infection*, *141*(2013), 1–10. http://doi.org/10.1017/S0950268812001744

Tenover, F. C., Gilbert, T., & O'Hara, P. (1989). Nucleotide sequence of a novel kanamycin resistance gene, aphA-7, from *Campylobacter jejuni* and comparison to other kanamycin phosphotransferase genes. *Plasmid*, *22*(1), 52–58. http://doi.org/10.1016/0147-619X(89)90035-8

US Food and Drug Administration. (2014). *NARMS Integrated Report: 2014*. Retrieved from https://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm059103.htm

Wallace, J. S., Stanley, K. N., & Jones, K. (1998). The colonization of turkeys by thermophilic campylobacters. *Journal of Applied Microbiology*, *85*(2), 224–230. http://doi.org/10.1046/j.1365-2672.1998.00470.x

Wright, S. L., Carver, D. K., Siletzky, R. M., Romine, S., Morrow, W. E. M., & Kathariou, S. (2008). Longitudinal study of prevalence of *Campylobacter jejuni* and *Campylobacter coli* from turkeys and swine grown in close proximity. *Journal of Food Protection*, *71*(9), 1791–1796.

Zirnstein, G., Helsel, L., Li, Y., Swaminathan, B., & Besser, J. (2000). Characterization of gyrA mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. *FEMS Microbiology Letters*, *190*, 1–7. http://doi.org/Doi 10.1016/S0378-1097(00)00306-2

Berndtson, E. (1996). *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. *International Journal of Food Microbiology*, *32*, 35–47.

Berndtson, E., Emanuelson, U., Engvall, A., & Danielsson-Tham, M. L. (1996). A 1-year epidemiological study of campylobacters in 18 Swedish chicken farms. *Preventive Veterinary Medicine*, *26*(3–4), 167–185. http://doi.org/10.1016/0167-5877(95)01008-4

Bolinger, H., & Kathariou, S. (2017). The current state of macrolide resistance in *Campylobacter* : A review of trends and impacts of resistance mechanisms. *Applied and Environmental Microbiology*, http://doi.org/10.1128/AEM.00416-17

Buswell, C. M., Herlihy, Y. M., Lawrence, L. M., McGuiggan, J. T., Marsh, P. D., Keevil, C. W., & Leach, S. A. (1998). Extended survival and persistence of *Campylobacter* spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining. *Applied and Environmental Microbiology*, *64*(2), 733–41. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9464415

European Food Safety Authority. (2011). Scientific opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal*, *9*(4), 1–141. http://doi.org/10.2903/j.efsa.2011.2105.

Engberg, J., Aarestrup, F. M., Taylor, D. E., Gerner-Smidt, P., & Nachamkin, I. (2001). Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: Resistance mechanisms and trends in human isolates. *Emerging Infectious Diseases*. http://doi.org/10.3201/eid0701.700024

European Food Safety Authority. (2014). EFSA explains zoonotic diseases. *Campylobacter*. Fact Sheet (2014) http://doi.org/10.2805/59450

Fawzy, M., Metwaly, E., Adawy, H. El, Hotzel, H., & Tomaso, H. (2016). Prevalence , genotyping and risk factors of thermophilic *Campylobacter* spreading in organic turkey farms in Germany. *Gut Pathogens*, *8*(28), 1–9. http://doi.org/10.1186/s13099-016-0108-2

Food and Agriculture Organization. (2017). Data on live animals. Retrieved June 9, 2017, from http://www.fao.org/faostat/en/#data/QA

Gill, C. ;, Bahrndorff, S. ;, Lowenberger, C., Version, D., Gill, C. ;, Bahrndorff, S. ;, & Lowenberger, C. (2016). *Campylobacter jejuni* in Musca domestica : An examination of survival and transmission potential in light of the innate immune responses of the house flies. *Insect Science*, *24*(4), 584–598. http://doi.org/10.1111/1744-7917.12353

Gillespie, I. A., O'Brien, S. J., Frost, J. A., Adak, G. K., Horby, P., Swan, A. V, … Neal, K. R. (2002). A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: a tool for generating hypotheses. *Emerging Infectious Diseases*, *8*(9), 937–942. http://doi.org/10.3201/eid0809.010187

Greenberg, B., & Bornstein, A. A. (1964). Fly disperstion from a rural mexican slaughterhouse*. *American Journal of Tropical Medicine and Hygiene*, *13*, 881–886. Retrieved from http://www.ajtmh.org.prox.lib.ncsu.edu/docserver/fulltext/14761645/13/6/TM0130060881.pdf?expires=1489174472&id=id&accname=12094&checksum=BFBDB8F99059604CCFDBF0E19EA15913

Gregory, E., Barnhart, H., Dreesen, D. W., Stern, N., & Corn, J. L. (1997). Epidemiological study of *Campylobacter* spp . in broilers: Source, time of colonization, and prevalence. *Avian Diseases*, *41*(4), 890–898. Retrieved from http://www.jstor.org/stable/1592343?seq=1#page_scan_tab_contents

Gu, W., Siletzky, R. M., Wright, S., Islam, M., & Kathariou, S. (2009). Antimicrobial

susceptibility profiles and strain type diversity of *Campylobacter jejuni* isolates from turkeys in eastern North Carolina. *Applied and Environmental Microbiology*, *75*(2), 474–482. http://doi.org/10.1128/AEM.02012-08

Hald, B., Skovgård, H., Bang, D. D., Pedersen, K., Dybdahl, J., Jespersen, J. B., & Madsen, M. (2004). Flies and *Campylobacter* infection of broiler flocks. *Emerging Infectious Diseases*, *10*(8), 1490–1492. http://doi.org/10.3201/eid1008.040129

Hald, B., Skovgård, H., Pedersen, K., Bunkenborg, H., Skovgard, H., Pedersen, K., & Bunkenborg, H. (2008). Influxed insects as vectors for *Campylobacter jejuni* and *Campylobacter coli* in Danish broiler houses. *Poultry Science*, *87*(7), 1428–1434. http://doi.org/10.3382/ps.2007-00301

Hald, B., Sommer, H. M., & Skovgard, H. (2007). Use of fly screens to reduce *Campylobacter* spp. introduction in broiler houses. *Emerging Infectious Diseases*, *13*(12), 1951–1953. http://doi.org/10.3201/eid1312.070488

Hald, B., Wedderkopp, A., & Madsen, M. (2000). Thermophilic *Campylobacter* spp. in Danish broiler production: a cross-sectional survey and a retrospective analysis of risk factors for occurrence in broiler flocks. *Avian Pathology*, *29*(2), 123–31. http://doi.org/10.1080/03079450094153

Havelaar, A. H., Kirk, M. D., Torgerson, P. R., Gibb, H. J., Hald, T., Lake, R. J., … (2015). World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLOS Medicine*, *12*(12), e1001923. http://doi.org/10.1371/journal.pmed.1001923

Hermans, D., Pasmans, F., Messens, W., Martel, A., Van Immerseel, F., Rasschaert, G., … Haesebrouck, F. (2012). Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*.

Vector-Borne and Zoonotic Diseases, *12*(2), 89–98. http://doi.org/10.1089/vbz.2011.0676

Humphrey, T., O'Brien, S., & Madsen, M. (2007). Campylobacters as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology*, *117*(3), 237–257. http://doi.org/10.1016/j.ijfoodmicro.2007.01.006

Jacobs-Reitsma, W. F., van de Giessen, A. W., Bolder, N. M., & Mulder, R. W. (1995). Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. *Epidemiology and Infection*, *114*(3), 413–21. http://doi.org/10.1017/S0950268800052122

Jesse, T. W., Englen, M. D., Pittenger-Alley, L. G., & Fedorka-Cray, P. J. (2006). Two distinct mutations in *gyrA* lead to ciprofloxacin and nalidixic acid resistance in *Campylobacter coli* and *Campylobacter jejuni* isolated from chickens and beef cattle. *Journal of Applied Microbiology*, *100*(4), 682–688. http://doi.org/10.1111/j.1365-2672.2005.02796.x

Korhonen, L. K., & Martikainen, P. J. (1991). Comparison of the survival of *Campylobacter jejuni* and *Campylobacter coli* in culturable form in surface water. *Canadian Journal of Microbiology*, *37*(7), 530–533. http://doi.org/10.1139/m91-089

Lambert, T., Gerbaud, G., Trieu-Cuot, P., Courvalin, P. (1985). Structural relationship between the genes encoding 3'-aminoglycoside phosphotransferases in *Campylobacter* and in Gram-positive cocci. *Annales de l'Institut Pasteur / Microbiologie*, 135–150.

Liebert, M. A., Kanamycin, P., Gibreel, A., Sköld, O. L. A., & Taylor, D. E. (2004). Characterization of plasmid-mediated aphA-3 kanamycin resistance in *Campylobacter jejuni*, *10*(2), 98–105.

Luangtongkum, T., Morishita, T. Y. T., Ison, A. J., Huang, S., McDermott, P. F., & Zhang, Q. (2006). Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. *Applied and Environmental*

Microbiology…, *72*(5), 3600–3607. http://doi.org/10.1128/AEM.72.5.3600

Miller, W. G., Englen, M. D., Kathariou, S., Wesley, I. V., Wang, G., Pittenger-Alley, L., … Mandrell, R. A. (2006). Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. *Microbiology*, *152*(1), 245–255. http://doi.org/10.1099/mic.0.28348-0

Miller, W. G., Huynh, S., Parker, C. T., & Niedermeyer, J. A. (2016). Complete genome sequences of multidrug-resistant *Campylobacter jejuni* strain 14980A (turkey feces) and *Campylobacter coli* strain 14983A (housefly from a turkey farm), harboring a novel gentamicin resistance mobile element. *Genome Announcement*, *4*(5), 16–17. http://doi.org/10.1128/genomeA.01175-16.

Nauta, M., Johannessen, G., Laureano Adame, L., Williams, N., & Rosenquist, H. (2016). The effect of reducing numbers of *Campylobacter* in broiler intestines on human health risk. *Microbial Risk Analysis*. http://doi.org/10.1016/j.mran.2016.02.001

Newell, D. G., & Fearnley, C. (2003). Sources of *Campylobacter* colonization in broiler chickens. *Applied and Environmental Microbiology*, *69*(8), 4343–4351. http://doi.org/10.1128/AEM.69.8.4343-4351.2003

Pava-Ripoll, M., Pearson, R. E. G., Miller, A. K., Tall, B. D., Keys, C. E., & Ziobro, G. C. (2015). Ingested *Salmonella enterica, Cronobacter sakazakii, Escherichia coli* O157:H7, and *Listeria monocytogenes*: transmission dynamics from adult house flies to their eggs and first filial (F1) generation adults. *BMC Microbiology*, *15*(1), 150. http://doi.org/10.1186/s12866-015-0478-5

Peyrat, M. B., Soumet, C., Maris, P., & Sanders, P. (2008). Recovery of *Campylobacter jejuni* from surfaces of poultry slaughterhouses after cleaning and disinfection procedures:

Analysis of a potential source of carcass contamination. *International Journal of Food Microbiology*, *124*(2), 188–194. http://doi.org/10.1016/j.ijfoodmicro.2008.03.030

Rosef, O., & Kapperud, G. (1983). House flies (Musca domestica) as possible vectors of *Campylobacter fetus* subsp. *jejuni*. *Applied and Environmental Microbiology*, *45*(2), 381–383. Retrieved from http://aem.asm.org.prox.lib.ncsu.edu/content/45/2/381.short

Rosenquist, H., Sommer, H. M., Nielsen, N. L., & Christensen, B. B. (2006). The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*. *International Journal of Food Microbiology*, *108*(2), 226–232. http://doi.org/10.1016/j.ijfoodmicro.2005.12.007

Royden, a, Wedley, a, Merga, J. Y., Rushton, S., Hald, B., Humphrey, T., & Williams, N. J. (2016). A role for flies (Diptera) in the transmission of *Campylobacter* to broilers? *Epidemiology and Infection*, *144*(15), 3326–3334. http://doi.org/10.1017/S0950268816001539

Ruiz-palacios, G. M. (2007). The health burden of *Campylobacter* infection and the impact of antimicrobial resistance : playing chicken. *Clinical Infectious Diseases*, *44*, 701–703. http://doi.org/10.1086/509936

Sahin, O., Morishita, T. Y., & Zhang, Q. (2002). *Campylobacter* colonization in poultry: sources of infection and modes of transmission. *Animal Health Research Reviews*, *3*(2), 95–105. http://doi.org/10.1079/AHRR200244

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., … Griffin, P. M. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. http://doi.org/10.3201/eid1701.091101p1

Scallan, E., Hoekstra, R. M., Mahon, B. E., Jones, T. F., & Griffin, P. M. (2015). An assessment

of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiology and Infection*, *143*(13), 2795–2804. http://doi.org/10.1017/S0950268814003185

Shane, S. M., Montrose, M. S., & Harrington, K. S. (1985). Transmission of *Campylobacter jejuni* by the Housefly (Musca domestica). *Source: Avian Diseases*, *29*(2), 384–391. Retrieved from http://www.jstor.org/stable/1590499

Skovgård, H., & Kristensen, K. (2017). Retention of *Campylobacter* (Campylobacterales: Campylobacteraceae) in the house fly (Diptera: Muscidae). *J Med Entomol*, 48 (6), 1202–1209.

Smith, K., Reimers, N., Barnes, H. J., Lee, B. C., Siletzky, R., & Kathariou, S. (2004). *Campylobacter* colonization of sibling turkey flocks reared under different management conditions. *Journal of Food Protection*, *67*(7), 1463–1468.

Solow, B. T., Cloak, O. M., & Fratamico, P. M. (2003). Effect of temperature on viability of *Campylobacter jejuni* and *Campylobacter coli* on raw chicken or pork skin. *Journal of Food Protection*, *66*(11), 2023–2031. Retrieved from http://jfoodprotection.org/doi/pdf/10.4315/0362-028X-66.11.2023

Szalanski, A. L., Owens, C. B., McKay, T., & Steelman, C. D. (2004). Detection of *Campylobacter* and *Escherichia coli* O157:H7 from filth flies by polymerase chain reaction. *Med Vet Entomol*, *18*(3), 241–246. http://doi.org/10.1111/j.0269-283X.2004.00502.x

Taylor, D. E., & Chau, A. (1996). Tetracycline resistance mediated by ribosomal protection. *Antimicrobial Agents and Chemotherapy*, *40*(1), 1–5.

Taylor, D. E., & Courvalin, P. (1988). Mechanisms of antibiotic resistance in *Campylobacter* species. *Antimicrobial Agents and Chemotherapy*, *32*(8), 1107–1112.

Taylor, E. V., Herman, K. M., Ailes, E. C., Fitzgerald, C., Yoder, J. S., Mahon, B. E., & Tauxe, R. V. (2012). Common source outbreaks of *Campylobacter* infection in the USA, 1997–2008. *Epidemiology and Infection*, *141*(2013), 1–10. http://doi.org/10.1017/S0950268812001744

Tenover, F. C., Gilbert, T., & O'Hara, P. (1989). Nucleotide sequence of a novel kanamycin resistance gene, *aphA-7*, from *Campylobacter jejuni* and comparison to other kanamycin phosphotransferase genes. *Plasmid*, *22*(1), 52–58. http://doi.org/10.1016/0147-619X(89)90035-8

US Food and Drug Administration. (2014). *NARMS Integrated Report: 2014*. Retrieved from https://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm059103.htm

Wallace, J. S., Stanley, K. N., & Jones, K. (1998). The colonization of turkeys by thermophilic campylobacters. *Journal of Applied Microbiology*, *85*(2), 224–230. http://doi.org/10.1046/j.1365-2672.1998.00470.x

Wright, S. L., Carver, D. K., Siletzky, R. M., Romine, S., Morrow, W. E. M., & Kathariou, S. (2008). Longitudinal study of prevalence of *Campylobacter jejuni* and *Campylobacter coli* from turkeys and swine grown in close proximity. *Journal of Food Protection*, *71*(9), 1791–1796.

Zirnstein, G., Helsel, L., Li, Y., Swaminathan, B., & Besser, J. (2000). Characterization of gyrA mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. *FEMS Microbiology Letters*, *190*, 1–7. http://doi.org/Doi 10.1016/S0378-1097(00)00306-2

Table 4-1 Two-way contingency table of species vs. sample type

Sample Source	Species n (%)		Total
	C. coli	*C. jejuni*	
Fecal	105 (48.84)	110 (51.16)	215
Fly	87 (53.05)	77 (46.95)	164
Total	192	187	379

Table 4-2 Antimicrobial resistances of each isolate by source and species. Strains only recovered from either feces or flies are highlighted in bold

C. jejuni				*C. coli*			
Fecal	ST (n)	Flies	ST (n)	Fecal	ST (n)	Flies	ST (n)
TSKQG (99)	1839 (9) 7730 (1)	TSKQG (53)	1839 (5)	TSEKQG (58)	889 (1), 1101 (1)	TSEKQG (41)	1017 (10), 1067 (3), 1101 (1), 8551 (1)
TSKQ (5)	1839 (1)	TSKQ (6)	1839 (1)	TEKQG (17)	1101 (2)	TEKQG (21)	1101 (1), 1192 (1)
TKG (4)	2934 (1)	TKG (6)	1839 (1), 2934 (1)	TKQG (13)	8213 (2)	TKQG (14)	1192 (1), 8224 (1)
TKQG (2)		TKQG (5)	1839 (2), 8227 (1)	TSKQG (5)		TS (3)	8218 (1)
TKQ (1)	1839 (1)	TKQ (5)	1839 (1)	T (5)	1833 (1)	TKG (3)	1833 (2), 8213 (1)
		TG (1)		TKG (3)	1833 (2)	TSKQG (2)	1101 (1)
		T (1)	2934 (1)	TK (2)		TKQ (1)	
				TEK (1)	1833 (1)	TSKQ (1)	
				SEKQG (1)		TK (1)	1192 (1)

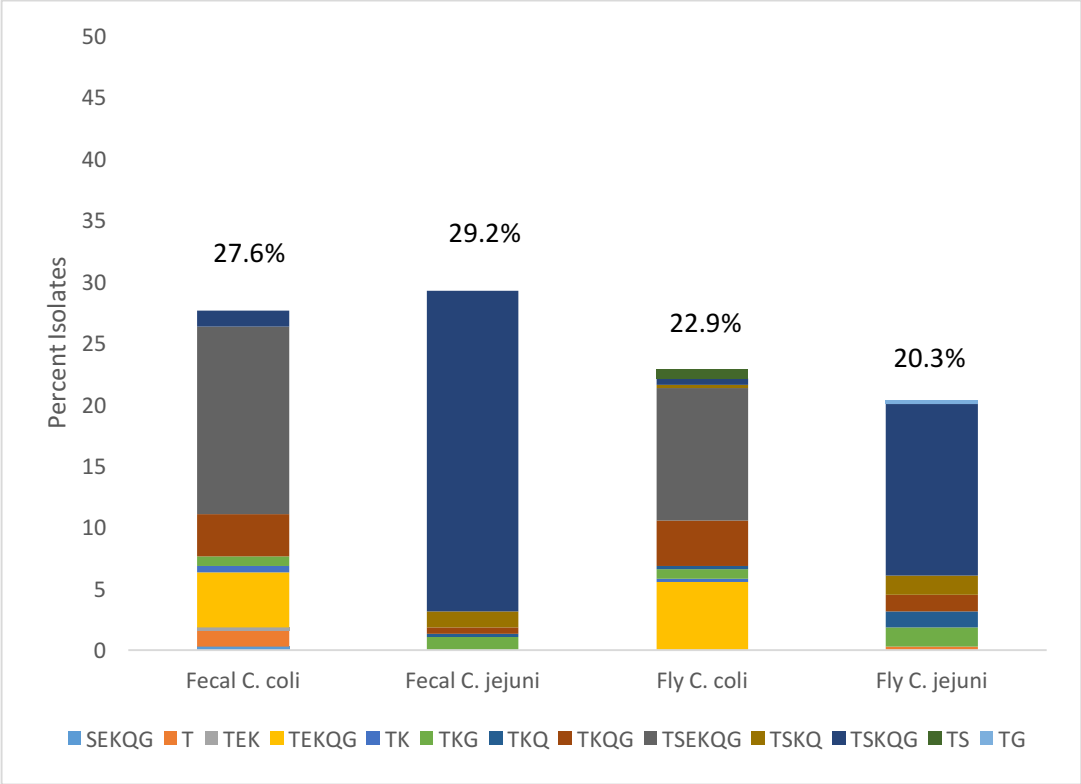


Figure 4-1 Percent contribution of each sample type to the AMR phenotypes

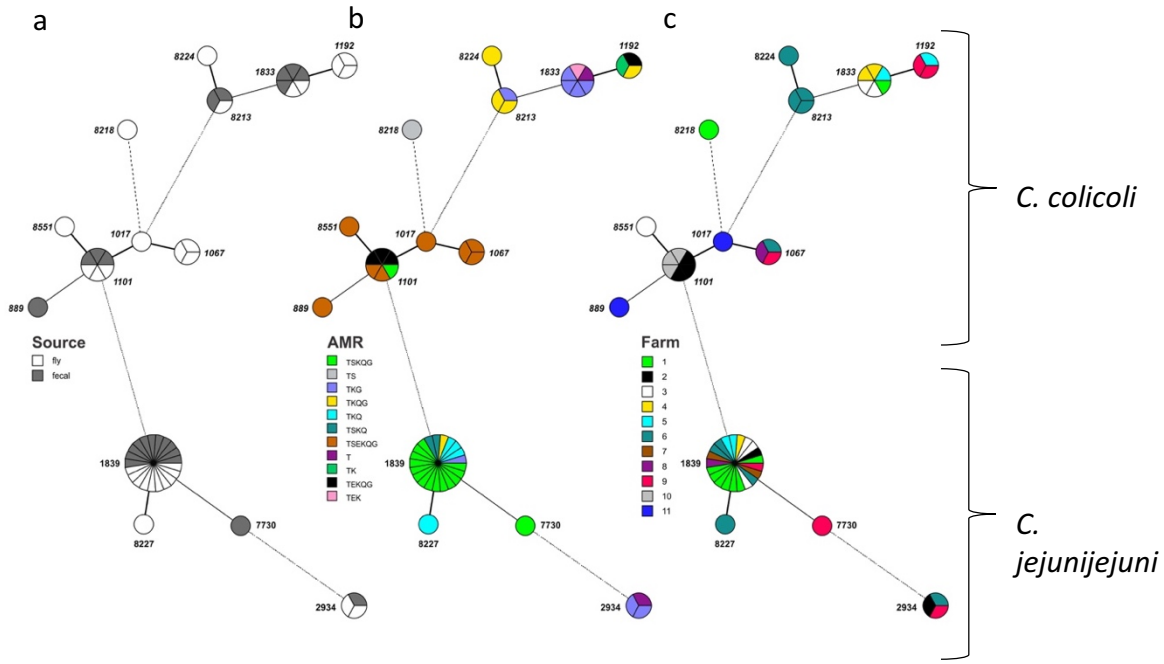


Figure 4-2 *Minimum Spanning Tree depicting the clustering of 14 identified STs shown by sample a) source, b) AMR profile, c) and farm. The tree was created as previously described (Miller et al., 2006) with each ST represented by a circle. Thick, short lines* connect single single locus variants, while longer, thin lines connect double locus variants, black dashed lines represent three or more allele differences, and gray dashed lines represent five or more allele differences.

Chapter 5 On-farm Risk Assessment and the Effects of Weather in Production of *Campylobacter*-Negative Turkey Flocks

SUMMARY

This study sought to identify risk factors that affect the *Campylobacter* status of turkey flocks in North Carolina. Because season has been shown to be strongly associated with risk for *Campylobacter* infection, specific weather changes related to season were assessed in order to further define the specifics of 'season' that affect *Campylobacter* status. Additionally, risk factors related to farm practices were screened for correlation with *Campylobacter* status. To assess the effect of climate, cecal and fecal samples from 309 brooder flocks were evaluated and corresponding weather data was collected for a period of five weeks prior to sampling. For farm-related risk factor analysis, cecal and fecal samples were evaluated from 152 brooder flocks and information on farm management and layout was gathered via on-farm audits and surveys. Of the 309 brooder flocks sampled for the weather study, 260 (84.1%) were found to carry *Campylobacter,* while 138 (90.8%) of the 152 brooder farms for the farm-level risk factor study were positive. The average monthly air temperature was used to determine hot and cold seasons and a strong effect of season on *Campylobacter* status was identified ($p < 0.0001$). The average dew point temperature and cloud coverage were determined to be significantly associated with flock outcome. A mixed multivariate logistic model containing a random farm effect and fixed effects for season as well as an indication of the presence or absence of a barrier around the farm was created although the strong correlation with the implementation of other biosecurity practices may indicate that barrier is an indicator of the adherence to biosecurity protocols. The results of this study both confirm and add to the list of *Campylobacter*-risk factors that have been identified in the past with poultry including the role that family plays, who is allowed in the turkey houses, and the hygiene practices of visitors. This study specifically speaks to risk in turkeys, a major gap in the literature regarding *Campylobacter* and poultry.

INTRODUCTION

Campylobacter spp. are important foodborne pathogens in the United States, being responsible for 850,000 human illnesses each year (Elaine Scallan et al., 2011). Most illnesses are sporadic and handling or consuming raw or undercooked poultry has been identified in several studies to be a leading risk factor for illness. (Hopkins & Scott, 1983; Kapperud, Skjerve, Bean, Ostroff, & Lassen, 1992; Potter, Kaneene, & Hall, 2003). Although *Campylobacter* is fastidious in nature, it is difficult to remove in the processing environment, thus sending flocks to slaughter that are free of *Campylobacter* is a high public health priority (Boysen et al., 2013; Hopkins & Scott, 1983; Kapperud et al., 1992; Potter et al., 2003). Lowering the prevalence of campylobacteriosis can be accomplished by reducing the number of flocks that carry this organism as a linear relationship exists between the prevalence of *Campylobacter*-positive broiler flocks and the risk to human health (Efsa, 2011; Nauta et al., 2016; Organization, 2012). For that reason, much effort has gone into identifying risk factors that increase a flock's risk of carrying *Campylobacter* (Agunos et al., 2014; Callicott et al., 2006; Cox et al., 2012).

Seasonal trends in the prevalence of human campylobacteriosis have been documented in countries all over the world, with more infections during warm, summer months (Bi, Cameron, Zhang, & Parton, 2008; Jore et al., 2010; Nylen et al., 2002; Patrick et al., 2004; Wagenaar, J.A. Newell, D.G., Kalupahana, R.S., Mughini-Gras, 2015). These trends are reflected in the prevalence of *Campylobacter* in poultry flocks, as more poultry carry *Campylobacter* in the warmer summer months (Sommer et al., 2016; Wallace, Stanley, Currie, Diggle, & Jones, 1997). Most studies that have investigated the weather's effect on *Campylobacter* have focused on broilers and have identified relationships between weather conditions such as temperature and hours of sunlight and the prevalence of *Campylobacter*-positive flocks in broiler production

(Jonsson et al., 2012; Wallace et al., 1997). To the best of the author's knowledge, no such study has been published about *Campylobacter* in turkey production.

Similarly, most studies seeking to identify on-farm risk factors for flock colonization have focused on broiler chickens, and many of these studies have taken place in countries other than the US, which may not reflect the same risks as poultry produced domestically (Birthe Hald et al., 2004b; S. Smith et al., 2016; A. van de Giessen et al., 1992; a W. van de Giessen et al., 1996). Turkey production is quite different than broiler production, and so results from broilers cannot simply be applied to the turkey industry. Identified risk factors in turkey production include foot traffic between flocks, manure management, and chlorination of the drinking water (Arsenault et al., 2007; K. Smith et al., 2004). Identifying risk factors is critical for developing effective interventions for prevention. The goal of this study was to identify farm risk factors, including specifics of season, and the *Campylobacter* status in order to better understand the introduction and transmission of *Campylobacter* spp. to commercially produced turkey flocks.

MATERIALS and METHODS

Sample Collection and *Campylobacter* isolation

In the United States, it is common practice for commercial turkey companies to keep turkeys on brooder farms from day of hatch to 4-5 weeks. At that point, brooder flocks are typically split into multiple growout flocks and transported to growout farms. A total of 311 flocks (n=309 brooder flocks, n=2 growout flocks) from 37 farms (n=35 brooder farms, n=2 growout farms) in North Carolina were sampled from June, 2012 to July, 2016 for inclusion in the weather analysis. Both brooder and growout farms were visited in this study, however because both growout farms were positive only weather data from brooder farms were used in the analysis and the birds

ranged from 11 to 39 days old. For the on-farm risk analysis, a total of 152 brooder flocks from 14 farms from 2013-2016 were sampled. The age of these brooder flocks ranged from 21 to 36 days old with an average of 30.8 days. Eighteen growout flocks were also sampled, but all were positive and so excluded from the analysis.

Samples were collected in two ways: first, samples of feces and flies were collected during on-farm visits. Second, cecal samples were shipped directly to North Carolina State University by the collaborating turkey production company as described in chapters three and four. Briefly, three seemingly fresh fecal samples were collected from each quadrant of the turkey house for a total of 12 fecal samples. Freshly voided fecal droppings were collected using sterile swabs, placed into sterile 15-mL polypropylene Corning tubes (Fisher Scientific, Pittsburgh, PA) transported to the laboratory on ice. Up to 10 live flies were also collected from the same turkey house, transported to the laboratory on ice and typically processed *for Campylobacter* on the same day. Fecal samples were combined in sterile Mueller-Hinton broth (MHB; Difco, Becton Dickinson, Franklin Lakes, NJ), serially diluted, and plated onto modified Charcoal-cefoperazone-deoxycholate agar (mCCDA; Oxoid, Hampshire, UK; SE 155, Oxoid) and incubated microaerobically at 42°C for 48 hours in anaerobic jars containing CampyPak Plus microaerobic sachets (Becton Dickinson, Sparks, MD). A total of six colonies per composite sample were purified on Mueller-Hinton agar (MHA; Difco, Becton Dickinson, Franklin Lakes, NJ), and pure cultures were preserved in Brain Heart Infusion broth (Becton Dickinson, Franklin Lakes, NJ) supplemented with 20% glycerol at -80°C.To isolate and enumerate *Campylobacter* spp. from flies, each fly was homogenized in 1 ml of sterile Phosphate Buffered Saline and the suspension was plated (100 μl) onto mCCDA. subsequent incubations and purifications were as described for the fecal samples.

Weekly shipments of ceca from randomly selected flocks were received from a collaborating company. Six ceca from each flock were shipped overnight on ice to North Carolina State University and processed within 24 hours. From each cecum, 0.1 g of internal contents was aseptically transferred to sterile MHB and serially diluted. From November 5, 2014 and onward, 100 μl of the cecal composite was also enriched in Bolton Broth (Oxoid, Cambridge, United Kingdom), serially diluted, and plated onto CCDA. Colonies were purified and frozen in the same manner as the fecal samples. Flocks were considered positive when either the ceca or feces were positive for *Campylobacter*. Comparisons between quantitative *Campylobacter* counts were difficult between feces and cecal isolate as the vast majority of flocks only had one sample type. *Campylobacter* counts from feces are generally 1-2 log CFUs lower than that of ceca, which contributes to the difficulty in making quantitative comparisons between these two sample types.

Weather data collection

The North Carolina Institute for Climate Studies collected historical weather data from regional airports closest to the farm coordinates provided. For each flock sample, data including hourly measures of the air temperature (°C), the dew point temperature (°C), cloud coverage (okta), wind speed (m/s), and liquid precipitation for a one and six hour duration (millimeters) was recorded for a period of five weeks prior to the sampling date. For measures of liquid precipitation, values of -1 indicate that there was non-measurable trace precipitation. These values were converted to zero so that they did not skew the average precipitation calculations. Zero also indicated that there was precipitation observed rather than the value of -9999 which was recorded when there was actually no precipitation events.

Survey development

An on-farm assessment and a survey were created to gather material about the farm layout, operations, and biosecurity protocols. The survey instrument was developed using questions on common farm practices that have the potential to affect the *Campylobacter* status (as well as other organisms) of each farm. The instrument was then reviewed, by the veterinary staff representing the company, for comments on possible omissions and also for questions that are not applicable due to no variation in the practice across farms (eg. all flocks are on the same vaccine schedule). Once the survey instrument was finalized, members of the research team and veterinary staff decided to have the instrument administered by the flock technicians. These are company-employed personnel who visit their assigned flocks at least two times per week and are very familiar with the farm practices. The research team initially accompanied the flock technicians to farms to administer the surveys in order to detect and clarify questions that were not clear. Once the farm technicians were familiar with the intent of the survey questions they administered the surveys independently. The survey instruments were collected by the veterinary staff and forwarded to the research team. Not all farm technicians returned the surveys resulting in participation by only 14 farms, but on those farms data were collected on multiple flocks.

Statistics

All statistics were calculated using Microsoft Excel and R: A Language and Environment for Statistical Computing (Vienna, Austria). Packages used were: dplyr, epitools, and lme4. Because the response variable, *Campylobacter* status of the flocks, is binary logistic regression was used to model the response against each predictor. Additionally, the farm that the flock was sourced from was included as a random effect as all farms, with the exception of one, was sampled more

than one time to create a mixed logistic model for the probability of a flock being positive for *Campylobacter* using the glmer function of the package lme4 of R. The response variable, μ, is the probability of success (or failure) for a given set of predictors and is constrained between 0 and 1. For regression, the probability (p) of success is converted to odds and then to log odds in the following manner:

$$odds = \frac{p}{1-p}$$

$$\log odds = \ln [odds]$$

which is analogous to

$$logit(\mu) = \ln\left(\frac{\mu}{1-\mu}\right)$$

The results from the univariate analysis were then used to create a multilogistic model of the following manner:

$$logit(\mu) = \beta_\circ + \beta_1 + \beta_2 \ldots \beta_n$$

First, the effect of season was investigated by grouping the months into two seasons, warm and cold. Average monthly temperatures were recorded over this study period and plotted to see if there was a natural break in the data. The distribution of these temperatures indicated that November through April were cold months and that May through October were hot months. Flocks were designated as being produced either in cold or hot months depending on which season the majority of their production took place in. For example, if a flock was sampled on October 1st, a "cold" month, it would actually be designated as "hot" because the majority of its production took place during the hot season. Fisher's exact test was used to investigate the relationship between season and flock status.

The second approach was to build a model that reflected the contribution of individual climactic variables. Hourly data were used to create variables such as: average daily temperature (°C), average daily wind speed, average daily cloud coverage, average rainfall and average dew point temperature as well as the number of days that it rained per flock. These averages were calculated from data three and five weeks prior to flock sampling to determine what time span best represented the likelihood of a flock being positive for *Campylobacter*. Potential collinearity among the candidate factors was evaluated by Spearman's rank correlation coefficient test using a cutoff value of 0.7 as too highly correlated. Model selection was performed by forward and backward selection and Akaike's Information Criteria (AIC). Associations between the season variable and each individual weather variable were then tested by analysis of variance to determine if the mean of each variable was different for each of the two seasons.

For the farm-level risk factor analysis, variables were first screened and reduced. For example, a variable was excluded from the analysis when all flocks responded in the same manner. Continuous variables were divided above and below the mean to fit either "high" or "low" categories. All factor variables were analyzed by chi-square test without Yate's continuity correction and kept for subsequent analysis if $p \leq 0.1$. Flocks within farms were not independent; therefore, the farm name was included in the model as a random effect. Potential collinearity was determined by calculating the Phi coefficient for two by two tables or Cramer's V for larger tables using a cutoff value of 0.5. Multivariable models were built which included the effect of season and were assessed by Akeike's information criterion.

RESULTS AND DISCUSSION

Prevalence of *Campylobacter*-positive turkey flocks

During the weather analysis, 260 brooder flocks of 309 (84.1%) flocks were positive for *Campylobacter.* From the 152 flocks used in the farm-level risk factor analysis, 138 (90.8%) were positive. These flocks came from 14 separate farms with an average of 10.9 flocks tested per farm. The reports on the prevalence of *Campylobacter* in turkeys varies widely. While the 2014 NARMS report was only able to find *Campylobacter* in 6.1% of turkey ceca, other studies from Canada and the US reported prevalence at 46% and 35%, respectively (Arsenault et al., 2007; Logue, Sherwood, Elijah, Olah, & Dockter, 2003; US Food and Drug Administration, 2014). The authors of the Canadian study cite technical issues and unexpected freezing of the cecal samples from the first 13 of 58 turkey flocks at -70°C for 8 months, which likely reduced the *Campylobacter* populations in those samples (Arsenault et al., 2007). However, other studies have reported prevalence as high as 87% of flocks when testing plant carcass rinses or turkey fecal samples (Berghaus et al., 2013; Wright et al., 2008). It is also important to emphasize that the true prevalence of *Campylobacter* in turkeys is unknown as most studies regarding *Campylobacter* have focused on chickens. To highlight the scarcity of studies on turkeys, a 2011 study of the risk factors for *Campylobacter* colonization of turkeys was only able to identify seven primary research articles (Newell et al., 2011). Additionally, the risk factors for *Campylobacter* colonization may vary widely due to farm layout, weather, and geographic location.

Seasonal effect

Table 5-1 shows the average air temperature for each month of sampling and was used to split the year into cold and warm months. January through April, and November and December were the six coldest months. Table 5-2 shows a two-by-two table and the results of fisher's exact test of the total number of positive flocks for warm and cold months as determined by table 5-1. These results, as reported in previous studies, indicate a strong effect of season on the prevalence of positive flocks ($p < 0.0001$).

Unilogistic regression of weather variables

Average values were calculated for wind speed, air temperature, dew point temperature, rainfall as measured in one and six hour increments, and cloud coverage for three and five weeks prior to sampling to determine which time period best represented the risk of colonization. Additionally, the number of days that it rained per flock was calculated. A mixed, univariate regression of each variable against flock status indicated that the average air temperature over three and five weeks, the average wind speed over three and five weeks, the cloud coverage over three and five weeks, and the average dew point temperature over three and five weeks were significantly ($p \leq 0.05$) associated with the probability of a flock being positive for *Campylobacter (table 5-3)*. The five week average was slightly more significantly associated with the flock outcome for the air temperature, cloud coverage, and dew point temperature compared to the three week average. The three week average of wind speed was more significantly associated with flock outcome than the five week average. Correlation was found between the dew point temperature and air temperature, thus air temperature was excluded from the analysis due to it having a less significant effect on flock status.

Average air temperature is strongly associated with the probability of flock being *Campylobacter* positive

The average air temperatures for three and five weeks prior to the sample-collection date were found to be significant as a predictor of the log-odds of a flock being positive (table 5-3). This agrees with previous reports in broiler flocks which have found increasing prevalence of *Campylobacter* in broiler flocks during warmer weather (Patrick et al., 2004; Sommer et al., 2016; Wallace et al., 1997). Additionally, increases in temperature have also been found to be associated with increases in the occurrence of human cases of campylobacteriosis (Bi et al., 2008; Nichols, 2005; Patrick et al., 2004). As warmer weather also initiates the development of fly eggs into adults, higher temperature may result in larger fly populations which are known to transmit bacteria like (Nichols, 2005; Rosef & Kapperud, 1983; Shane et al., 1985).

Wind is associated with an increased probability of a flock carrying *Campylobacter*

Average wind speed prior to sampling was found to be significantly associated with the probability that a flock was *Campylobacter*-positive. The average wind speed (m/s) for the three weeks prior to sampling was slightly more significantly than the five week average ($p = 0.00593$ vs $p = 0.0318$, respectively) (table 5-3). Increases in wind speed were found to result in a decreased risk of a flock being colonized (OR = 0.39, 95% CI 0.19 – 0.73). The findings on wind speed may be especially interesting as there have not been many studies published regarding wind speed and poultry production. Wind speed during transport of turkeys to slaughter was found to be associated with the prevalence of *Salmonella* but not *Campylobacter* on the carcasses (Arsenault et al., 2007). The authors of that study hypothesize that higher winds may dry the

crates thus killing the *Salmonella* on those surfaces, however as *Campylobacter* is known to be susceptible to dehydration it is puzzling that this did not have the same effect for this organism. It may be that higher winds decrease fly populations, dry out potential environmental niches, or have another unexplained effect.

Increasing cloud coverage increases the risk of a flock carrying *Campylobacter*

The average cloud coverage, measured in oktas, or the fraction of the total celestial dome covered by clouds or other obscuring phenomena, for the three and five weeks prior to sampling was found to be highly associated with the probability of a flock carrying *Campylobacter* ($p<0.001$). The results from the three week average cloud coverage indicate that for each okta increase in cloud coverage results in nearly four times the odds of a flock being positive (OR= 3.0, 95% CI 1.87 – 9.94). Cloud coverage is particularly interesting as studies done in broilers have produced mixed results regarding the effect of sunshine on *Campylobacter* prevalence (Hansson et al., 2007; Sommer et al., 2016; Wallace et al., 1997). The results of one study found that the number of hours of sunshine was positively correlated with the carriage rate in broiler ceca likely reflect the increased length of days during the summer months (Wallace et al., 1997). In the case of our results, lower cloud coverage may allow the sun to dry the ground decreasing possible environmental niches for *Campylobacter*.

Rain has little effect on the odds of *Campylobacter* being in a flock

Data on rainfall were measured as both one-hour accumulation periods and six-hour accumulation periods over three and five weeks. Values recorded as NA can also indicate that there was no rainfall and so were recoded as 0. None of the rainfall estimates were significantly

correlated with the outcome of the flocks. Additionally, the number of days that it rained per flock was calculated and also found to be insignificant (table 5-3). The results of our analysis on rain appear to be in agreement with what has been found in broiler flocks, although one study has found that *Campylobacter* recovery outside of broiler houses is highest just after a rain event (Hansson et al., 2007). However, other studies have found that rainfall was not associated with the status of broiler flocks or with human campylobacteriosis (Jonsson et al., 2012; Patrick et al., 2004; Tam et al., 2006).

Dew point temperature is highly associated with flock outcome

Dew point temperature was used to calculate relative humidity with the following conversion: Relative humidity = $100 - 5(t - t_d)$ where t is the air temperature and td is the dew point temperature. However, this equation is only valid for moist air (RH≥50%) and there was 48,107 data points that did not meet this criteria. For that reason, the dew point temperature and not relative humidity was used in all calculations. The average dew point temperature over both three and five weeks prior to sampling was found to be highly associated with the odds of a flock carrying ($p<0.001$).

Other studies have reported relationships between humidity or dew point temperature and the status of broiler flocks (Tam et al., 2006). While temperature control in poultry houses is emphasized during summer months, during the rest of year keeping a favorable humidity is likely more critical for bird health and fly control as houses with higher relative humidity have been found to become colonized with *Campylobacter* more quickly than birds in houses with lower relative humidity (Line, 2006; Ward & Lachance, 2015). Finally, humidity has been found to have a weak association with the prevalence of human campylobacteriosis (Bi et al., 2008;

Patrick et al., 2004). The dew point temperature outside likely affects the humidity inside the turkey houses and most conventional houses are open to the outside. Higher humidity can allow the litter to become more moist and allow for higher fly populations (Ward & Lachance, 2015).

Multilogistic regression

The most significant average of each variable (three weeks versus five week average) was kept for inclusion in the multilogistic regression. Significant variables kept for inclusion in the multilogistic model were: average air temperature over five weeks, the average wind speed over three weeks, the amount of rain measured by 6 hour increments over three weeks, the average cloud coverage in five weeks, and the average dew point temperature over five weeks. The average air temperature was removed for inclusion in a multilogistic model due to a high degree of collinearity with the dew point temperature (table 5-4). The same occurred for the one-hour and six-hour estimates of rain fall. Based on the lower p-value, the six-hour estimate was kept for model inclusion.

The final multivariate model is one containing the fixed effects of average dew point temperature (T_d) for the five weeks prior to sampling and the average cloud coverage for the five weeks prior to sampling(S), and the random effects of flock (f) (table 5-5):

$$logit(\mu) = \beta_{\circ} + T_d + S + f + \varepsilon$$

This was the favored model by both forward and back selection of significant variables from the univariate analysis. The results of table 5-5 indicate that both dew point temperature and cloud coverage are positively associated with the probability of a flock testing positive for *Campylobacter*. This makes some intuitive sense as *Campylobacter* is known to be susceptible to dehydration. Increased dew point temperature may increase the moisture inside the turkey houses

allowing greater survival and transmission. Similarly, increased cloud coverage may allow the ground to remain wetter and perhaps even limit UV exposure to bacterial cells on the ground.

Season as a predictor of weather variables

An analysis of variance (ANOVA) was used to determine if there was a difference in the mean weather variables between the cold and hot seasons (table 5-6). Season was found to be highly associated with all tested variables other than rainfall, which was not significantly associated with the log odds of a flock being *Campylobacter*-positive. Since there was not a complete set of weather data for all flocks used in the risk factor analysis, season was determined based on the month in which sampling took place and used as a surrogate as found to be appropriate by the results of the ANOVA.

RISK FACTOR ANALYSES

Numerous variables were highly correlated

The results of the Chi-square tests identified twelve categorical variables that were significantly associated with *Campylobacter* farm status (p-values ≤ 0.1). However, evaluation of Phi and Cramer's V coefficient indicated correlation between numerous variables . Further evaluation of odds ratios and 95% confidence intervals resulted in the rejection of all variables other than barrier i.e. was there a barrier around the farm? Collinearity was found among a) barrier around the farm (no barrier or barrier around entire perimeter), b) the litter disposal method (either on the farm or off the farm), c) who was responsible for removing the litter from the farm (the poultry company, a contractor, or the farm owner) d) the use of hand sanitizer, and e) the presence of a locked gate at farm entrances. However, the barrier variable was chosen to be kept

due to its substantially smaller p-value and the fact that the litter disposal method, the person responsible for removing litter, the use of hand sanitizer, and the presence of a gate were all found to be collinear with each other and other additional variables as well while Barrier was associated only with who removed the litter and what method was used. Additionally, having family members that work with poultry or keep birds as pets was associated with the use of hand sanitizer on the farm as was requiring visitors to wear plastic overboots. Keeping the doors to the poultry house was associated with fumigating the houses between flocks. Although these variables were not kept for analysis in the multivariate model, their effects on flock status are shown in table 5-7 and discussed individually below with the exception of litter removal method as this is considered as a standard practice in our collaborating poultry company which is vertically integrated.

Barrier around the farm

The variable 'Barrier' was used to indicate if there was a fence around just the poultry houses, around other areas, around the entire perimeter of the farm, or no barrier at all. All responses fit either no barrier (n=144) or a barrier around the entire perimeter of the farm (n=8, with all eight flocks being produced on the same farm). Those flocks that had no fence around the entire perimeter were 13.4 times more likely to be positive than were flocks from farms with a fence around the perimeter (p-value = 0.0000415) (table 5-7). It may be that fencing deters some animal or human traffic from accessing the farm and introducing *Campylobacter*. Or, this variable may be an indication of commitment to biosecurity, farm maintenance, privacy, or other protective factor.

Entity responsible for removing used litter

Litter was removed from the poultry houses by either an outside contractor (n=124), the farm owner or manager (n=14), or the poultry company (n=14). Farms where an outside contractor was responsible for removing used litter had 1.1 times the odds of producing a positive flock than farms where the owner or manager was responsible (OR = 1.1, table 5-7). These odds increase by a factor of 8.06 when an outside contractor removes the litter compared to when the poultry company does (OR=8.06). Although other studies have investigated and identified litter management as a risk factor no other study was found that investigated who removed the litter as a risk factor (Berndtson et al., 1996; K. Smith et al., 2004). It may be that outside contractors are not cleaning and disinfecting their equipment, clothing, or personnel when they move between farms and so may be bringing *Campylobacter* onto farms. While equipment from the poultry company would also be moving between farms, they may recognize the importance of disinfecting that equipment and be stringent in their adherence to biosecurity protocols. Who removes the litter was found to be highly correlated with barrier.

Tools

Farms that owned all of the tools they needed were asked if other farms ever used their tools. Farms that responded that did allow other farms to use their tools had 3.47 times the odds of producing a *Campylobacter*-positive flock than did farms which did not allow other farms to use their tools (OR = 3.47). This is in agreement with a past study that found that designating tools to specific broiler houses resulted in lowered risk of *Campylobacter* colonization across six European countries (Sommer et al., 2016). However, no past studies could be found where this

variable was investigated on turkey farms. Like litter removal, it appears that controlling the traffic of people and equipment onto the turkey farms has a protective effect against *Campylobacter*.

Keeping the doors to the turkey house locked

Flocks that were produced on farms where the doors to the turkey houses were kept locked had a reduction in the likelihood of the flock being positive for *Campylobacter.* The odds of producing a positive flock for those farms which kept the doors locked were 0.37 times the odds of those that did not. One other study was found that had also identified locking doors to the poultry house as protective, although that study investigated broiler flocks (Arsenault et al., 2007). This study proposes the idea that this variable is a "surrogate variable for the quality of biosecurity measures implemented by producers" (Arsenault et al., 2007). Keeping the doors locked was highly correlated with fumigating between flocks.

Hand sanitizer

Whether a hand sanitizing station was provided (n=28) or not (n=124) at each poultry house was assessed. The presence of a hand sanitizing station was found to be a protective effect as the results indicate that farms that provide a hand sanitizing station at each poultry house are 0.25 times as likely to produce a *Campylobacter*-positive flock than those who do not. This is in agreement with what has been found in broiler production. Hygiene-based interventions, including providing hand sanitizer or washing hands before tending to flocks, has been shown in other studies to reduce the risk of a flock carrying *Campylobacter* (Gibbens, Pascoe, Evans, Davies, & Sayers, 2001; A. van de Giessen et al., 1992; a W. van de Giessen et al., 1996).

Additionally, other studies have found that broilers were more likely to become colonized once neighboring houses on the same farm were positive (Jacobs-Reitsma et al., 1995). These findings emphasize the importance of preventive hygienic steps when entering turkey houses including moving between houses on the same farm. The presence of hand sanitizer at each poultry house was associated with the use of gates on the farm.

Using fogs to treat for flies

Controlling fly populations on the poultry farm has been identified in several studies to impact the risk of a flock becoming colonized by *Campylobacter* (Birthe Hald et al., 2004b; Rosef & Kapperud, 1983; Royden et al., 2016). Using fogs to treat against flies was assessed and found to have a protective effect (OR = 0.36). Fly fogs have been cited as being the most effective and economic method of controlling fly populations (Williams & Entomologist, 2010).

Allowing people into the turkey houses

Allowing repairmen into the turkey houses was found be protective (OR = 0.4) as was allowing family members into the turkey houses was also found to be protective (OR = 0.32). It may be that turkey houses of farms that allow repairmen into houses are in better repair. Family members may be a better option for work on the farm as hiring outside employees was found to be a risk factor for *Campylobacter* in turkey flocks (OR = 2.29).

Multivariate logistic regression

Because each farm was sampled several times, a multivariate logistic model that included the farm identifier as a random effect was evaluated. Mixed models also included the seasonal effect.

The output of the mixed model indicate that flocks produced on farms without a barrier were 8.41 times more likely to be positive *for Campylobacter* (table 5-8). The final model chosen was one containing only season as a predictor of flock status (table 5-9). Barrier is really a farm-level indicator and does not vary from flock to flock at the same farm.

CONCLUSIONS

This study investigated recent weather trends on the seasonal effects and *Campylobacter* outcomes of commercially-produced turkey flocks in North Carolina. The strong seasonal effect, as shown by the simple fisher's exact test, confirms what has been seen in the past in broiler production (Hansson et al., 2007; Jonsson et al., 2012). Warmer months result in a greater number of flocks that are *Campylobacter*-positive. Season may encompass several weather variables such as humidity and temperature, but may also affect other potential risk factors such as insect populations and litter moisture, which may explain why the seasonal effect on the *Campylobacter* status of flocks is so clearly evident. Additionally, these results have identified several individual variables that are associated with the *Campylobacter* status of the flocks, which may allow effective intervention strategies to be created for example, limiting traffic into turkey houses during high risk periods.

The low prevalence of negative flocks in this study likely hampered our ability to detect significant risk factors on the farms. For this reason, we chose to include table 5-7 to describe variables that we were unable to include in the multivariate model, but that may have an impact on the outcome of the turkey flocks as assessed by odds ratio. The variable of barrier could be indicative of the farm's commitment to strict biosecurity controls. In this case, only one brooder farm had a fence all the way around its perimeter. The flocks from this farm were negative 50%

of the time and produced during both hot (n=3) and cold (n=5) months. This farm also had gates that were kept locked and posted "No Visitor" signs with contact information of the grower. The grower provided coveralls, rubber boots, and a shower for visitors and employees. There were no outside employees and the grower reported using a clean pair of coveralls every day. Additionally, control programs were in place for darkling beetles, flies, and rodents and no tools were loaned to other farms.

These results may indicate that controlling the traffic of people and equipment onto turkey brooder farms results in a reduced likelihood of flocks being colonized by *Campylobacter*. The longer life span and increased exposure to people, equipment, and multiple farms means that turkeys will have more opportunity for colonization by this human pathogen. However, as many brooder flocks are already colonized by the time they are sent to growout, it is clear that the first step in producing *Campylobacter*-free flocks will be to master the production of brooder flocks that are *Campylobacter*-negative. Greater diligence in biosecurity which may include fencing farms, providing a hand sanitizing station at every turkey house with the expectation that it is consistently used, and limiting the introduction of people and materials onto the farms are all steps that can be taken toward this aim.

REFERENCES

Agunos, A., Waddell, L., Léger, D., & Taboada, E. (2014). A systematic review characterizing on-farm sources of *Campylobacter* spp. for broiler chickens. *PloS One*, *9*(8), e104905. http://doi.org/10.1371/journal.pone.0104905

Arsenault, J., Letellier, A., Quessy, S., Normand, V., & Boulianne, M. (2007). Prevalence and risk factors for Salmonella spp. and *Campylobacter* spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. *Preventive Veterinary Medicine*, *81*(4), 250–64. http://doi.org/10.1016/j.prevetmed.2007.04.016

Berghaus, R. D., Thayer, S. G., Law, B. F., Mild, R. M., Hofacre, C. L., & Singer, R. S. (2013). Enumeration of *Salmonella* and *Campylobacter* spp. in environmental farm samples and processing plant carcass rinses from commercial broiler chicken flocks. *Applied and Environmental Microbiology*, *79*(13), 4106–4114. http://doi.org/10.1128/AEM.00836-13

Berndtson, E., Emanuelson, U., Engvall, A., & Danielsson-Tham, M. L. (1996). A 1-year epidemiological study of campylobacters in 18 Swedish chicken farms. *Preventive Veterinary Medicine*, *26*(3–4), 167–185. http://doi.org/10.1016/0167-5877(95)01008-4

Bi, P., Cameron, A. S., Zhang, Y., & Parton, K. A. (2008). Weather and notified *Campylobacter* infections in temperate and sub-tropical regions of Australia: An ecological study. *Journal of Infection*, *57*(4), 317–323. http://doi.org/10.1016/j.jinf.2008.08.004

Boysen, L., Rosenquist, H., Larsson, J. T., Nielsen, E. M., Sørensen, G., Nordentoft, S., & Hald, T. (2013). Source attribution of human campylobacteriosis in Denmark. *Epidemiology and Infection*, (2014), 1–10. http://doi.org/10.1017/S0950268813002719

Callicott, K. A., Frioriksdóttir, V., Reiersen, J., Lowman, R., Bisaillon, J. R., Gunnarsson, E., … Stern, N. J. (2006). Lack of evidence for vertical transmission of *Campylobacter* spp. in

chickens. *Applied and Environmental Microbiology*, *72*(9), 5794–5798. http://doi.org/10.1128/AEM.02991-05

Cox, N. A., Richardson, L. J., Maurer, J. J., Berrang, M. E., Fedorka-Cray, P. J., Buhr, R. J., … Doyle, M. P. (2012). Evidence for horizontal and vertical transmission in *Campylobacter* passage from hen to her progeny. *Journal of Food Protection*, *75*(10), 1896–902. http://doi.org/10.4315/0362-028.JFP-11-322

European Food Safety Authority. (2011). Scientific opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal*, *9*(4), 1–141. http://doi.org/10.2903/j.efsa.2011.2105.

Gibbens, J. C., Pascoe, S. J. S., Evans, S. J., Davies, R. H., & Sayers, a. R. (2001). A trial of biosecurity as a means to control *Campylobacter* infection of broiler chickens. *Preventive Veterinary Medicine*, *48*, 85–99. http://doi.org/10.1016/S0167-5877(00)00189-6

Hald, B., Skovgård, H., Bang, D. D., Pedersen, K., Dybdahl, J., Jespersen, J. B., & Madsen, M. (2004). Flies and *Campylobacter* Infection of Broiler Flocks. *Emerging Infectious Diseases*, *10*(8), 1490–1492. http://doi.org/10.3201/eid1008.040129

Hansson, I., Vågsholm, I., Svensson, L., & Olsson Engvall, E. (2007). Correlations between *Campylobacter* spp. prevalence in the environment and broiler flocks. *Journal of Applied Microbiology*, *103*(3), 640–649. http://doi.org/10.1111/j.1365-2672.2007.03291.x

Hopkins, R. S., & Scott, A. S. (1983). Handling Raw Chicken as a Source for Sporadic *Campylobacter jejuni* Infections. *Jounral of Infectious Diseases*, *148*(4), 770.

Jacobs-Reitsma, W. F., van de Giessen, A. W., Bolder, N. M., & Mulder, R. W. (1995). Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. *Epidemiology and Infection*, *114*(3), 413–21. http://doi.org/10.1017/S0950268800052122

Jonsson, M. E., Chriél, M., Norström, M., & Hofshagen, M. (2012). Effect of climate and farm environment on *Campylobacter* spp. colonisation in Norwegian broiler flocks. *Preventive Veterinary Medicine*, *107*(1–2), 95–104. http://doi.org/10.1016/j.prevetmed.2012.05.002

Jore, S., Viljugrein, H., Brun, E., Heier, B. T., Borck, B., Ethelberg, S., … Hofshagen, M. (2010). Trends in *Campylobacter* incidence in broilers and humans in six European countries, 1997–2007. *Preventive Veterinary Medicine*, *93*(1), 33–41. http://doi.org/10.1016/j.prevetmed.2009.09.015

Kapperud, G., Skjerve, E., Bean, N. H., Ostroff, S. M., & Lassen, J. (1992). Risk factors for sporadic *Campylobacter* infections: Results of a case- control study in southeastern Norway. *Journal of Clinical Microbiology*, *30*(12), 3117–3121.

Line, J. E. (2006). Influence of relative humidity on transmission of *Campylobacter jejuni* in broiler chickens. *Poultry Science*, *85*, 1145–1150. Retrieved from https://watermark.silverchair.com/api/watermark?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAfkwggH1BgkqhkiG9w0BBwagggHmMIIB4gIBADCCAdsGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMavz4evRSNX_dBjk1AgEQgIIBrA0Q9HHzkTXmwr0Vm4a3AAvxNxDhiEyEgHg2i27v33MDI

Logue, C. M., Sherwood, J. S., Elijah, L. M., Olah, P. A., & Dockter, M. R. (2003). The incidence of *Campylobacter* spp. on processed turkey from processing plants in the midwestern United States*. *Journal of Applied Microbiology*, *95*(2), 234–241. http://doi.org/10.1046/j.1365-2672.2003.01969.x

Nauta, M., Johannessen, G., Laureano Adame, L., Williams, N., & Rosenquist, H. (2016). The effect of reducing numbers of *Campylobacter* in broiler intestines on human health risk. *Microbial Risk Analysis*. http://doi.org/10.1016/j.mran.2016.02.001

Newell, D. G., Elvers, K. T., Dopfer, D., Hansson, I., Jones, P., James, S., … Allen, V. M. (2011). Biosecurity-based interventions and strategies to reduce *Campylobacter* spp. on poultry farms. *Applied and Environmental Microbiology*, *77*(24), 8605–8614. http://doi.org/10.1128/AEM.01090-10

Nichols, G. L. (2005). Fly transmission of *Campylobacter*. *Emerging Infectious Diseases*, *11*(3), 361–364. http://doi.org/10.3201/eid1103.040460

Nylen, G., Dunstan, F., Palmer, S. R., Andersson, Y., Bager, F., Cowden, J., … Ruutu, P. (2002). The seasonal distribution of *Campylobacter* infection in nine European countries and New Zealand. *Epidemiol Infect*, *128*(3), 383–390. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12113481

World Health Organization. (2012). The global view of campylobacteriosis: report of an expert consultation. World Health Organization, Utrecht, The Netherlands. *http://apps.who.int/iris/bitstream/10665/80751/1/9789241564601_eng.pdf*

Patrick, M. E., Christiansen, L. E., Wainø, M., Ethelberg, S., Madsen, H., & Wegener, H. C. (2004). Effects of climate on incidence of *Campylobacter* spp. in humans and prevalence in broiler flocks in Denmark. *Applied and Environmental Microbiology*, *70*(12), 7474–80. http://doi.org/10.1128/AEM.70.12.7474-7480.2004

Potter, R. C., Kaneene, J. B., & Hall, W. N. (2003). Risk factors for sporadic *Campylobacter jejuni* infections in rural michigan: A prospective case–control study. *American Journal of Public Health*, *93*(12), 2118–2123. http://doi.org/10.2105/AJPH.93.12.2118

Rosef, O., & Kapperud, G. (1983). House flies (Musca domestica) as possible vectors of *Campylobacter fetus* subsp. *jejuni*. *Applied and Environmental Microbiology*, *45*(2), 381–

383. Retrieved from http://aem.asm.org.prox.lib.ncsu.edu/content/45/2/381.short

Royden, a, Wedley, a, Merga, J. Y., Rushton, S., Hald, B., Humphrey, T., & Williams, N. J. (2016). A role for flies (Diptera) in the transmission of *Campylobacter* to broilers? *Epidemiology and Infection*, *144*(15), 3326–3334. http://doi.org/10.1017/S0950268816001539

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., … Griffin, P. M. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. http://doi.org/10.3201/eid1701.091101p1

Shane, S. M., Montrose, M. S., & Harrington, K. S. (1985). Transmission of *Campylobacter jejuni* by the Housefly (Musca domestica). *Source: Avian Diseases*, *29*(2), 384–391. Retrieved from http://www.jstor.org/stable/1590499

Smith, K., Reimers, N., Barnes, H. J., Lee, B. C., Siletzky, R., & Kathariou, S. (2004). *Campylobacter* colonization of sibling turkey flocks reared under different management conditions. *Journal of Food Protection*, *67* (7), 1463–1468.

Smith, S., Meade, J., Gibbons, J., McGill, K., Bolton, D., & Whyte, P. (2016). The impact of environmental conditions on *Campylobacter jejuni* survival in broiler faeces and litter. *Infection Ecology & Epidemiology*, *6*(0), 31685. http://doi.org/10.3402/iee.v6.31685

Sommer, H. M., Hog, B. B., Larsen, L. S., Sorensen, A. I. V, Williams, N., Merga, J. Y., … Rosenquist, H. (2016). Analysis of farm specific risk factors for *Campylobacter* colonization of broilers in six European countries. *Microbial Risk Analysis*, *2–3*, 16–26. http://doi.org/10.1016/j.mran.2016.06.002

Tam, C. C., Rodrigues, L. C., O'Brien, S. J., & Hajat, S. (2006). Temperature dependence of reported *Campylobacter* infection in England, 1989-1999. *Epidemiol Infect*, *134*(1), 119–

125. http://doi.org/10.1017/S0950268805004899

US Food and Drug Administration. (2014). *National Antimicrobial Resistance Monitoring System: Integrated Report: 2014*. Retrieved from https://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm059103.htm

van de Giessen, A., Mazurier, S. I., Jacobs-Reitsma, W., Jansen, W., Berkers, P., Ritmeester, W., & Wernars, K. (1992). Study on the epidemiology and control of *Campylobacter jejuni* in poultry broiler flocks. *Appl. Environ. Microbiol.*, *58*(6), 1913–1917. Retrieved from http://aem.asm.org/cgi/content/abstract/58/6/1913

van de Giessen, a W., Bloemberg, B. P., Ritmeester, W. S., & Tilburg, J. J. (1996). Epidemiological study on risk factors and risk reducing measures for *Campylobacter* infections in Dutch broiler flocks. *Epidemiology and Infection*, *117*(2), 245–250. http://doi.org/10.1017/S0950268800001412

Wagenaar, J.A. Newell, D.G., Kalupahana, R.S., Mughini-Gras, L. (2015). *Zoonoses-Infections Affecting Humans and Animals: Focus on Public Health Aspects*. Retrieved from: http://doi.org/10.1007/978-94-017-9457-2

Wallace, J. S., Stanley, K. N., Currie, J. E., Diggle, P. J., & Jones, K. (1997). Seasonality of thermophilic *Campylobacter* populations in chickens. *Journal of Applied Microbiology*, *82*(2), 219–224. http://doi.org/DOI 10.1111/j.1365-2672.1997.tb02854.x

Ward, D., & Lachance, S. (2015). *House Fly Control in Poultry Barns*. Ontario Ministry of Agriculture, Food and Rural Affairs. *www.omafra.gov.on.ca/english/engineer/housefly.pdf*

Williams, R. E., & Entomologist, E. (2010). Control of Poultry Pests. *Livestock & Poultry*. Retrieved from https://extension.entm.purdue.edu/publications/E-3.pdf

Wright, S. L., Carver, D. K., Siletzky, R. M., Romine, S., Morrow, W. E. M., & Kathariou, S. (2008). Longitudinal study of prevalence of *Campylobacter jejuni* and *Campylobacter coli* from turkeys and swine grown in close proximity. *Journal of Food Protection*, *71*(9), 1791–1796.

Table 5-1 Average temperature (°C) for each month

Month	Average Temperature (°C)
1	5.43
2	5.04
3	10.71
4	16.92
5	20.84
6	25.30
7	26.09
8	24.65
9	22.37
10	17.05
11	11.83
12	11.35

Table 5-2 Two-by-two table of the number of positive and negative flocks per season. Fisher's exact test resulted in a p-value of 4.049e-05 indicating a strong association between season and flock status

	Negative	Positive
Cold	40	111
Hot	9	149

Table 5-3 Table of univariate analyses

Variable	Odds Ratio	95% CI	p-value
Average Air Temperature (3 weeks)	1.17	1.11-1.24	6.530E-08
Average Air Temperature (5 weeks)	1.19	1.12-1.27	3.330E-08
Average wind speed (3 weeks)	0.392	0.19-0.73	0.00593
Average wind speed (5 weeks)	0.414	0.17 - 0.88	0.03180
Amount of rain measured in 1 hour increments (3 weeks)	1	1.0-1.01	0.27300
Amount of rain measured in 1 hour increments (5 weeks)	1	1.0-1.01	0.33600
Amount of rain measure in 6 hour increments (3 weeks)	1.01	1.0-1.01	0.17900
Amount of rain measure in 6 hour increments (5 weeks)	1	1.0-1.01	0.30200
Number of days it rained per flock	0.95	0.87 - 1.03	0.26600
How much of the sky is covered (3 weeks)	3.9	1.87 - 9.94	0.00098
How much of the sky is covered (5 weeks)	4.16	1.87 - 9.42	0.00077
Average dew point temperature (3 weeks)	1.16	1.10 - 1.23	3.900E-08
Average dew point temperature (5 weeks)	1.18	1.12 - 1.26	1.850E-08

Table 5-4 Spearman's rank correlation between all variables

	Avg_Sky5	dTemp3	mTemp3	Rain6	Avg_Sky3	Avg_Wind3	n	Rain1	mTemp5	Avg_Wind5	dTemp	Rain6_5	Rain1_5
Avg_Sky5	1.00	0.41	0.42	0.10	**0.93**	-0.09	0.06	0.12	0.39	-0.06	0.36	0.17	0.16
dTemp3	0.41	1.00	**0.98**	0.13	0.36	-0.44	0.03	0.16	**0.98**	-0.43	**0.98**	0.17	0.18
mTemp3	0.42	0.98	1.00	0.13	0.38	-0.39	-0.01	0.16	**0.98**	-0.37	**0.95**	0.16	0.17
Rain6	0.10	0.13	0.13	1.00	0.11	-0.06	0.65	**0.93**	0.10	-0.03	0.09	**0.86**	**0.84**
Avg_Sky3	0.93	0.36	0.38	0.11	1.00	-0.01	0.03	0.13	0.32	0.01	0.28	0.13	0.13
Avg_Wind3	-0.09	-0.44	-0.39	-0.06	-0.01	1.00	-0.07	-0.05	-0.43	**0.94**	-0.48	-0.11	-0.09
n	0.06	0.03	-0.01	0.65	0.03	-0.07	1.00	0.68	-0.04	-0.03	-0.01	0.71	0.74
Rain1	0.12	0.16	0.16	0.93	0.13	-0.05	0.68	1.00	0.13	-0.02	0.12	**0.81**	**0.87**
mTemp5	0.39	0.98	0.98	0.10	0.32	-0.43	-0.04	0.13	1.00	-0.41	**0.98**	0.14	0.15
Avg_Wind5	-0.06	-0.43	-0.37	-0.03	0.01	0.94	-0.03	-0.02	-0.41	1.00	-0.47	-0.07	-0.05
dTemp	0.36	0.98	0.95	0.09	0.28	-0.48	-0.01	0.12	0.98	-0.47	1.00	0.14	0.15
Rain6_5	0.17	0.17	0.16	0.86	0.13	-0.11	0.71	0.81	0.14	-0.07	0.14	1.00	**0.94**
Rain1_5	0.16	0.18	0.17	0.84	0.13	-0.09	0.74	0.87	0.15	-0.05	0.15	0.94	1.00

Table 5-5 Results of multilogistic regression of weather variables

	Estimate	Standard Error	Odds Ratio	95% CI	p-value
(Intercept)	0.19951	0.38065			0.6002
dew temperature	0.16363	0.03079	1.18	1.13 - 1.26	1.07E-07
Average Sky Coverage	0.89187	0.33036	2.44	1.36 - 5.05	0.00694

Table 5-6 Results of analysis of variance of the mean of each weather variable between the two seasons, hot and cold

Response	DF	Sum of Squares	Mean Square	F-value	P-value
Air Temperature					
Season	1	10316573	10316573	218402	< 2.2e-16 ***
Residuals	262438	12396665	47		
Dew Temperature					
Season	1	11816351	11816351	232888	< 2.2e-16 ***
Residuals	262300	13308691	51		
Wind Speed					
Season	1	12104	12103.7	3346.8	< 2.2e-16 ***
Residuals	263136	951637	3.6		
Sky Coverage					
Season	1	1020	1020.40	245.55	< 2.2e-16 ***
Residuals	171777	713845	4.16		
Response: Rainfall (as measured in 6 hour increments)					
Season	1	2	1.7592	1.38	0.2401
Residuals	263208	335529	1.2748		

Table 5-7 Results of univariate analyses from risk factor analysis of flock-level factors

Variable	Result	Positive	Negative	Odds Ratio	95% CI	P-value
Are visitors required to wear plastic overboots?	Yes No	50 88	9 5	0.32	0.81 – 8.03	0.040
Do you loan tools to other farms?	Yes No	48 76	2 11	3.47	0.74 – 16.4	0.096
Who is responsible for removing litter?	Outside contractor Owner/manager Poultry company	116 13 9	8 1 5	1.11 8.06	0.13 – 9.64 2.18 – 29.9	0.92 0.00038
Fog Fly	Yes No	45 93	8 6	0.36	0.12 – 1.11	0.066
Do you hire outside employees	Yes No	96 42	7 7	2.29	0.75 – 6.93	0.14
Are repairmen allowed in the turkey houses?	Yes No	69 69	10 4	0.4	0.12 – 1.34	0.13
Are the doors to the turkey house locked when no one is inside?	Yes No	30 108	6 8	0.37	0.12 – 1.15	0.077
Is hand sanitizer provided at each turkey house?	Yes No	22 116	6 8	0.25	0.08 – 0.80	0.013
Are the houses fumigated between flocks?	Yes No	28 110	6 8	0.34	0.11 – 1.06	0.054
Do you have family that works with poultry or keeps a pet bird?	Yes No	39 89	7 6	0.38	0.12 – 1.19	0.087
What kind of barrier is on your farm?	None Entire perimeter	134 4	10 4	13.4	2.91 – 61.7	0.000042
Are family members allowed in the houses?	Yes No	50 88	9 5	0.32	0.11 – 1.17	0.040
Are entrances gated and kept closed?	Yes No	23 115	5 9	0.36	0.11 – 1.17	0.080

Table 5-8 Results of multivariate mixed analysis

	Estimate	Std. Error	Z value	OR	p-value
(Intercept)	-1.0086	0.9208	-1.095	-	0.27336
Season - Hot	3.0784	1.0932	2.816	21.72	0.00486 **
Barrier - None	2.7387	0.9636	2.842	8.47	0.00448 **

Table 5-9 Results of Final mixed model

	Estimate	Std. Error	Z value	OR	p-value
(Intercept)	1.4628	0.3678	3.977	-	6.98e-05 ***
Season - Hot	3.0150	1.0532	2.863	20.39	0.0042 **

Chapter 6 Outreach to Increase Knowledge about *Campylobacter*-associated Foodborne Illness

SUMMARY

Campylobacter is one of the top foodborne illness-causing pathogens worldwide. In order to assess knowledge gaps regarding *Campylobacter* in young adults, an assessment was distributed to students taking a Food Microbiology course. The results of this assessment indicated that 67.5% of students had heard of *Campylobacter*. Of the 68 students indicating they had heard of *Campylobacter,* 63 knew it was a bacterium. Nearly half (44.6%) of students knew that poultry was commonly associated with *Campylobacter*-related illness, however 23% of students erroneously chose produce as another risky food item. When asked where they heard about foodborne outbreaks, the greatest number of students (62.1%) responded that they heard about foodborne outbreaks via word of mouth which includes social media. To share information regarding *Campylobacter* with other researchers and food safety professionals, outreach programs were created. A Twitter campaign was started in November 2015 under the handle "CampyPack" and as of November 2017, the campaign had gathered 230 followers and had made nearly 30,000 impressions. A website, campylobacter.fbns.ncsu.edu, was created in March 2016 to try to reach poultry producers, consumers, and food safety professionals and researchers. Content relevant to consumers and poultry producers had garnered 3,100 page views as of October 2017. Finally, multiple articles were written and published in poultry trade journals and food safety magazines to educate poultry producers on what they can do to reduce the risk of their flocks carrying this organism. Collectively, these efforts worked to increase the public, the industry, and researchers' knowledge of *Campylobacter.*

INTRODUCTION

Campylobacter is one of the five foodborne illness-causing pathogens worldwide (Elaine Scallan et al., 2011). Despite this, it is one of the least well-known foodborne pathogens with recent estimates indicating that only 7% of U.S. consumers have even heard of this pathogen compared to approximately 94% and 90% for *Salmonella* and *E. coli,* respectively (Jordan Lin, Jensen, & Yen, 2005). This is despite the fact that *Campylobacter* is responsible for more illnesses (90% CI=845,000) per year than *E. coli* (90% CI=205,000) and nearly as many as *Salmonella* (90% CI=1,027,500) (Elaine Scallan et al., 2011). Approximately 17% of campylobacteriosis cases require hospitalization, and illness may be followed by severe sequelae such as reactive arthritis or Guillaine-Barre syndrome (Elaine Scallan et al., 2011; Yang et al., 2016; Yuki & Hartung, 2012). However, unlike *E. coli* and *Salmonella* which have made recent headlines for major outbreaks or food recalls, *Campylobacter* is generally associated with sporadic outbreaks which possibly receive less media attention (Centers for Disease Control and Prevention, 2014a). Previous research has indicated that having heard of *E. coli* or *Campylobacter* was associated with less risky behavior such as serving more well-done hamburgers (Jordan Lin et al., 2005). The goals of this project were to evaluate a group of university students for their knowledge regarding *Campylobacter* as well as investigating where these students received information about food safety. At the same time, we sought to increase awareness and elevate the knowledge of *Campylobacter* in the public and in poultry producers by initiating an outreach campaign in the form of a website, a social media campaign, and publishing in food safety or poultry-oriented magazines.

MATERIALS AND METHODS

Assessment design and analysis

An assessment was created as the collective effort of NCSU professors from the Food Science and Communication departments and graduate students in the Food Science department. It was distributed to two classes of students taking the Food Microbiology course at North Carolina State University to assess their knowledge of *Campylobacter*. The class was composed mostly of students majoring in Food Science (denoted FS) and Microbiology (MB) enrolled as either an undergraduate (405) or graduate (505) student. There were some differences between the assessments distributed in 2016 and 2017 which will be explained below. The response rate for the assessment was determined as the number of assessments collected from the number that were sampled. This response rate is the result of enrollment data which may not accurately reflect the enrollment numbers at the time the assessment was distributed, however it is the closest estimate possible from the existing data

The first question on the assessment asked the students if they had ever heard of *Campylobacter.* If participants responded "yes" to the first question, they were then asked what percentage of foodborne illnesses *Campylobacter* is responsible for. In the second year of the assessment the ability to respond "unsure" was included. Next, the participants were asked if *Campylobacter* was a virus, a bacterium, or chemical, and again the opportunity to respond "unsure" was included in the assessment's second year. The participants were then asked where one would be most likely to come into contact with *Campylobacter* with the ability to choose as many answers as they wished. The possible responses were: "raw poultry", "eggs", "cheese", "fresh produce", "unpasteurized milk", or "a sick friend", and

again the ability to respond "unsure" was included in the second year. Finally, participants were asked where they get information about foodborne outbreaks with the option to respond: "TV news", "print news", "radio talk shows", "social media government websites", "other websites", "word of mouth", "other", or "I never heard about foodborne outbreaks or recalls". In the second year, the options of "social media", "other", and "word of mouth" were combined into one response labeled "word of mouth, (e.g. social media)" and the option to respond "radio talk show" was discarded as a response. Responses were analyzed using Excel and R.

An assessment of a public communication outreach effort which comprised two components (Twitter and website)

To share information regarding *Campylobacter* with other researchers and food safety professionals, outreach programs were created, which comprised two components, Twitter and a website. Twitter was used to target the general public as well as other researchers interested in food safety, particularly *Campylobacter*. This was done as a means of engaging other researchers to further highlight and promote conversations and issues relevant to this research project. The Twitter campaign, under the handle @CampyPack, was launched in November 2015 and was used to retweet information about ongoing outbreaks, federal and state public health news, recent research findings, as well as general information about *Campylobacter.* The efficacy of the twitter campaign was determined through the retweets our posts received. There are numerous paid options to determine these statistics, however we used the free version from www.retweetrank.com. Additionally, Twitter was used as a

way to bring an audience to the website that was also started as part of the outreach campaign. Images were used for tweets were either taken in our lab, sourced from government websites, or pulled from the copyright-free image site www.unsplash.com. Twitter Analytics was used to track the success of tweets. A website to disseminate information and news regarding *Campylobacter* was developed through WordPress with a theme provided by North Carolina State University. Search engine optimization was enhanced using the plugin Yoast SEO and the traffic to the website was monitored using Google Analytics. Articles were written by undergraduate and graduate students in Dr. Sophia Kathariou's laboratory.

Outreach articles in trade magazines

Four articles (appendices 1-4) highlighting campylobacteriosis, routes of transmission, and antibiotic resistance were published in various food technology and safety, poultry, and university online publications. These articles were mainly published in venues that target food industry professionals and specifically poultry-focused professionals.

RESULTS AND DISCUSSION

Most students have heard of *Campylobacter* but have knowledge gaps

Of the 101 (56 in 2016 and 45 in 2017) students who participated in the assessment there was an average estimated response rate of 96.4% for the two years. A yearly average percent of 67.5%) indicated that "yes", these students had heard of *Campylobacter* (figure 1*)*. One student did not respond to this question although they did provide answers for all follow up

questions. The data was further divided and Fisher's Exact tests were performed to see if the students' major (Food Science or Microbiology) or rank (undergraduate or graduate) affected the outcome of having heard of this pathogen. There was no statistically significant effect from major (food science or microbiology) or status (graduate vs. undergraduate) ($p=0.3716$ and 0.2808, respectively).

All the 68 students that responded that they had heard of *Campylobacter* and the student that skipped the first question responded to the follow up question asking what *Campylobacter* actually is (figure 2). The question was correctly answered as bacterial by 90.6% of the 69 respondents. Responses of "virus" and "unsure" were selected by 4.5% and 4.8% of participants. A little more than half (59.5%) of the students wrongly responded that they believed that *Campylobacter* was responsible for 25% of foodborne outbreaks. The correct response of 9% was selected by 21.3% of the assessment participants. The remaining choices of 3% and 58% were selected by 9.4% and 4.1% of students, respectively. The ability to state that they were unsure of the correct response was only included in the second year of the assessment, however it still accounted for 12.9% of responses.

"Poultry" was correctly selected as a likely agent in campylobacteriosis by 44.6% of respondents as was "raw milk" by 19.5% (figure 5). However, "eggs", "cheese", "produce", and "a sick friend" were mistakenly selected by 16.6%, 8.5%, 23.0%, and 3.2% of assessment participants, respectively. Again, the ability to choose "unsure" was only included in the assessment's second year and was selected by 9.7% of participants.

The results of the assessments indicate that the majority (67.5%) of participants have heard of *Campylobacter* which is well above a recent estimate which indicates that only 7%

of the American public has heard of *Campylobacter* (Jordan Lin et al., 2005). It may not necessarily be surprising that so many of the students taking Food Microbiology have heard of *Campylobacter.* These students have usually taken a general microbiology course by the time they enter this class, and they could have been exposed to common foodborne pathogens at that time or during discussions in other food science classes. One might also assume that these students would have an interest in foodborne pathogens.

It does appear that students are overestimating the burden of reported foodborne diseases from *Campylobacter* spp. in the U.S and do not have a clear understanding of the routes of transmission of this and possibly other enteric pathogens. As this assessment was administered at the beginning of the semester it may be useful to professors in identifying gaps in students' knowledge regarding *Campylobacter* or the routes of transmission of this and other enteric pathogens that are transmitted via the fecal-oral route through the food chain. For example, students should understand that Norovirus is responsible for many more illnesses than *Campylobacter* or any of the other bacterial pathogens.

Students get most of their information regarding foodborne outbreaks from social media or word of mouth

Both students who had and had not heard of *Campylobacter* were asked where they received information of foodborne outbreaks. Because the responses "word of mouth" and "social media" were separate in the assessment's first year, but combined in the second year, these two responses were combined into a single response for all data. The results of the assessment indicate that 62.1% of the students use this combined response to get information

of foodborne outbreaks (figure 6). The next most popular response was "TV" (51.4%) followed by "other websites" (28.3%), "print news" (18.7%), and "government websites" (13.2%). Only one of the 101 responses collected indicated that they never hear about outbreaks. Since *Campylobacter* is rarely responsible for large outbreaks, it may not make headline news on social media or TV which is where these young participants were getting much of their outbreak information. Since these participants had likely taken microbiology and many were in the Food Science program, they likely represent an over-estimation of the general public's knowledge. Additionally, the results of the assessment suggested that social media could be a good way to communicate food safety messages to this population.

Twitter campaign results

Since beginning the @Campypack Twitter campaign in November 2015, 112 original tweets have been sent out as well as retweeting 63 times. Additionally, 40 photos and videos were included in the original tweets. As of October 26, 2017, 230 twitter users were following @Campypack, and the twitter campaign had made 29,965 impressions. On average, 4.9 tweets were posted every month resulting in an average 1,302.8 monthly impressions. As would be expected there does appear to be a general trend of increasing impressions with increasing tweets (figure 7). While other food safety microbiologists and technology companies did re-tweet and "like" our content, there was not a lot of interaction with the general public or poultry producers as far as the authors could tell. It can be difficult to tell what impact tweets have on their audience, however tracking the number of re-tweets is one method to do so.

The results from www.retweetrank.com indicated that @campypack's rank was 557,709, so @campypack was the 557,709th most influential twitter handle on Twitter of the 326 million active users on Twitter as reported by the company after determining they had been overestimating their number of monthly users (Bray, 2017). The retweet rank percentile was determined by www.retweetrank.com to be 92.81%, however more specific data regarding retweets was unobtainable in the free version of the service. Retweet rank percentile is determined as such:

$$\frac{(Total\ Users\ on\ Retweet\ Rank - User\ Rank) * 100}{Total\ Users\ on\ Retweet\ Rank}$$

In the future, a social media campaign would likely greatly benefit from paid advertisements and promotions. There are also additional social media venues such as Facebook, or even email campaigns through MailChimp which could be utilized.

Website results

The website was started in March 2016, and since that time has had more than 3,100 page views by 1,169 users. Within these 1,169 users there has been a 60.9% bounce rate. Google defines the bounce rate as "a session that triggers only a single request to the Analytics server, such as when a user opens a single page on the site and then exits without triggering any other requests to the Analytics server during that session" (Google Analytics, 2017). This means that the user navigated away from the website from the page that they landed on

without further exploring the website. However approximately 26.9% of the users to the website were recurring and a user spent an average of 2 minutes, 5 seconds on the site. The most successful article on the website has been a post written about whole-genome sequencing of *Campylobacter* *(https://campylobacter.fbns.ncsu.edu/2016/10/09/campylobacter-wgs-review/)* followed by one about antibiotic resistance with an additional focus on *Clostridium difficile* *(https://campylobacter.fbns.ncsu.edu/2017/02/08/antibiotic-resistance-trifecta/).* The majority of users to the website were either from the U.S. (39.5%) or the United Kingdom (21.2%). The remaining countries in the top ten were Russia, South Korea, Brazil, Canada, Germany, Poland, and not defined. The website may have been more successful with paid promotions and more knowledgeable administrators. Search engine optimization, which is key for bringing people to the site, was a challenge on the website although the Yoast plug-in was helpful.

Trade journal publications

The first article, "The Bugs and Drugs in Your Birds", was published in March 2015 in the food technology journal New Foods and sought to educate those in animal agriculture about the routes of *Campylobacter* transmission through the food chain and possible food safety implications of antibiotic resistance. The second article, "Food Safety 101: What is *Campylobacter* (and What Are We Doing About It)?" was published in North Carolina State University's own NC State News and was picked up by Foodsafetynews.com. This article brought attention to research being done at North Carolina State University as well as

discussing the epidemiology, routes of transmission, and best ways to prevent illness with *Campylobacter*. The third article was published in the magazine MeatingPlace which serves those in the meat and poultry processing industries. This article discussed some of the major managerial differences between *Campylobacter*-negative and *Campylobacter*-positive flocks in an attempt to education poultry producers on simple steps they can take to minimize the risk of their flocks carrying this organism. Finally, the last article was published in Poultry Times which is a magazine geared toward those in the poultry industry. The Poultry Times article discussed differences between what biosecurity practices are being recommended in the literature versus what is actually being done on farms as assessed during our on-farm assessments at commercial turkey farms. While data on the number of readers for these articles is not available, New Foods magazine estimates that they have a bi-monthly readership of approximately 34,000. Magazine editors could likely supply more data on how the article was received and who read it. More articles like these can be written in the future to continue the outreach efforts to consumers and producers.

CONCLUSIONS

While awareness of *Campylobacter* remains low, expanded outreach campaigns could decrease the gaps in knowledge between researchers, the general population, and poultry producers. With limited resources, as many as 60,000 people were exposed to information on *Campylobacter* as a result of the outreach program through Dr. Kathariou's lab, and this number could greatly increase through Twitter advertising, improved search engine

optimization, and a presence on additional social media platforms like Facebook and Instagram.

REFERENCES

Bray, C. (2017). Twitter Says It Overstated Monthly-User Figures for 3 Years. *New York Times*. Retrieved from https://www.nytimes.com/2017/10/26/technology/twitter-q3-earnings-trolls.html

Centers for Disease Control and Prevention. (2014). *Campylobacter*. Retrieved February 20, 2016, from http://www.cdc.gov/foodsafety/diseases/campylobacter/index.html

Google Analytics. (2017). Web statistics for www.campylobacter.fbns.ncsu.edu. Retrieved July 18, 2017, from https://support.google.com/analytics/answer/1009409?hl=en

Jordan Lin, C.-T., Jensen, K. L., & Yen, S. T. (2005). Awareness of foodborne pathogens among US consumers. *Food Quality and Preference*, *16*, 401–412. http://doi.org/10.1016/j.foodqual.2004.07.001

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., … Griffin, P. M. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. http://doi.org/10.3201/eid1701.091101p1

Yang, B., Lian, Y., Liu, Y., Wu, B.-Y., & Duan, R.-S. (2016). A retrospective analysis of possible triggers of Guillain–Barre syndrome. *Journal of Neuroimmunology*, *293*, 17–21. http://doi.org/10.1016/j.jneuroim.2016.02.003

Yuki, N., & Hartung, H.-P. (2012). Guillain-Barré syndrome. *The New England Journal of Medicine*, *366*(24), 2294–2304. http://doi.org/10.1056/NEJMra1114525

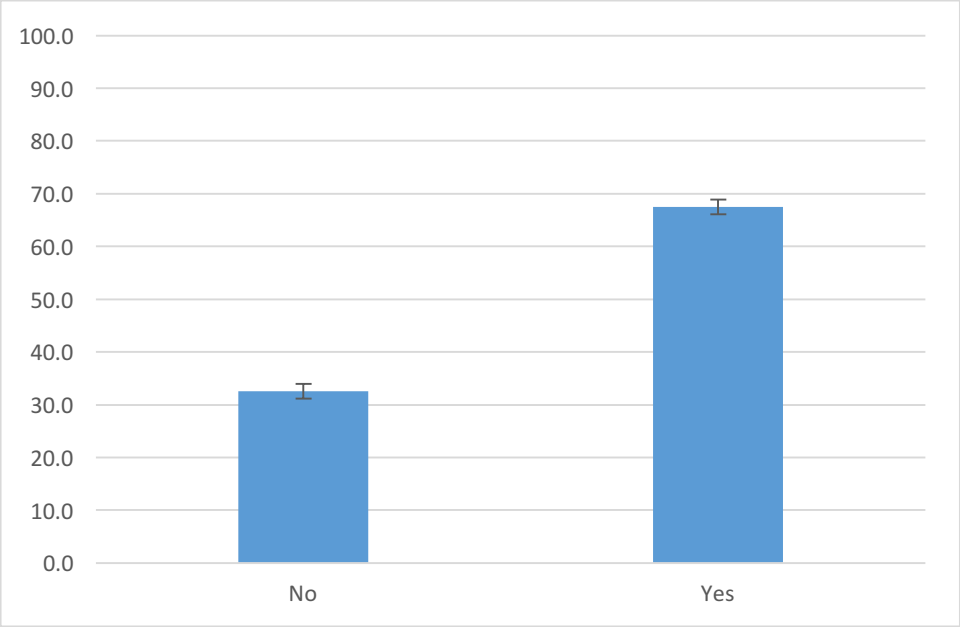


Figure 6-1. Have you heard of *Campylobacter*? Average yearly percent +/- standard deviations

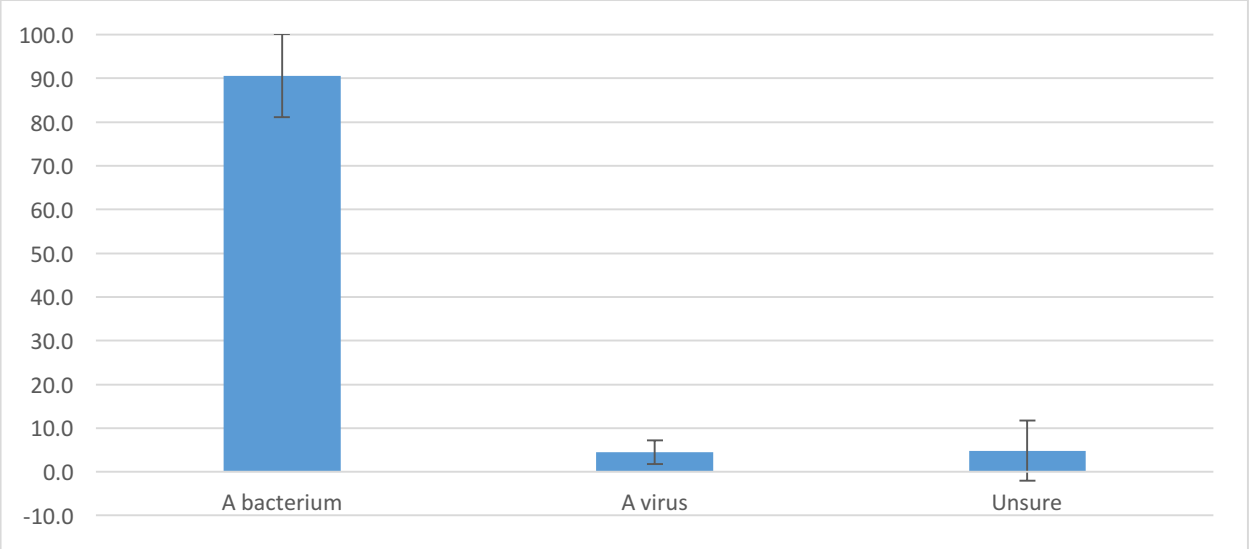


Figure 6-2. What is *Campylobacter*? Average yearly percent +/- standard deviations

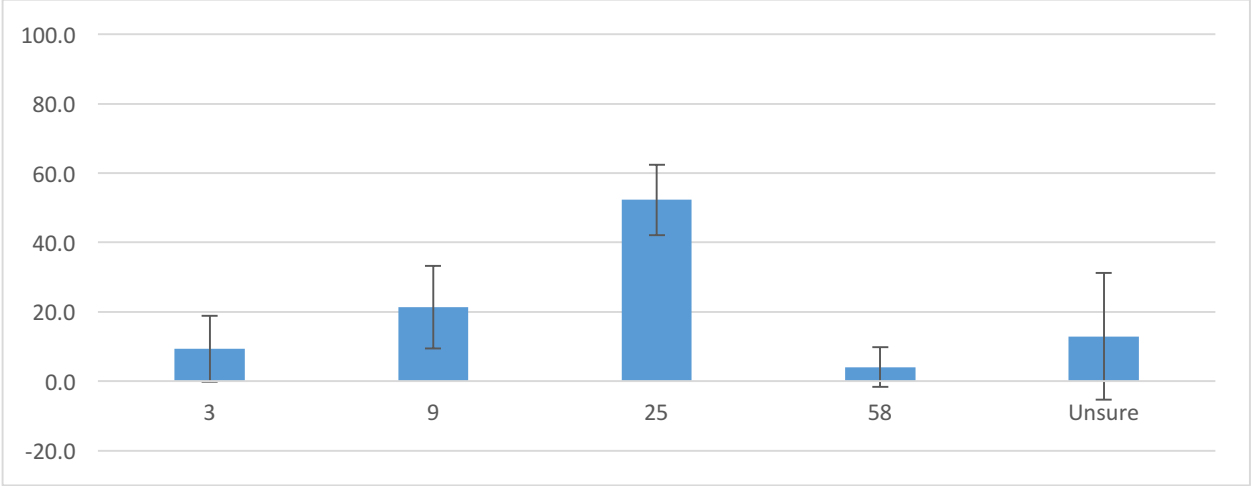


Figure 6-3. What percentage of foodborne illness is *Campylobacter* responsible for? Average yearly percent +/- standard deviations

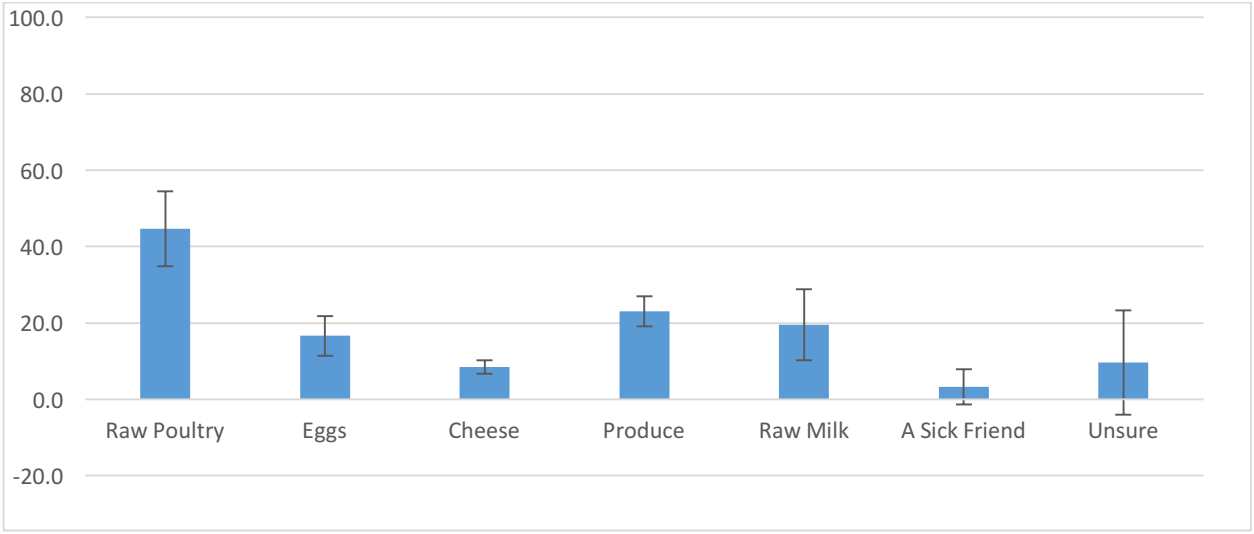


Figure 6-4. What items are most commonly implicated in cases of campylobacteriosis? Average yearly percent +/- standard deviations

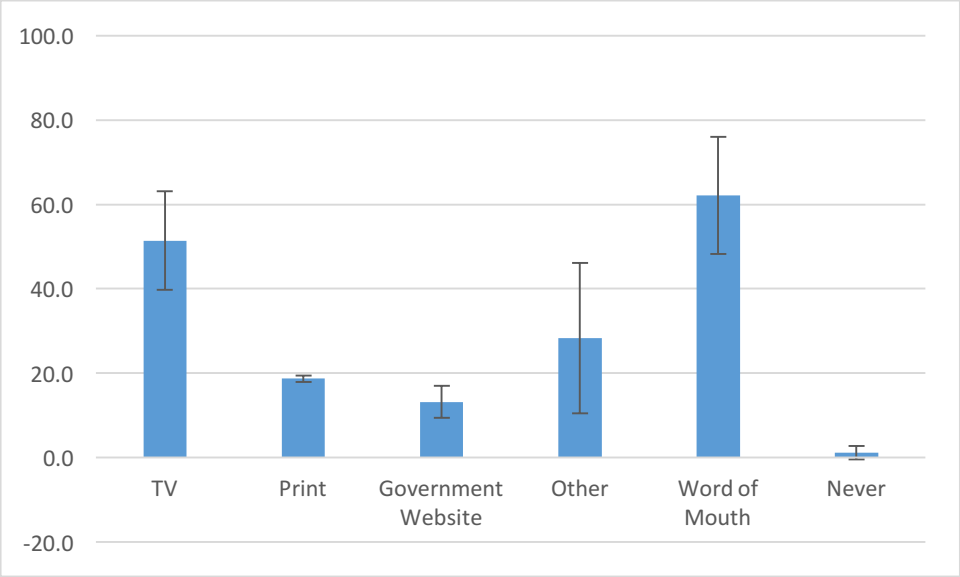


Figure 6-5. Where do you normally hear about foodborne outbreaks and recalls?

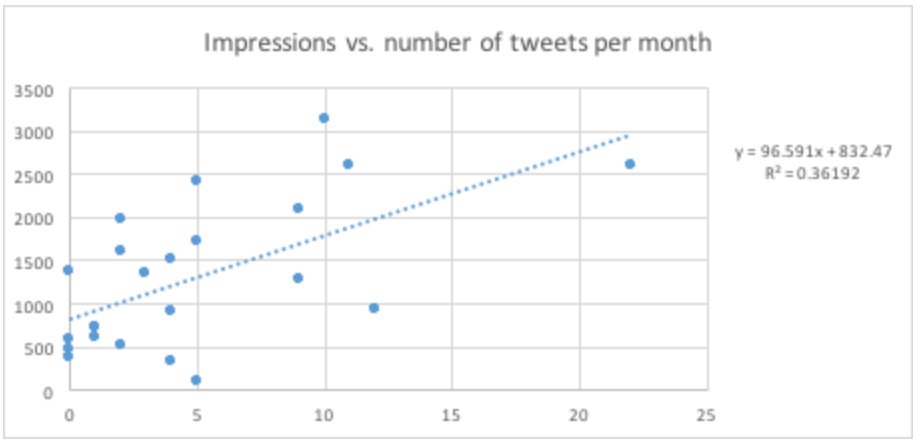


Figure 6-6. *Impressions versus the monthly number of tweets posted*

APPENDICES

Appendix A

List of isolates used in the evaluation of gentamicin resistance determinants.

ID	Received	Source	State	abbreviation	ST	Species	gene	GentR	HasGene
12548	7/6/12	fecal	NC	TKQG	NA	*C. coli*	f	R	Yes
12573	7/6/12	fecal	NC	TSKQ	NA	*C. jejuni*	-	S	NO
12590	7/6/12	fecal	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
12594	7/6/12	fecal	NC	T	NA	*C. coli*	-	S	NO
12837	8/9/12	fecal	NC	TKQ	NA	*C. jejuni*	-	S	NO
12924	9/6/12	fecal	NC	TKQG	NA	*C. coli*	f	R	Yes
13067	9/12/12	fecal	NC	TSKQ	NA	*C. jejuni*	-	S	NO

13112	9/14/12	fecal	NC	TSEKQG	NA	*C. coli*	f	R	Yes
13157	9/28/12	fecal	NC	TSEKQG	NA	*C. coli*	f	R	Yes
13271	10/18/12	fecal	NC	TKG	NA	*C. coli*	f	R	Yes
13571	12/11/12	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
13693	2/7/13	Ceca	NC	TKQG	NA	*C. coli*	f	R	Yes
13694	2/7/13	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
13761	2/28/13	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
14148-2	7/23/13	fly	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14156-1	7/26/13	fecal	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
14158-1	7/26/13	fly	NC	TS	2869	*C. coli*	-	S	NO
14159-1	7/26/13	fly	NC	TSKQG	NA	*C. coli*	f	R	Yes

14159-2	7/26/13	fly	NC	TKQG	NA	*C. jejuni*	f	R	Yes
14160-1	7/26/13	fly	NC	TKG	8224	*C. coli*	f	R	Yes
14160-2	7/26/13	fly	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14160-3	7/26/13	fly	NC	TKQG	8227	*C. jejuni*	f	R	Yes
14161-1	7/26/13	fly	NC	TKQG	1839	*C. jejuni*	f	R	Yes
14162-1	7/26/13	fly	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
14162-3	7/26/13	fly	NC	TKQG	1839	*C. jejuni*	f	R	Yes
14163-2	7/26/13	fly	NC	TKQG	NA	*C. coli*	f	R	Yes

14165-1	7/26/13	fly	NC	TKG	2934	*C. jejuni*	f	R	Yes
14166-1	7/26/13	fly	NC	TKQG	NA	*C. jejuni*	f	R	Yes
14167-2	7/26/13	fly	NC	TSKQG	8215	*C. jejuni*	f	R	Yes
14192-5	8/5/13	fecal	NC	TSEKQG	1101	*C. coli*	f	R	Yes
14228-4	8/14/13	fecal	NC	TKQG	1839	*C. jejuni*	f	R	Yes
14228-5	8/14/13	fecal	NC	TSKQG	8218	*C. jejuni*	f	R	Yes
14228-6	8/14/13	fecal	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14229-2	8/14/13	fecal	NC	TKQG	1192	*C. coli*	f	R	Yes

14229-5	8/14/13	fecal	NC	TKG	2934	*C. jejuni*	f	R	Yes
14229-6	8/14/13	fecal	NC	TKQG	8217	*C. coli*	f	R	Yes
14233	8/14/13	fly	NC	TKQG	1833	*C. coli*	f	R	Yes
14234	8/14/13	fly	NC	TKQG	1839	*C. jejuni*	f	R	Yes
14236	8/14/13	fly	NC	TKQG	NA	*C. coli*	f	R	Yes
14240-5	8/14/13	fecal	NC	TSKQ	1839	*C. jejuni*	-	S	NO
14240-6	8/14/13	fecal	NC	T	8219	*C. coli*	-	S	NO
14241-5	8/14/13	fecal	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
14243	8/14/13	fly	NC	TK	8220	*C. coli*	-	S	NO
14248	8/14/13	fly	NC	TKQG	NA	*C. coli*	f	R	Yes

14249	8/14/13	fly	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
14250	8/14/13	fly	NC	TKQG	NA	*C. coli*	f	R	Yes
14253-6	8/15/13	fecal	NC	TKQG	NA	*C. coli*	f	R	Yes
14327-2	9/2/13	fecal	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14329	9/2/13	fly	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14338	9/2/13	fly	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
14351-1	9/5/13	fecal	NC	TEKQG	1101	*C. coli*	f	R	Yes
14351-3	9/5/13	fecal	NC	TEKQG	NA	*C. coli*	f	R	Yes
14354	9/5/13	fly	NC	TEKQG	8212	*C. coli*	f	R	Yes
14355	9/5/13	fly	NC	TEKQG	NA	*C. coli*	f	R	Yes
14358	9/5/13	fly	NC	TEKQG	NA	*C. coli*	f	R	Yes

14385-3	9/13/13	fecal	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14385-4	9/13/13	fecal	NC	TKQG	NA	*C. coli*	f	R	Yes
14386-1	9/13/13	fecal	NC	TSEKQG	NA	*C. coli*	f	R	Yes
14409-1	9/19/13	fecal	NC	TKQG	NA	*C. jejuni*	f	R	Yes
14410-5	9/19/13	fecal	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14413	9/19/13	fly	NC	TSEKQG	NA	*C. coli*	f	R	Yes
14521-3	10/14/13	fecal	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14521-6	10/14/13	fecal	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14522-1	10/14/13	fecal	NC	TSKQG	NA	*C. jejuni*	f	R	Yes

14584-2	10/23/13	fly	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14750	12/6/13	Ceca	NC	TKQG	NA	*C. coli*	f	R	Yes
14945 E	5/16/14	Ceca	NC	T	7634	*C. jejuni*	-	S	NO
14946 D	5/16/14	Ceca	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
14948C	5/16/14	Ceca	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
14952A	5/30/14	Ceca	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
14955A	6/20/14	Ceca	NC	TKG	2934	*C. jejuni*	f	R	Yes
14956F	6/20/14	Ceca	NC	TSKQG	1839	*C. jejuni*	f	R	Yes

14963A	7/23/14	Ceca	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
14969A	8/22/14	Ceca	NC	TSEKQG	1149	*C. coli*	f	R	Yes
14974A	8/29/14	Ceca	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
14975D	9/5/14	Ceca	NC	TSEKQG	1067	*C. coli*	f	R	Yes
14977A	9/17/14	Ceca	NC	TEKQG	1161	*C. coli*	f	R	Yes
14987E	7/17/14	fecal	NC	TSKQG	NA	*NA*	f	R	Yes
14988E	7/17/14	fecal	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14995A	7/17/14	fly	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
14997C	7/30/14	fecal	NC	TSKQG	NA	*C. jejuni*	f	R	Yes

14998B	7/30/14	fecal	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15025A	9/10/14	fly	NC	TSEKQG	1017	*C. coli*	f	R	Yes
15030-B	9/30/14	Ceca	AR	TKG	939	*C. jejuni*	g	R	Yes
15062C	10/8/14	Ceca	NC	TSEKQG	7728	*C. coli*	f	R	Yes
15065A	10/8/14	Ceca	NC	TEKQG	7729	*C. jejuni*	f	R	Yes
15081E	10/15/14	Ceca	NC	TSEKQG	1101	*C. coli*	f	R	Yes
15090B	10/21/14	Ceca	NC	TQ	2083	*C. jejuni*	-	S	NO
15093C	10/22/14	fecal	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15093F	10/22/14	fecal	NC	TSKQG	7730	*C. jejuni*	f	R	Yes
15095A	10/22/14	fly	NC	TEKQG	8213	*C. coli*	f	R	Yes

15098A	10/22/14	fly	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
15101A	10/22/14	fly	NC	T	2934	*C. jejuni*	-	NA	NO
15103B	10/22/14	fly	NC	TKQG	1192	*C. coli*	f	R	Yes
15163B	12/3/14	Ceca	AR	TKQG	NA	*C. coli*	g	R	Yes
15170B	12/3/14	Ceca	AR	TKG	NA	*C. coli*	g	R	Yes
15181A	12/10/14	Ceca	AR	TKG	NA	*C. jejuni*	-	R	NO
15196B	12/18/14	Ceca	AR	TKG	NA	*C. coli*	g	R	Yes
15199A	12/18/14	Ceca	AR	TKG	NA	*C. coli*	-	R	NO
15208B	1/6/15	Ceca	AR	TKG	NA	*C. coli*	g	R	Yes
15210E	1/8/15	Ceca	AR	TKQG	NA	*C. coli*	g	R	Yes
15213C	1/8/15	Ceca	AR	TKG	NA	*C. coli*	g	R	Yes
15215F	1/13/15	Ceca	AR	TKG	NA	*C. coli*	g	R	Yes

15323 BBE A	4/29/15	Ceca	NC	TG	NA	*C. jejuni*	f	R	Yes
15324 A	5/7/15	Ceca	NC	TKCG	NA	*C. jejuni*	f	R	Yes
15362A	5/29/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15363A	5/29/15	Ceca	NC	TSEKQG	1839	*C. jejuni*	f	R	Yes
15363B	5/29/15	Ceca	NC	TEKQG	1839	*C. jejuni*	f	R	Yes
15365 E	5/29/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15365 F	5/29/15	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
15365BBE-A	5/29/15	Ceca	NC	TKG	NA	*C. coli*	f	R	Yes

15366 A	5/29/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15366 B	5/29/15	Ceca	NC	TKQG	NA	*C. jejuni*	f	R	Yes
15367 A	6/3/15	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15368 C	6/3/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15368 E	6/3/15	Ceca	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
15368 F	6/3/15	Ceca	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
15369 A	6/3/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15370A	6/10/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15370BBE-A	6/10/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15371A	6/10/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15371BBE-B	6/10/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15373A	6/24/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15374A	6/24/15	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15375B	6/24/15	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
15376A	6/24/15	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
15377 BBE A	7/1/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15377A	7/1/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15378B	7/1/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15379 BBEA	7/1/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15379A	7/1/15	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15379B	7/1/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15380 BBEA	7/1/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15380A	7/1/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15381 BBEA	7/8/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15381A	7/8/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15382A	7/8/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15383 BBEA	7/8/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15383A	7/8/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15384 BBEA	7/8/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15385 BBEA	7/8/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15385A	7/8/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15385C	7/8/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15386A	7/15/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15386B	7/15/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15386BBE-A	7/15/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15387A	7/15/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15387BBE-A	7/15/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15388A	7/15/15	Ceca	NC	KQG	NA	*C. jejuni*	f	R	Yes
15388B	7/15/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15388BBE-A	7/15/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15389A	7/15/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15389B	7/15/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15389BBE-A	7/15/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15390A	7/15/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15391 BBE A	7/22/15	Ceca	NC	TKG	2934	*C. jejuni*	f	R	Yes
15391B	7/22/15	Ceca	NC	TKQG	NA	*C. coli*	f	R	Yes
15392A	7/22/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15392C	7/22/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15394A	7/22/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15394E	7/22/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15395A	7/22/15	Ceca	NC	TSKQG	8085	*C. jejuni*	f	R	Yes
15395C	7/22/15	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
15395D	7/22/15	Ceca	NC	TSEKQG	8086	*C. coli*	f	R	Yes

15396A	7/22/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15397 BBEA	7/29/15	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15397B	7/29/15	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15398 BBEA	7/29/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15398A	7/29/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15398C	7/29/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15399 BBEA	7/29/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes

15399C	7/29/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15400 BBEA	7/29/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15400A	7/29/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15400B	7/29/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15401 BBEA	7/29/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15401A	7/29/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15402 BBEA	7/29/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15402A	7/29/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15403 BBEA	8/12/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes

15403A	8/12/15	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15403D	8/12/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15404 BBE A	8/12/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15404 BBE B	8/12/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15404A	8/12/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15404B	8/12/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15405A	8/12/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15406A	8/12/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15408A	8/12/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15408D	8/12/15	Ceca	NC	TKQG	NA	*C. jejuni*	f	R	Yes
15411A	8/24/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15415BBE-A	8/25/15	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
15417A	8/26/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15418B	8/26/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15419A	8/26/15	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15422A	8/27/15	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
15423A	8/27/15	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
15428A	9/4/15	Ceca	NC	TKG	NA	*C. coli*	f	R	Yes
15428B	9/4/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15429D	9/4/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15436C	9/10/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15441C	9/16/15	Ceca	NC	KQG	NA	*C. jejuni*	f	R	Yes
15442A	9/16/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15443B	9/16/15	Ceca	NC	TKQG	NA	*C. jejuni*	f	R	Yes
15443C	9/16/15	Ceca	NC	TKQG	NA	*C. jejuni*	f	R	Yes
15448B	9/18/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15448E	9/18/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15449A	9/23/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15450C	9/23/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15451B	9/23/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15452A	9/30/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15453A	9/30/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15453BBE-A	9/30/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15454A	9/30/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15454BBE-A	9/30/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15456BBE-A	10/9/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15456C	10/9/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15457A	10/9/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15457BBE-A	10/9/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15458A	10/9/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15458E	10/9/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15460A	10/14/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15460BBE-B	10/14/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15461A	10/14/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15461BBE-A	10/14/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15461BBE-B	10/14/15	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15464BBE-A	10/21/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15465A	10/21/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15467C	10/21/15	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes

15467D	10/21/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15503A	12/16/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15504A	12/16/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15505A	12/16/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15506C	12/16/15	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15507A	1/7/16	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15507B	1/7/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15508A	1/7/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15509A	1/7/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15509C	1/7/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15510A	1/7/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15511A	1/7/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15512B	1/13/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15513A	1/13/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15513BBE-A	1/13/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15514A	1/13/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15515A	1/13/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15515BBE-A	1/13/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15516C	1/13/16	Ceca	NC	TSEKQG	1839	*C. jejuni*	f	R	Yes
15517A	1/20/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes

15518A	1/20/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15519A	1/20/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15519C	1/20/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15520A	1/20/16	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
15520BBE-B	1/20/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15520C	1/20/16	Ceca	NC	TSEKQG	NA	*C. jejuni*	f	R	Yes
15521B	1/20/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15522A	1/20/16	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
15522C	1/20/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15523A	1/20/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes

15523F	1/20/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15525A	2/3/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15525BBE-A	2/3/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15525E	2/3/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15527A	2/3/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15527B	2/3/16	Ceca	NC	TEKG	NA	*C. coli*	f	R	Yes
15527BBE-A	2/3/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15527BBE-B	2/3/16	Ceca	NC	TSKQG	NA	*C. coli*	f	R	Yes
15527D	2/3/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15528A	2/3/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15528BBE-A	2/3/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15529C	2/3/16	Ceca	NC	TQ	NA	*C. jejuni*	-	S	NO
15529E	2/3/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15530A	2/10/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15531A	2/10/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15532A	2/10/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15533A	2/10/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15534BBE-B	2/17/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15535A	2/17/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15535B	2/17/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15536BBE-A	2/17/16	Ceca	NC	TSEKQG	NA	*C. jejuni*	f	R	Yes
15537BBE-A	2/17/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15538BBE-A	2/17/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15538BBE-B	2/17/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15541A	3/2/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15542A	3/9/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15542BBE-A	3/9/16	Ceca	NC	TKQG	NA	*C. coli*	f	R	Yes
15543C	3/9/16	Ceca	NC	TKQG	NA	*C. jejuni*	f	R	Yes

15544A	3/9/16	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15546A	3/16/16	Ceca	NC	TKG	NA	*C. coli*	f	R	Yes
15546B	3/16/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15547A	3/16/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15547B	3/16/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15548A	3/16/16	Ceca	NC	TKQG	NA	*C. jejuni*	f	R	Yes
15549A	3/24/16	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15550A	3/24/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15551A	3/24/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15552A	3/24/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15554A	3/24/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15554B	3/24/16	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15555A	3/30/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15555D	3/30/16	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15556A	3/30/16	Ceca	NC	TKG	NA	*C. coli*	f	R	Yes
15558A	3/30/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15559A	4/6/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15560D	4/6/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15561A	4/6/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15561B	4/6/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15562A	4/6/16	Ceca	NC	TKQG	NA	*C. coli*	f	R	Yes
15563A	4/6/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15564B	4/19/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15564F	4/19/16	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
15565A	4/19/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
15566A	4/19/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15567A	4/19/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15588A	5/25/16	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
15588D	5/25/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15589B	5/25/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

15589D	5/25/16	Ceca	NC	TKQG	NA	*C. coli*	f	R	Yes
15590A	5/25/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15590C	5/25/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
15590F	5/25/16	Ceca	NC	TSEKCG	NA	*C. coli*	f	R	Yes
15593D	6/1/16	Ceca	NC	TQ	NA	*C. coli*	-	S	NO
15624F	7/27/16	Ceca	NC	T	NA	*C. coli*	-	S	NO
F1C1W3A	5/11/16	Ceca	NC	TSEKQG	889	*C. coli*	f	R	Yes
F2C1W3D	5/11/16	Ceca	NC	TSEKQG	889	*C. coli*	f	R	Yes
F2C5W4D	5/18/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
F3C1W2D	5/11/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
F3C1W4A	5/25/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
F3C4W5A	6/1/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
F4C9W3A	5/18/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes

F4C6W4B	5/25/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
F4C8W5B	6/1/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
F1C5W5D	5/25/16	Ceca	NC	TSEKQG	NA	*C. coli*	f	R	Yes
F2C2W2B	5/4/16	Ceca	NC	TKQG	NA	*C. coli*	f	R	Yes
F2C5W3C	5/11/16	Ceca	NC	TKQG	NA	*C. coli*	f	R	Yes
F3C5W4A	5/25/16	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
F3C6W4A	5/25/16	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
F3C6W4B	5/25/16	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
F4J3W4B	5/25/16	jejunum	NC	TEKQG	NA	*C. coli*	f	R	Yes
F4J3W4A	5/25/16	jejunum	NC	TEKQG	NA	*C. coli*	f	R	Yes
F4C9W5B	6/1/16	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
F2C3W3C	5/11/16	Ceca	NC	TEKQG	NA	*C. coli*	f	R	Yes
F1J4W4A	5/18/16	jejunum	NC	TEKG	NA	*C. coli*	f	R	Yes

F3C9W3C	5/18/16	Ceca	NC	TSEQG	NA	*C. coli*	f	R	Yes
F1C5W4C	5/18/16	Ceca	NC	TKG	NA	*C. coli*	f	R	Yes
F4C1W5A	6/1/16	Ceca	NC	TKG	NA	*C. coli*	f	R	Yes
F4C7W4B	5/25/16	Ceca	NC	TKG	1192	*C. coli*	f	R	Yes
F1C8W3D	5/11/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
F4J5W4A	5/25/16	jejunum	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
F4C7W5B	6/1/16	Ceca	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
F3C6W2E	5/11/16	Ceca	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
F3J7W4A	5/25/16	jejunum	NC	TSKQG	1839	*C. jejuni*	f	R	Yes

F3C2W5A	6/1/16	Ceca	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
F4J8W3C	5/18/16	jejunum	NC	TSKQG	8227	*C. jejuni*	f	R	Yes
F1J1W5D	5/25/16	jejunum	NC	TSKQG	8227	*C. jejuni*	f	R	Yes
F2J10W3C	5/11/16	jejunum	NC	TSKQG	8227	*C. jejuni*	f	R	Yes
F2C4W4A	5/18/16	Ceca	NC	TSKQG	8227	*C. jejuni*	f	R	Yes
F1C10W4C	5/18/16	Ceca	NC	TKQG	NA	*C. jejuni*	f	R	Yes
F4J1W4A	5/25/16	jejunum	NC	TKQG	NA	*C. jejuni*	f	R	Yes

F4J2W4D	5/25/16	jejunum	NC	TKQG	NA	*C. jejuni*	f	R	Yes
F3J2W3C	5/18/16	jejunum	NC	TKQG	1839	*C. jejuni*	f	R	Yes
F3C7W5A	6/1/16	Ceca	NC	TKQG	8227	*C. jejuni*	f	R	Yes
F3J2W4B	5/25/16	jejunum	NC	TKQG	8227	*C. jejuni*	f	R	Yes
F2C2W4C	5/18/16	Ceca	NC	TKQG	NA	*C. jejuni*	f	R	Yes
F2J1W3A	5/11/16	jejunum	NC	TKQG	NA	*C. jejuni*	f	R	Yes
F4C5W5A	6/1/16	Ceca	NC	TSKQG	NA	*C. jejuni*	f	R	Yes

F1J7W3C	5/11/16	jejunum	NC	TSKQG	NA	*C. jejuni*	f	R	Yes
F1J8W5A	5/25/16	jejunum	NC	TSKQG	1839	*C. jejuni*	f	R	Yes
F1C2W4A	5/18/16	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
F1C1W5A	5/25/16	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
F1C3W3D	5/11/16	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
F4C2W4D	5/25/16	Ceca	NC	TKG	NA	*C. jejuni*	f	R	Yes
F4J7W5A	6/1/16	jejunum	NC	TKG	NA	*C. jejuni*	f	R	Yes

F3J7W3A	5/18/16	jejunum	NC	TKG	NA	*C. jejuni*	f	R	Yes
F2C3W4C	5/18/16	Ceca	NC	TKQG	8227	*C. jejuni*	f	R	Yes
F1J3W3E	5/11/16	jejunum	NC	TKG	NA	*C. jejuni*	f	R	Yes

Appendix B

Isolates that were included in MLST analysis of chapter 4

Isolate	ST	Source	Species	Farm	AMR	aspA	glnA	gltA	glyA	pgm	tkt	uncA
15021B	889	Feces	*coli*	11	TSEKQG	33	39	30	82	113	47	41
15025A	1017	Fly	*coli*	11	TSEKQG	33	39	30	82	104	43	41
15239B	1067	Fly	*coli*	6	TSEKQG	33	39	30	140	104	43	41
14983A	1067	Fly	*coli*	8	TSEKQG	33	39	30	140	104	43	41
15023	1067	Fly	*coli*	9	TSEKQG	33	39	30	140	104	43	41
14351-1	1101	Feces	*coli*	10	TEKQG	33	39	30	82	189	43	41
14352-1	1101	Feces	*coli*	10	TEKQG	33	39	30	82	189	43	41
14192-5	1101	Feces	*coli*	2	TSEKQG	33	39	30	82	189	43	41
14354	1101	Fly	*coli*	10	TEKQG	33	39	30	82	189	43	41
14199-2	1101	Fly	*coli*	2	TSEKQG	33	39	30	82	189	43	41

14199-1	1101	Fly	*coli*	2	TSKQG	33	39	30	82	189	43	41
15095A	1192	Fly	*coli*	9	TEKQG	103	110	103	172	188	169	79
14243	1192	Fly	*coli*	5	TK	103	110	103	172	188	169	79
15103B	1192	Fly	*coli*	9	TKQG	103	110	103	172	188	169	79
14240-6	1833	Feces	*coli*	5	T	103	110	103	172	188	261	79
15230C	1833	Feces	*coli*	4	TEK	103	110	103	172	188	261	79
14459-4	1833	Feces	*coli*	3	TKG	103	110	103	172	188	261	79
15231C	1833	Feces	*coli*	4	TKG	103	110	103	172	188	261	79
14160-1	1833	Fly	*coli*	1	TKG	103	110	103	172	188	261	79
14462	1833	Fly	*coli*	3	TKG	103	110	103	172	188	261	79
14229-2	8213	fecal	*coli*	6	TKQG	103	110	103	171	104	261	79
14229-6	8213	fecal	*coli*	6	TKQG	103	110	103	171	104	261	79
14237	8213	fly	*coli*	6	TKG	103	110	103	171	104	261	79
14158-1	8218	fly	*coli*	1	TS	33	38	44	82	104	44	17

14233	8224	fly	*coli*	6	TKQG	103	110	103	171	104	169	79
14467	8551	fly	*coli*	3	TSEKQG	33	39	103	82	189	43	41
14228-4	1839	fecal	*jejuni*	6	TKQ	2	222	29	250	303	25	35
14240-5	1839	fecal	*jejuni*	5	TSKQ	2	222	29	250	303	25	35
14156-1	1839	fecal	*jejuni*	1	TSKQG	2	222	29	250	303	25	35
14192-1	1839	fecal	*jejuni*	2	TSKQG	2	222	29	250	303	25	35
14459-2	1839	fecal	*jejuni*	3	TSKQG	2	222	29	250	303	25	35
14459-3	1839	fecal	*jejuni*	3	TSKQG	2	222	29	250	303	25	35
15230D	1839	fecal	*jejuni*	4	TSKQG	2	222	29	250	303	25	35
14241-5	1839	fecal	*jejuni*	5	TSKQG	2	222	29	250	303	25	35
14228-5	1839	fecal	*jejuni*	6	TSKQG	2	222	29	250	303	25	35
14328-6	1839	fecal	*jejuni*	7	TSKQG	2	222	29	250	303	25	35
14980A	1839	fecal	*jejuni*	8	TSKQG	2	222	29	250	303	25	35
14165-1	1839	fly	*jejuni*	1	TKG	2	222	29	250	303	25	35
14161-1	1839	fly	*jejuni*	1	TKQ	2	222	29	250	303	25	35

14162-3	1839	fly	*jejuni*	1	TKQ	2	222	29	250	303	25	35
14160-3	1839	fly	*jejuni*	1	TKQG	2	222	29	250	303	25	35
14162-1	1839	fly	*jejuni*	1	TSKQ	2	222	29	250	303	25	35
14167-2	1839	fly	*jejuni*	1	TSKQG	2	222	29	250	303	25	35
14470	1839	fly	*jejuni*	3	TSKQG	2	222	29	250	303	25	35
14249	1839	fly	*jejuni*	6	TSKQG	2	222	29	250	303	25	35
14338	1839	fly	*jejuni*	7	TSKQG	2	222	29	250	303	25	35
15098A	1839	fly	*jejuni*	9	TSKQG	2	222	29	250	303	25	35
14229-5	2934	fecal	*jejuni*	6	TKG	7	112	5	2	167	67	6
15101A	2934	fly	*jejuni*	9	T	7	112	5	2	167	67	6
14203-3	2934	fly	*jejuni*	2	TKG	7	112	5	2	167	67	6
15093F	7730	fecal	*jejuni*	9	TSKQG	7	1	5	250	303	25	35
14234	8227	fly	*jejuni*	6	TKQ	2	608	29	250	303	25	35

Appendix C

Isolates used in chapter 4.

ID	ST	Farm	Source	Strain
14156-1	1839	1	fecal	TSKQGCj
14156-2	NA	1	fecal	TSKQGCj
14156-5	NA	1	fecal	TSKQGCj
14157-1	NA	1	fecal	TSKQGCj
14157-2	NA	1	fecal	TSKQGCj
14157-3	NA	1	fecal	TSKQGCj
14157-4	NA	1	fecal	TSKQGCj
14192--2	NA	2	fecal	TSKQGCj
14192-1	1839	2	fecal	TSKQGCj
14192-5	1101	2	fecal	TSEKQGCc
14192-6	NA	2	fecal	TSKQGCj
14193-1	NA	2	fecal	TSKQGCj
14193-4	NA	2	fecal	TSKQGCj
14193-5	NA	2	fecal	TSKQGCj
14193-6	NA	2	fecal	TSKQGCj
14459-1	NA	3	fecal	TSKQGCj
14459-2	1839	3	fecal	TSKQGCj

14459-3	1839	3	fecal	TSKQGCj
14459-4	1833	3	fecal	TKGCj
14459-5	NA	3	fecal	TSKQGCj
14459-6	NA	3	fecal	TSKQGCj
14460-1	NA	3	fecal	TSKQGCj
14460-2	NA	3	fecal	TSKQGCj
14460-3	NA	3	fecal	TSKQGCj
14460-4	NA	3	fecal	TSKQGCj
14460-5	NA	3	fecal	TSKQGCj
14460-6	NA	3	fecal	TSKQGCj
152302F	NA	4	fecal	TSKQGCj
15230C	1833	4	fecal	TEKCc
15230D	1839	4	fecal	TSKQGCj
15231A	NA	4	fecal	TSEKQGCc
15231B	NA	4	fecal	TSKQGCj
15231C	1833	4	fecal	TKGCc
15231D	NA	4	fecal	TSEKQGCc
15231E	NA	4	fecal	TSEKQGCc
15231F	NA	4	fecal	TSEKQGCc
14240-2	NA	5	fecal	TCc
14240-3	NA	5	fecal	TCc

14240-4	NA	5	fecal	TCc
14240-5	1839	5	fecal	TSKQCj
14240-6	1833	5	fecal	TCc
14241--3	NA	5	fecal	TKCc
14241-1	NA	5	fecal	TKCc
14241-5	1839	5	fecal	TSKQGCj
14241-6	NA	5	fecal	TCc
14228-4	1839	6	fecal	TKQCj
14228-5	1839	6	fecal	TSKQGCj
14228-6	NA	6	fecal	TSKQGCj
14229-1	NA	6	fecal	TKQGCc
14229-2	1192	6	fecal	TKQGCc
14229-5	2934	6	fecal	TKGCj
14229-6	8213	6	fecal	TKQGCc
14327-2	NA	7	fecal	TSKQGCj
14327-3	NA	7	fecal	TSKQGCj
14328-1	NA	7	fecal	TSKQGCj
14328-2	NA	7	fecal	TSKQGCj
14328-3	NA	7	fecal	TSKQGCj
14328-5	NA	7	fecal	TSKQGCj
14328-6	1839	7	fecal	TSKQGCj

14980A	1839	8	fecal	TSKQGCj
14980B	NA	8	fecal	TSKQGCj
14980C	NA	8	fecal	TSKQGCj
14980D	NA	8	fecal	TSKQGCj
14980E	NA	8	fecal	TSKQGCj
14980F	NA	8	fecal	TSKQGCj
15093A	NA	9	fecal	TSKQGCj
15093B	NA	9	fecal	TSKQGCj
15093C	NA	9	fecal	TSEKQGCc
15093D	NA	9	fecal	TSKQGCj
15093E	NA	9	fecal	TSKQGCj
15093F	7730	9	fecal	TSKQGCj
15094B	NA	9	fecal	TSKQGCj
15094D	NA	9	fecal	TSKQGCj
15094E	NA	9	fecal	TSKQGCj
15094F	NA	9	fecal	TSKQGCj
14351-1	1101	10	fecal	TEKQGCc
14351-2	NA	10	fecal	TEKQGCc
14351-3	NA	10	fecal	TEKQGCc
14351-4	NA	10	fecal	TEKQGCc
14351-5	NA	10	fecal	TEKQGCc

14351-6	NA	10	fecal	TEKQGCc
14352-1	1101	10	fecal	TEKQGCc
14352-2	NA	10	fecal	TEKQGCc
14352-3	NA	10	fecal	TEKQGCc
14352-4	NA	10	fecal	TEKQGCc
14352-5	NA	10	fecal	TEKQGCc
14352-6	NA	10	fecal	TEKQGCc
15021A	NA	11	fecal	TKGCc
15021B	889	11	fecal	TSEKQGCc
15021C	NA	11	fecal	TSKQGCj
15021D	NA	11	fecal	TSEKQGCc
15021E	NA	11	fecal	TSEKQGCc
15022A	NA	11	fecal	TSEKQGCc
15022B	NA	11	fecal	TSEKQGCc
15022E	NA	11	fecal	TKGCc
15022F	NA	11	fecal	TSEKQGCc
14987A	1839	12	fecal	TSKQGCj
14987B	NA	12	fecal	TSKQGCj
14988A	NA	12	fecal	TSKQGCj
14988E	NA	12	fecal	TSKQGCj
14988F	NA	12	fecal	TSKQGCj

14997C	NA	13	fecal	TSKQGCj
14998A	NA	13	fecal	TSKQGCj
14998B	NA	13	fecal	TSKQGCj
15009A	NA	14	fecal	TSKQGCj
15009B	NA	14	fecal	TSKQGCc
15009C	NA	14	fecal	TSKQGCj
15009D	NA	14	fecal	TSKQGCj
15009E	NA	14	fecal	TSKQGCj
15009F	NA	14	fecal	TSEKQGCc
15010A	NA	14	fecal	TSKQGCc
15010B	NA	14	fecal	TSKQGCj
15010C	NA	14	fecal	TSKQGCj
15010D	NA	14	fecal	TSKQGCj
15010E	NA	14	fecal	TSKQGCc
15010F	NA	14	fecal	TSKQGCc
15218A	NA	15	fecal	TSEKQGCc
15218B	NA	15	fecal	TSEKQGCc
15218C	NA	15	fecal	TSEKQGCc
15218D	NA	15	fecal	TSEKQGCc
15218E	NA	15	fecal	TSEKQGCc
15219A	NA	15	fecal	TSEKQGCc

15219B	NA	15	fecal	TSEKQGCc
15219C	NA	15	fecal	TSEKQGCc
15219D	NA	15	fecal	TSEKQGCc
15219E	NA	15	fecal	TSEKQGCc
15219F	NA	15	fecal	TSEKQGCc
15245A	NA	16	fecal	TSEKQGCc
15245B	NA	16	fecal	TSEKQGCc
15245C	NA	16	fecal	SEKQGCc
15245D	NA	16	fecal	TSEKQGCc
15245F	NA	16	fecal	TSEKQGCc
15246A	NA	16	fecal	TSEKQGCc
15246B	NA	16	fecal	TSEKQGCc
15246C	NA	16	fecal	TSEKQGCc
15246E	NA	16	fecal	TSEKQGCc
15246F	NA	16	fecal	TSEKQGCc
15026-2A	NA	17	fecal	TSEKQGCc
15026-2B	NA	17	fecal	TSEKQGCc
15026-2C	NA	17	fecal	TSEKQGCc
15026-2D	NA	17	fecal	TSEKQGCc
15026-2E	NA	17	fecal	TSEKQGCc
15026-2F	NA	17	fecal	TSEKQGCc

15026A	NA	17	fecal	TSEKQGCc
15026B	NA	17	fecal	TSEKQGCc
15026C	NA	17	fecal	TSEKQGCc
15026D	NA	17	fecal	TSEKQGCc
15026E	NA	17	fecal	TSEKQGCc
15026F	NA	17	fecal	TSEKQGCc
14181-2	NA	18	fecal	TSKQGCj
14181-3	NA	18	fecal	TKGCj
14181-4	NA	18	fecal	TSKQGCj
14363-1	NA	19	fecal	TSEKQGCc
14363-2	NA	19	fecal	TSKQGCj
14363-3	NA	19	fecal	TSKQGCj
14363-3	NA	19	fecal	TSKQGCj
14363-4	NA	19	fecal	TSEKQGCc
14363-4	NA	19	fecal	TSEKQGCc
14363-5	NA	19	fecal	TSEKQGCc
14363-5	NA	19	fecal	TSEKQGCc
14363-6	NA	19	fecal	TSKQGCj
14363-6	NA	19	fecal	TSKQGCj
14364-1	NA	19	fecal	TKQGCc
14364-2	NA	19	fecal	TSKQGCj

14252-1	NA	20	fecal	TKQGCc
14252-2	NA	20	fecal	TSEKQGCc
14252-3	NA	20	fecal	TSEKQGCc
14252-5	NA	20	fecal	TSEKQGCc
14252-6	NA	20	fecal	TSEKQGCc
14253-1	NA	20	fecal	TSEKQGCc
14253-2	NA	20	fecal	TKQGCc
14253-3	NA	20	fecal	TSEKQGCc
14253-4	NA	20	fecal	TSEKQGCc
14253-5	NA	20	fecal	TSEKQGCc
14253-6	NA	20	fecal	TKQGCc
14132-1	NA	21	fecal	TSKQGCj
14132-2	NA	21	fecal	TSKQGCj
14132-3	NA	21	fecal	TSKQGCj
14132-4	NA	21	fecal	TEKQGCc
14132-5	NA	21	fecal	TKGCj
14132-6	NA	21	fecal	TSKQGCj
14133-1	NA	21	fecal	TEKQGCc
14133-2	NA	21	fecal	TSKQGCj
14133-3	NA	21	fecal	TEKQGCc
14133-4	NA	21	fecal	TSKQGCj

14133-5	NA	21	fecal	TEKQGCc
14133-6	NA	21	fecal	TEKQGCc
14385-1	NA	22	fecal	TKQGCc
14385-3	NA	22	fecal	TSKQGCj
14385-4	NA	22	fecal	TKQGCc
14385-6	NA	22	fecal	TSKQGCc
14386-2	NA	22	fecal	TSKQGCj
14386-3	NA	22	fecal	TSKQGCj
14409-1	NA	23	fecal	TKQGCj
14409-3	NA	23	fecal	TKQGCc
14409-5	NA	23	fecal	TKQGCc
14410-2	NA	23	fecal	TSKQGCj
14410-3	NA	23	fecal	TKQGCc
14410-4	NA	23	fecal	TKQGCc
14410-5	NA	23	fecal	TSKQGCj
14410-6	NA	23	fecal	TSKQGCj
14521-1	NA	24	fecal	TSKQGCj
14521-2	NA	24	fecal	TKQGCj
14521-3	NA	24	fecal	TSKQCj
14521-4	NA	24	fecal	TSKQGCj
14521-5	NA	24	fecal	TSKQGCj

14521-6	NA	24	fecal	TSKQGCj
14522-1	NA	24	fecal	TSKQGCj
14522-2	NA	24	fecal	TSKQGCj
14522-3	NA	24	fecal	TSKQGCj
14522-4	NA	24	fecal	TSKQGCj
14522-5	NA	24	fecal	TSKQGCj
14522-6	NA	24	fecal	TSKQGCj
14565-3	NA	25	fecal	TSKQCj
14565-4	NA	25	fecal	TSKQCj
14565-5	NA	25	fecal	TSKQCj
14158-1	8218	1	fly	TSCc
14158-2	NA	1	fly	TSCc
14158-3	NA	1	fly	TSCc
14159-1	NA	1	fly	TSKQCc
14159-2	NA	1	fly	TKQGCj
14160-1	8224	1	fly	TKGCc
14160-3	8227	1	fly	TKQGCj
14161-1	1839	1	fly	TKQCj
14161-3	NA	1	fly	TKQGCc
14162-1	1101	1	fly	TSKQCj
14162-2	NA	1	fly	TSKQCj

14162-3	1839	1	fly	TKQCj
14163-1	NA	1	fly	TSKQCj
14163-2	NA	1	fly	TKQCc
14163-3	NA	1	fly	TSKQCj
14164-1	NA	1	fly	TSKQCj
14164-2	NA	1	fly	TSKQCj
14165-1	2934	1	fly	TKGCj
14165-2	NA	1	fly	TKGCj
14165-3	NA	1	fly	TKQGCj
14166-1	NA	1	fly	TKQGCj
14166-2	NA	1	fly	TKQCj
14166-3	NA	1	fly	TKQCj
14167-2	1839	1	fly	TSKQGCj
14167-3	NA	1	fly	TSKQGCj
14196-2	NA	2	fly	TSKQGCj
14197-1	NA	2	fly	TGCj
14199-1	1101	2	fly	TSKQGCj
14199-2	1101	2	fly	TSEKQGCc
14203-1	NA	2	fly	TKGCj
14203-2	NA	2	fly	TSEKQGCc
14203-3	2934	2	fly	TKGCj

14462	1833	3	fly	TKGCj
14467	8551	3	fly	TSEKQGCc
14469	NA	3	fly	TSKQGCc
14470	1839	3	fly	TSKQGCj
15238B	NA	4	fly	TSEKQGCc
15239B	1067	4	fly	TSEKQGCc
14243	1192	5	fly	TKCc
14248	NA	5	fly	TKQGCc
14249	1839	5	fly	TSKQGCj
14250	NA	5	fly	TKQGCc
14233	1833	6	fly	TKQGCc
14234	1839	6	fly	TKQCj
14236	NA	6	fly	TKQGCc
14237	8213	6	fly	TKGCc
14239	NA	6	fly	TKQGCc
14329	NA	7	fly	TSKQGCj
14330	NA	7	fly	TSKQGCj
14338	1839	7	fly	TSKQGCj
14982A	NA	8	fly	TSKQGCj
14983A	1067	8	fly	TSEKQGCc
14983B	NA	8	fly	TSEKQGCc

14984A	NA	8	fly	TSKQGCj
14984B	NA	8	fly	TSKQGCj
14985A	NA	8	fly	TSKQGCj
14985B	NA	8	fly	TSKQGCj
14986A	NA	8	fly	TSKQGCj
15095A	8213	9	fly	TEKQGCc
15095B	NA	9	fly	TEKQGCc
15096A	NA	9	fly	TEKQGCc
15096B	NA	9	fly	TSEKQGCc
15097A	NA	9	fly	TEKQGCc
15097B	NA	9	fly	TSKQGCj
15098A	8213	9	fly	TSKQGCj
15098B	NA	9	fly	TSKQGCj
15099A	NA	9	fly	TEKQGCc
15099B	NA	9	fly	TEKQGCc
15100B	NA	9	fly	TEKQGCc
15101A	2934	9	fly	TCj
15101B	NA	9	fly	TSKQGCc
15102A	NA	9	fly	TSKQGCj
15102B	NA	9	fly	TSEKQGCc
15103A	NA	9	fly	TSKQGCj

15103B	2934	9	fly	TKQGCc
15104A	NA	9	fly	TSKQGCj
15104B	NA	9	fly	TEKQGCc
14354	1101	10	fly	TEKQGCc
14355	NA	10	fly	TEKQGCc
14358	NA	10	fly	TEKQGCc
15023	1067	11	fly	TSEKQGCc
15025A	1017	11	fly	TSEKQGCc
15025B	NA	11	fly	TKGCc
14989B	NA	12	fly	TSKQGCj
14991A	NA	12	fly	TSKQGCj
14991B	1839	12	fly	TSKQGCj
14995A	NA	12	fly	TSKQGCj
14995B	NA	12	fly	TSKQGCj
14999A	NA	13	fly	TSEKQGCc
14999B	NA	13	fly	TSEKQGCc
15000A	NA	13	fly	TSKQGCj
15000B	NA	13	fly	TSKQGCj
15001B	NA	13	fly	TKGCj
15004A	NA	13	fly	TSEKQGCc
15004B	NA	13	fly	TSKQGCj

15006A	NA	13	fly	TSEKQGCc
15006B	NA	13	fly	TSEKQGCc
15007A	NA	13	fly	TSKQGCj
15007B	NA	13	fly	TSKQGCj
15012A	NA	14	fly	TSEKQGCc
15012B	NA	14	fly	TSKQGCj
15014A	NA	14	fly	TSEKQGCc
15014B	NA	14	fly	TSKQGCj
15015A	NA	14	fly	TKQGCc
15015B	NA	14	fly	TKQGCc
15017A	NA	14	fly	TSEKQGCc
15017B	NA	14	fly	TSEKQGCc
15221A	NA	15	fly	TSEKQGCc
15221B	NA	15	fly	TSEKQGCc
15222A	NA	15	fly	TSEKQGCc
15222B	NA	15	fly	TSEKQGCc
15225B	NA	15	fly	TSEKQGCc
15226A	NA	15	fly	TSEKQGCc
15227B	NA	15	fly	TSEKQGCc
15229A	NA	15	fly	TSEKQGCc
15229B	NA	15	fly	TSEKQGCc

15250A	NA	16	fly	TSEKQGCc
15027A	NA	17	fly	TSEKQGCc
15027B	NA	17	fly	TSEKQGCc
15028A	NA	17	fly	TSEKQGCc
15028B	NA	17	fly	TEKQGCc
15029A	NA	17	fly	TSEKQGCc
15029B	NA	17	fly	TSEKQGCc
14184-3	NA	18	fly	TSKQGCj
14186	NA	18	fly	TSKQGCj
14188-1	NA	18	fly	TSKQGCj
14188-2	NA	18	fly	TSKQGCj
14367	NA	19	fly	TKQGCc
14368	NA	19	fly	TSEKQGCc
14374-S2	NA	19	fly	TEKQGCc
14254	NA	20	fly	TKQGCc
14259	NA	20	fly	TKQGCc
14260	NA	20	fly	TSEKQGCc
14261	NA	20	fly	TKQGCc
14146-1	NA	21	fly	TEKQGCc
14147-1	NA	21	fly	TEKQGCc
14147-2	NA	21	fly	TEKQGCc

14147-3	NA	21	fly	TEKQGCc
14148-1	NA	21	fly	TEKQGCc
14148-2	NA	21	fly	TSKQGCj
14148-3	NA	21	fly	TSKQGCj
14149-1	NA	21	fly	TSEKQGCc
14149-2	NA	21	fly	TEKQGCc
14149-3	NA	21	fly	TSKQGCj
14150-1	NA	21	fly	TEKQGCc
14150-2	NA	21	fly	TEKQGCc
14150-3	NA	21	fly	TSKQGCj
14151-1	NA	21	fly	TSKQGCj
14151-2	NA	21	fly	TSKQGCj
14151-3	NA	21	fly	TKQGCj
14396-1A	NA	22	fly	TSKQGCj
14396-1B	NA	22	fly	TSKQGCj
14413	NA	23	fly	TSEKQGCc
14419	NA	23	fly	TKQGCc
14523-1	NA	24	fly	TSKQGCj
14523-2	NA	24	fly	TSKQGCj
14524-1	NA	24	fly	TSKQGCj
14525-1	NA	24	fly	TSKQGCj

14525-2	NA	24	fly	TSKQGCj
14584-2	NA	25	fly	TSKQGCj
14200-2	NA	2	fly	TSEKQGCc
14201-2	NA	2	fly	TSKQGCj
14201-3	NA	2	fly	TSKQGCj
14202-3	NA	2	fly	TSEKQGCc

Appendix D

A Snapshot of Management Practices in the American Turkey Industry: If We are to Reduce *Campylobacter* Should Some Practices Change?

By: Hannah Bolinger, Sophia Kathariou, & Donna Carver

Publication date Apr 10, 2017. Publication description Poultry Times.

The average American eats 15.8 pounds of turkey every year, and production has increased nearly 104% since 1970. Turkey meat exported from the U.S. reached nearly 800 million pounds in 2012 (1). With the increasing popularity of turkey worldwide comes the increasing responsibility to produce healthy birds as well as a safe product. Due to the difficulty in eliminating pathogens in the processing environment, it is imperative that growers do their best to keep their flocks free of undesirable microorganisms. Management practices can dramatically impact the health and performance of the birds as well as the risk for human pathogens such as *Salmonella* and *Campylobacter*. *Salmonella* and its associated disease salmonellosis are extensively recognized, but *Campylobacter* is a much less known pathogen. However, it is responsible for one of the most frequent human foodborne diseases, campylobacteriosis. The handling or consumption of raw or undercooked poultry is a leading risk factor for contracting campylobacteriosis. In the United States alone *Campylobacter* is responsible for approximately 850,000 cases of human disease annually and is likely under-reported (2, 3). It is also considered the most common human foodborne pathogen in Europe (4).

Campylobacter lives in the intestinal tract of mammals and poultry, with poultry considered to be a leading reservoir for strains causing human foodborne illness. *Campylobacter*-positive birds shed large numbers of the bacteria in the feces. Though much attention has been directed to broiler colonization with *Campylobacter*, turkeys are also frequently colonized. Unlike broilers which are typically processed at 4-6 weeks of age, turkeys are markedly older (typically 14-21 weeks) at processing, thus allowing more time and opportunity for *Campylobacter* colonization. Although most brooder turkeys (4-6 weeks old) will already be colonized, the standard American practice of brooders being moved by truck to other locations for growout provides additional opportunities for acquisition of *Campylobacter*, e.g. via exposure to contaminated vehicles, equipment or personnel or by enhanced susceptibility to colonization from transportation-related stresses.

Campylobacter is generally considered a harmless commensal organism to poultry. However, recent findings suggest that in fact *Campylobacter* infection of broiler chickens results in diarrhea and poorer bird health, with inflammation and damage to the mucosal lining of the intestine being noted (5, 6). Less work has been done in turkeys, and results from chickens cannot necessarily be extrapolated to turkeys. Turkeys and broilers are naturally coprophagic (will eat feces) and utilize communal feeders and waterers; thus, once one bird is colonized the bacterium spreads readily to the rest of the flock (7). Many efforts have focused on management practices and feed ingredients (e.g. competitive exclusion cultures) to keep *Campylobacter* out of poultry flocks, but the task has proven to be quite difficult.

Previous studies with turkeys have shown that although *Campylobacter* is frequently found to colonize turkey flocks it is by no means unavoidable, and flocks grown under different management conditions can vary markedly in *Campylobacter* levels (8, 9). In this article our objective was to identify which poultry management practices have been shown in the scientific literature to reduce food safety risks pre-harvest, and which are actually implemented in the turkey industry. We sent surveys regarding farm layout and operations to 27 commercial turkey farms (14 brooder and 13 growout farms) in the United States and fisher's exact test was used to determine whether a practice was significantly different between farm types.

Survey Results:

Perhaps the most critical variables in raising *Campylobacter*-free birds are the actions of the growers themselves and the employees they hire. Greater numbers of employees working on a turkey farm may constitute a risk factor for *Campylobacter*, as already shown for *Salmonella* (8). This may be partially due to the difficulty in guaranteeing that employees follow adequate biosecurity procedures (10, 11). In our study, we found that 71% of brooder and 23% of growout farms employed workers from outside the farm. Brooder farms are generally more labor-intensive which may explain this practice.

As with *Salmonella*, increased biosecurity may help with the control of *Campylobacter* (8). Key to biosecurity is the use of separate boots or fresh overboots for each poultry house. The rationale is that changing boots will reduce the likelihood that the bacteria will be tracked into subsequent poultry houses. *Campylobacter* found on boots is often genetically identical to that found in the birds' feces (10). Indeed, birds are at higher risk for

Campylobacter if adjacent houses are already positive (10). Although footbaths can help disinfect the bottoms of boots, it is difficult to guarantee sufficient contact time between the boots and sanitizer, or that the sanitizer concentration is appropriate. So, having a set of boots designated for each house and regularly disinfected may be a better strategy.

In our study, almost all farms (100% and 92% of brooder and growout farms, respectively) reported that rubber boots were required at the start of each work day (Fig. 1). Relatively few farms required the boots to be changed between houses but all farms required the use of a footbath when moving between turkey houses. We also asked whether farms require coveralls to be worn on the farm and how often these need to be cleaned. Separate clothing for each house was shown to have a protective effect against *Campylobacter* in broiler production (12).

Approximately 71% of the 14 brooder and 54% of the 13 growout farms required coveralls. Of farms that required coveralls, 20% of brooders required them to be changed between turkey houses; none of the growout farms reported such a requirement. Also, of farms requiring coveralls 90% of brooders and 57% of growouts required that the coveralls be washed daily. Most (86%) brooder farms provided coveralls (either reusable or disposable) to their employees, while this practice was less common (31%) among growouts. Provision and requirement of coveralls may promote their use.

Washing hands between poultry houses is another variable that has been shown to have a protective effect against *Campylobacter* colonization of the birds (12, 13). We asked whether farms required the use of hand sanitizer or gloves. We found that 50% of the 14 brooders and 38% of the 13 growouts were required to wear gloves, while 57% of brooders

and 15% of growouts were required to use hand sanitizer at the beginning of every work day (Fig. 1). Of the 8 brooders that required hand sanitizer, 6 (75%) required its use when moving between houses. One farm that did not require its use at the beginning of the day did require it when moving between houses. Few farms (2 of 7 brooder and 1 of 5 growout farms) that required gloves required them to be changed when moving between houses. Some farms indicated a requirement for hand sanitizer but not gloves, or vice versa.

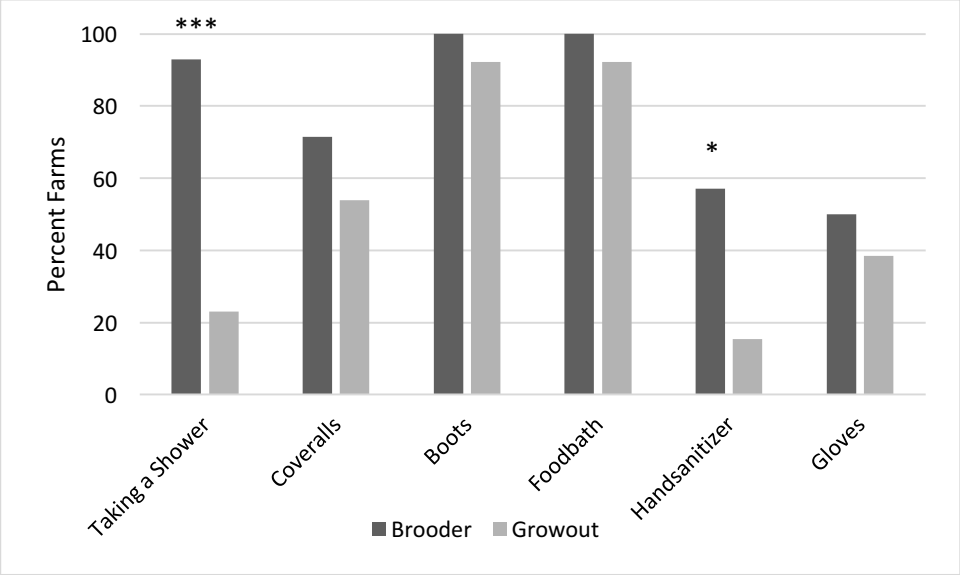


Figure 1: What is required each day before beginning work? (* ≤ 0.05, *** ≤ 0.001)

Campylobacter colonizes the intestinal tracts of many animals, and the presence of other animals on the farm has been identified as a risk factor for the spread of *Campylobacter*. Pigs, cattle, sheep, and other poultry have all been identified as risk factors for *Campylobacter* colonization of commercial broiler flocks (10, 12, 13). Our study showed that no brooder farms kept pigs, cattle, or other poultry. However, among the growouts an estimated 23% of the farms also raised pigs while 38% had cattle on the same farm.

Besides livestock, insects and other pests (e.g. rodents) can harbor *Campylobacter* as well as *Salmonella.* All surveyed brooder and growout farms stated that they had rodent control programs in place. Measures taken outside of the turkey houses to deter pests included keeping grass trimmed, creating a gravel buffer or concrete pads around the turkey houses and using rodent bait and traps (Fig. 2). All farms used bait and almost all reported that they kept the grass trimmed. Gravel buffers were used in 36% of brooders and 15% of growouts, while 57% of brooders and 8% of growouts had concrete pads around the turkey houses. This means that all but one brooder had a physical barrier around the houses, while less than 25% of growouts did. About half of the farms (57 and 54% of brooder and growouts, respectively) used rodent traps outside the turkey houses (Fig. 2).

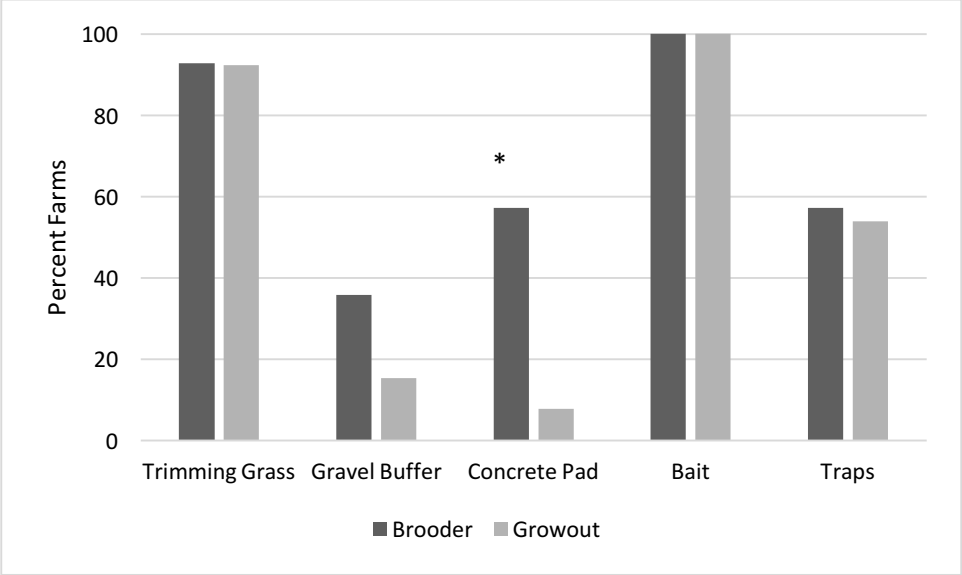


Figure 2: What measures are in place outside your poultry houses to control rodents? (* ≤ 0.05)

Insect controls were addressed in the surveys, and Figure 3 shows the results of the survey questions asking whether growers used sticky tape or traps, bait, surface sprays, litter treatments, or fogs or mists for fly control. Most farms reported using sticky tape or traps, bait, surface sprays, and litter treatments while fogs or mists were the least popular fly control measure (Fig. 3). However, if used properly, sprays, fogs, or mists can be highly effective for controlling an existing high population of flies. Additionally, surface sprays may be ineffective as they often get coated in dust and flies have built up resistance (15, 16). Fly-speck cards can be used to monitor the fly population in poultry houses and assess the need for corrective action (14).

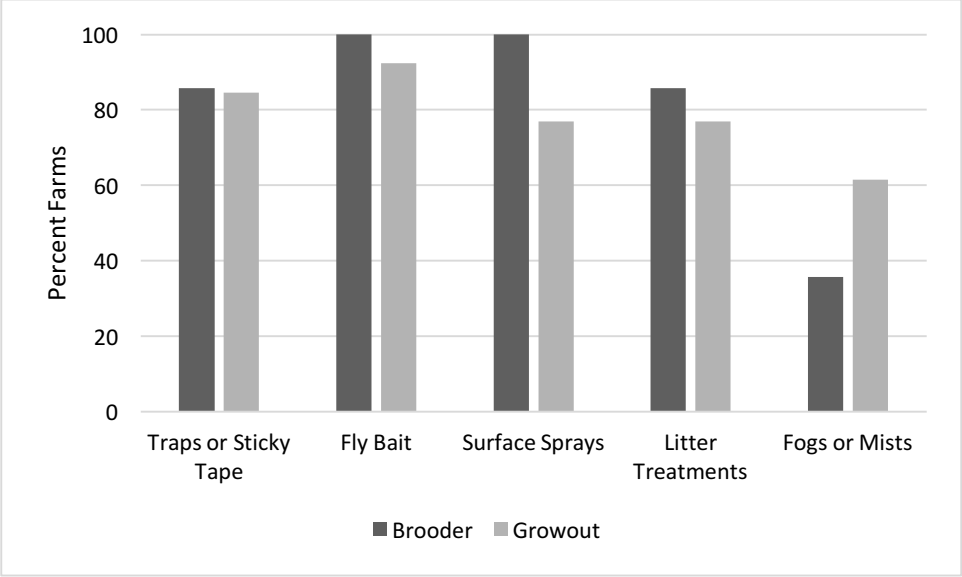


Figure 3: What Methods Do You Use to Control Flies?

The role of flies in *Campylobacter* transmission into poultry flocks may partially explain the seasonal spike in *Campylobacter* colonization of flocks during the warmer months since this is when flies are more prevalent (17, 18). Studies in Denmark showed that installation of fly screens was accompanied by reductions of *Campylobacter*-positive flocks from 51% to 15% (17). Poultry manure is an excellent breeding ground for insects such as flies and darkling beetles so keeping the litter in the houses dry and well-aerated is recommended to make it less suitable for flies. Additionally, manure piles outside of the houses can be treated or covered with tarps to prevent fly reproduction.

Litter management findings are shown below in Figures 4 and 5. The majority of farm (86% of brooders and 85% of growouts) reported that used litter was immediately removed at the end of each flock cycle. Almost half of brooders reported that litter from their farms was used to top-dress growout farms, which may not be ideal as *Campylobacter* can survive in litter. Some growouts report using litter from their houses as fertilizer on their own land or within half a mile of their farm. Pathogens may be able to survive in litter, which therefore should be properly composted before land applications (19).

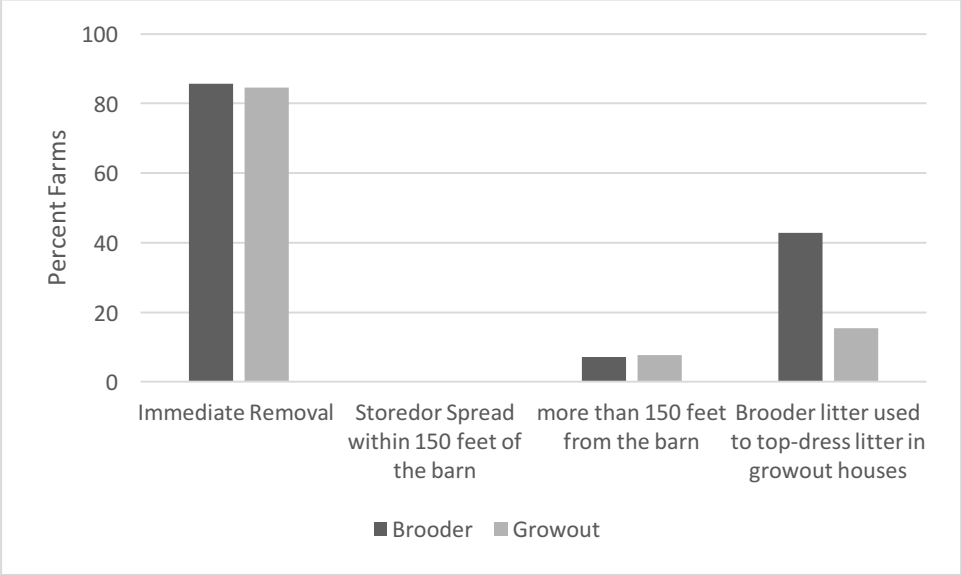


Figure 4: Once used, where is litter stored?

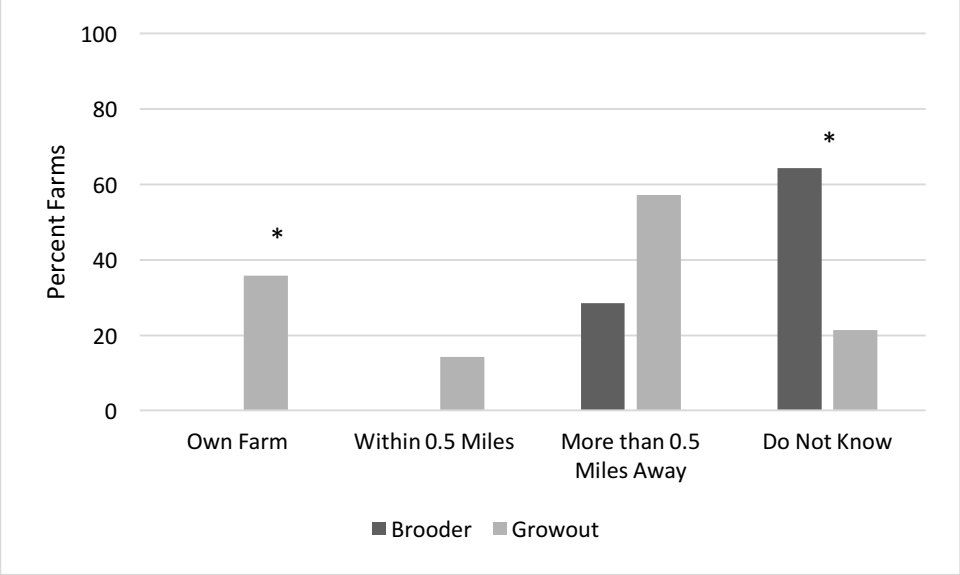


Figure 5: If the used litter is used as fertilizer, where is it used? (* $\leq$ 0.05)

Conclusions:

Our survey identified certain practices in the industry that can be improved upon to enhance the likelihood of producing *Campylobacter*-free flocks. It appears that a number of the important biosecurity behaviors used on brooder farms are no longer practiced at growout. Thus, even if brooders are *Campylobacter*-free, they can become colonized with *Campylobacter* during growout. If birds are going to remain *Campylobacter*-free continued vigilance is needed all the way through the growing chain and into the processing plant.

Designating separate boots or using new plastic over-boots for each poultry house as well as changing clothes between poultry houses may be a useful practice on all farms. Additionally, some sort of hand hygiene should be used when going from one turkey house to another. Fly populations should be monitored with the use of "fly speck" cards (really just 3" by 5" index cards) in a quantitative way as described here:

"*Once placed, cards should be left for a period of 7 days and replaced with new cards at the same place each week. The number of "fly specks" on the exposed side (one side) of each card should be counted and recorded in a record keeping notebook. Generally, 100 or more spots per card indicates the need for fly control measures."* (16)

Some of the recommended actions, such as changing boots and regular laundering of clothes used on the farm will require more time and effort. Smarter strategies need to be used against flies and other insect vectors such as darkling beetles. Beginning May 11, 2016 the USDA Food Safety and Inspection Service (FSIS) began assessing if food producers are meeting the recently approved performance standards to reduce *Salmonella* and

Campylobacter in ground chicken and turkey, as well as chicken parts. Improved management practices and continuing vigilance will contribute to helping American turkey producers meet these standards and produce products that fall within the performance standard guidelines.

REFERENCES:

1. **National Turkey Federation**. Statistics. < http://www.eatturkey.com/why-turkey/stats>
2. **Scallan E**, **Hoekstra RM**, **Angulo FJ**, **Tauxe R V**, **Widdowson M-A**, **Roy SL**, **Jones JL**, **Griffin PM**. 2011. Foodborne illness acquired in the United States--major pathogens. Emerg Infect Dis **17**:7–15.
3. 2014. CDC - *Campylobacter*, General Information - NCZVED.
4. **European Food Safety Authority**. 2014. EFSA explains zoonotic diseases. *Campylobacter*.
5. **Humphrey S**, **Chaloner G**, **Kemmett K**, **Davidson N**, **Williams N**, **Kipar A**, **Humphrey T**, **Wigley P**. 2014. *Campylobacter* jejuni is not merely a commensal in commercial broiler chickens and affects bird welfare. MBio **5**:1–7.
6. **Reid WDK**, **Close AJ**, **Humphrey S**, **Chaloner G**, **Lacharme-Lora L**, **Rothwell L**, **Kaiser P**, **Nicola J**, **Humphrey TJ**, **Wigley P**, **Rushton SP**. 2016. Cytokine responses in birds challenged with the human food-borne pathogen *Campylobacter* jejuni implies a Th17 response
7. **Wallace JS**, **Stanley KN**, **Jones K**. 1998. The colonization of turkeys by thermophilic campylobacters. J Appl Microbiol **85**:224–30.
8. **Arsenault J**, **Letellier A**, **Quessy S**, **Normand V**, **Boulianne M**. 2007. Prevalence and risk factors for *Salmonella* spp. and *Campylobacter* spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. Prev Vet Med **81**:250–64.

9. **Smith K**, **Reimers N**, **Barnes HJ**, **Lee BC**, **Siletzky R**, **Kathariou S**. 2004. *Campylobacter* colonization of sibling turkey flocks reared under different management conditions. J Food Prot **67**:1463–1468.

10. **Agunos A**, **Waddell L**, **Léger D**, **Taboada E**. 2014. A systematic review characterizing on-farm sources of *Campylobacter* spp. for broiler chickens. PLoS One **9**:e104905.

11. **Newell DG**, **Fearnley C**. 2003. Sources of *Campylobacter* Colonization in Broiler Chickens. Appl Environ Microbiol **69**:4343–4351.

12. **van de Giessen A**, **Mazurier SI**, **Jacobs-Reitsma W**, **Jansen W**, **Berkers P**, **Ritmeester W**, **Wernars K**. 1992. Study on the epidemiology and control of *Campylobacter* jejuni in poultry broiler flocks. Appl Environ Microbiol **58**:1913–1917.

13. **van de Giessen a W**, **Bloemberg BP**, **Ritmeester WS**, **Tilburg JJ**. 1996. Epidemiological study on risk factors and risk reducing measures for *Campylobacter* infections in Dutch broiler flocks. Epidemiol Infect **117**:245–250.

14. **Ward D (OMAFRA)**, **Lachance S (University of G**. 2015. House Fly Control in Poultry Barns.

15. **Kaufman PE**, **Rutz D**, **Pitts C**. 2000. Pest management recommendations for poultry. Cornell Penn State Univ Coop Ext Pub Univ Park PA **24**:1–16.

16. **Williams RE**. Control of Poultry Prests.

17. **Hald B**, **Sommer HM**, **Skovgard H**. 2007. Use of fly screens to reduce *Campylobacter* spp. introduction in broiler houses. Emerg Infect Dis **13**:1951–1953.

18. **Bahrndorff S**, **Rangstrup-Christensen, Lena Nordentoft S**, **Birthe H**. 2013. Foodborne Disease Prevention and Broiler Chickens with Reduced *Campylobacter* Infection. Emerg Infect Dis **19**:19–21.

19. **Chen Z**, **Jiang X**. 2014. Microbiological Safety of Chicken Litter or Chicken Litter-Based Organic Fertilizers: A Review. Agriculture **4**:1–29.

Appendix E

Biosecurity Practices in *Campylobacter*-Negative and Positive Commercial Turkey Farms

Hannah Bolinger, Kenneth M. Mann, Donna Carver, Sophia Kathariou

Publication date March 20, 2017. Publication description by MeatingPlace.

The human pathogen *Campylobacter* is not as well-known as others such as *Salmonella* or *E. coli,* but is responsible for almost 0.8 million illnesses in the U.S. alone every year. The handling or consumption of raw or undercooked poultry is considered the leading risk factor for contracting campylobacteriosis, the illness caused by *Campylobacter*. Poultry commonly carry this bacterium and typically acquire it from their environment. Usually the birds do not show any symptoms of illness, making it impossible to know whether or not they are colonized without specific laboratory tests. Because of this, growers must be diligent in their biosecurity protocols to keep *Campylobacter* and other undesirable microorganisms out of the poultry houses. The majority of commercial turkey flocks that our lab has tested have been positive for *Campylobacter,* but numerous scientific articles have documented the positive effect that management strategies can have on protecting poultry flocks against *Campylobacter*.

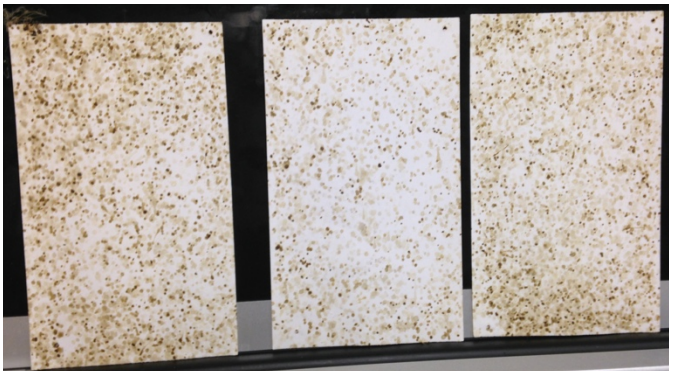
Biosecurity protocols are meant to provide a buffer or barrier between the inside of the poultry houses and the outside world. All sorts of animals, including insects, can carry *Campylobacter* and potentially spread it to the birds, so actions that deter pests are critical to successful biosecurity. At the same time equipment, clothing and humans can also carry

Campylobacter into flocks. The goal of this article is to highlight the biosecurity-related practices of one farm ('Farm A') that consistently tested negative for *Campylobacter* and to compare the practices to those at another farm ('Farm B') that always tested positive. The two farms were approximately 34 miles apart in the southeastern United States, and belonged to different growers who produced the birds under contract with the same vertical integrator. Both farms were visited in July 2013, while additional visits were made in November 2013 and December 2014 (Farm A) and June 2013 (Farm B).

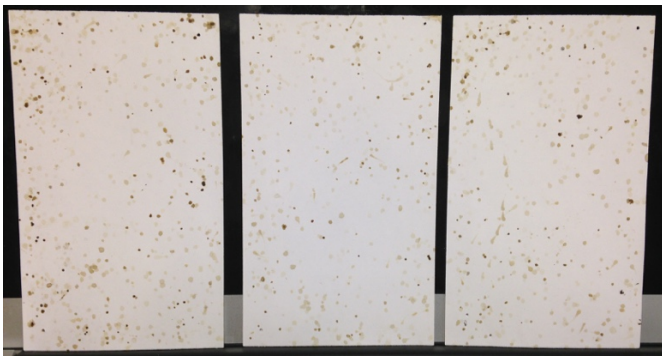
Observations of Farm A clearly indicate that this is a farm with excellent biosecurity. Upon pulling up to this farm it was noted that it was completely fenced in and locked, with posted signs indicating that unauthorized visitors were not allowed and highlighting the biosecurity procedures of that farm. For example, all visitors were required to wash their truck and take a shower. They were also provided with coveralls and rubber boots before entering the farm. In contrast, there was no fencing around Farm B, no signs posted, and no requirement for washing the truck. Visitors to Farm B were required to shower before entering the farm but were not required to wear coveralls. These farms also differed in the cleanliness of the lavatories and other general hygiene practices. While Farm A's lavatory was consistently recorded as being clean and well maintained, Farm B's lavatory was noted as marginally clean with a clogged sink with standing water. Eating and smoking was confined to the office on Farm A, while drinking cups were found in the turkey houses of Farm B.

Tall grass, which can provide a hiding place for pests, was kept trimmed at both farms and neither farm had a lot of trash or debris laying around, although some standing

water was noted on Farm B. Concrete or gravel buffers should be used around the turkey houses to deter pests from entering the house. Farm B did not have a buffer around the houses, but the status of this at Farm A is unknown. Inside the houses, the litter was tilled at least weekly and was in good condition on both farms. While both farms used fly mitigation strategies such as traps and bait, Farm A also used Neoprex to kill fly larvae. Fly speck cards are highly recommended inside the turkey houses to monitor the fly population, and examples of fly speck cards from representative houses with high and medium / low fly density are shown below. Extension offices provide a great deal of information on fly speck card and how fly control can be customized based on the results of the fly speck cards.



(High density fly population)



(Medium / low density fly population)

The grower for Farm A was recorded as wearing coveralls while the grower from Farm B did not wear coveralls. Neither grower wore plastic overboots in the houses. While both growers indicated that they used the footbath before entering the houses, it can be difficult to guarantee that footbaths contain the appropriate amount of sanitizer or that there has been enough contact time between the sanitizer and the boots for adequate disinfection. It is best to designate a set of boots for each turkey house and, if possible, to use disposable, plastic overboots in each house to avoid tracking organisms from one house into the next. The presence of other food animals on neighboring farms may increase the risk of a flock becoming colonized by *Campylobacter.* Farm A was a half mile away from a hog farm while Farm B was within a half mile of both hog and broiler farms; the latter may especially increase the *Campylobacter* risk to that farm. Companion animals should be kept out of commercial turkey farms, and no dogs were ever seen on Farm A while the grower's three dogs were recorded as running loose on farm B.

While no single action may be responsible for the differences in the *Campylobacter* status between these farms, consistent diligence regarding biosecurity practiced on Farm A may have allowed this farm to remain *Campylobacter*-free. It is extremely difficult to eliminate *Campylobacter* in the processing environment, and having more *Campylobacter*-negative flocks enter processing will undoubtedly result in food safety benefits for the public.

Table: Practices that may affect *Campylobacter* entry into turkey houses

	Farm A	Farm B
Companion Animals on farm	Yes	No
Visitors or Staff Wearing Coveralls	Yes	No
Farm is Fenced	Yes	No
Biosecurity Signs Posted	Yes	No
Truck Must Be Washed	Yes	No
Shower required for visitors	Yes	Yes
Grass Trimmed	Yes	Yes
Litter in Good Condition	Yes	Yes

Appendix F

Food Safety 101: What is *Campylobacter* and what are we doing about it? March 31, 2015 , NC State News and picked up by food safety news

Campylobacters are spiral- shaped bacteria frequently colonizing the intestines of animals grown for food (as well as other animals) and able to cause acute diarrheal disease (campylobacteriosis) in humans. These bacteria, especially the species *C. jejuni* and *C. coli*, are a leading bacterial cause of foodborne disease, resulting in an estimated 0.8 million cases of illness annually in the United States alone. Campylobacteriosis is most commonly attributed to the consumption of undercooked poultry, unpasteurized milk, and untreated water[1]. These products generally become vehicles for *Campylobacter* via fecal contamination- for instance *Campylobacter* in the intestines of poultry can contaminate poultry carcasses during the evisceration process at the slaughterhouse, and milk can become contaminated by animal feces as a result of unsanitary procedures during milking.

Symptoms of campylobacteriosis generally last from 2 to 10 days and include severe abdominal cramping, diarrhea (sometimes bloody) and vomiting. Symptoms usually subside without medical treatment but severe autoimmune complications such as reactive arthritis and Guillain-Barre syndrome- a form of paralysis may follow as many as 1 in every 1,000 *Campylobacter jejuni* infections[2] , making this pathogen less than innocuous. Immunocompromised patients or those in which the bacteria have entered the bloodstream can benefit from treatment with antibiotics including macrolides (e.g. erythromycin or

azithromycin) or fluoroquinolones (e.g. ciprofloxacin). However, we are observing increased resistance in *Campylobacter* to a number of antibiotics.

The best way to prevent campylobacteriosis is to practice safe food handling techniques in the kitchen (especially in regard to preventing cross-contamination of ready-to-eat foods with raw poultry), thoroughly cook (using a meat thermometer) poultry and other meat products and avoid unpasteurized dairy products and untreated water[3]. For tips on safe food handling techniques, you can visit this site:
http://www.foodsafety.gov/keep/basics/separate/

NCSU is actively involved in research and has partnered with the food industry to better understand and control *Campylobacter*. At the Department of Food, Bioprocessing and Nutrition Sciences at NCSU, the lab of Dr. Sophia Kathariou has partnered with Dr. Donna Carver, Extension Veterinarian at the Prestage Department of Poultry Science, to conduct research on how growers can produce turkeys that are free of *Campylobacter* or only carry the bacterium in small amounts. Between 60-80% of poultry flocks worldwide are positive for *Campylobacter* so simply reducing this number could have a major effect on public health[4]. Additionally, these NCSU researchers are investigating antibiotic resistance trends in *Campylobacter* from commercial turkey flocks. The research being done at NCSU will add to our base of knowledge about the genes responsible for resistance, how prevalent resistance is in *Campylobacter* found in our food supply, and how to eliminate *Campylobacter* from poultry flocks. Such knowledge is critically needed for development of science-based strategies to enhance the safety of food and reduce the public health threat posed by antimicrobial resistance in foodborne pathogens such as *Campylobacter*.

http://www.foodsafety.gov/poisoning/causes/bacteriaviruses/campylobacter/

http://wwwnc.cdc.gov/eid/pdfs/vol21no3_pdf-version.pdf

(http://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM297627.pdf

Hermans D, Pasmans F, Messens W, Martel A, Van Immerseel F, Rasschaert G, Heyndrickx M, Van Deun K, Haesebrouck F. 2012. Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*. Vector Borne Zoonotic Dis. 12(2):89-98. doi: 10.1089/vbz.2011.0676. Epub 2011

Appendix G

The Drugs and Bugs in your Birds: Understanding Food Safety Implications of Antibiotic Resistance in *Campylobacter*

By: Hannah Bolinger and Sophia Kathariou

Published *Campylobacter March 9, 2015. New Food Magazine*

The bacterium *Campylobacter* (see figure 1), especially *Campylobacter jejuni* and *C. coli*, is a leading cause of human foodborne illness and a major concern for the poultry industry. In 2012, *Campylobacter jejuni* and was the second most commonly reported bacterial food borne illness and was found to be more frequent in that year than in the period from 2006-2008. Humans with campylobacteriosis experience acute gastroenteritis with diarrhea, cramping, abdominal pain and fever, generally recovering within 5-7 days. However, a subset of patients can experience severe autoimmune sequelae such as arthritis and, in an estimated 1 of every 1,000 cases Guillain-Barré syndrome, a form of paralysis that can require months for resolution and leave life-long disability. Consumption and handling of undercooked poultry is one of the most commonly implicated transmission routes for the disease [1,2].

Up to 80% of poultry flocks at slaughter are positive for *Campylobacter*[3]. Although poultry-derived *Campylobacter* can make humans sick, the birds themselves typically do not exhibit signs of illness. Growers and inspection agents cannot tell which birds do or do not harbor the bacteria without laboratory testing. *Campylobacter* is carried in the intestinal tract

(especially the cecum) of chickens and turkeys at levels as high as 1,000,000,000 viable bacterial cells per gram of cecal content[4]. Poultry carcasses become contaminated during slaughter and processing and human illness can result following ingestion of as few as 400-500 bacteria[5].

Once *Campylobacter* enters a poultry house and some birds become colonized, further transmission within the flock is rapid and extensive due in part to the close quarters and shared space within the poultry houses (see figure 2)[6]. Within-flock transmission is primarily via ingestion of feces (coprophagy) or litter, feed and water contaminated with freshly excreted feces.

What is the source of *Campylobacter* for the poultry industry pre-harvest?

The ecology of this pathogen is complex and still poorly understood, but the following have been frequently implicated:

Insect vectors, primarily flies and darkling beetles

Other farm animals (e.g. poultry, cattle, swine) in the vicinity

Compromised biosecurity, resulting in farm employees, visitors and equipment tracking bacteria from one contaminated site and farm to another

Growers and farm personnel should be aware of their potential importance in keeping a flock *Campylobacter*-free. For instance, *Campylobacter* can survive in water for various lengths of time, especially at low temperatures, and one might easily track *Campylobacter* into the poultry house from water puddles outside. Putting on single-use plastic over boots just before entering the poultry house may be helpful in minimizing exposure to

Campylobacter; plastic over boots should be changed between poultry houses in case the birds in just one house are infected. Additionally, tools and equipment should always be cleaned and sanitized before being taken into a different house.

Antibiotic resistance in *Campylobacter*

In 2013, the CDC released a list of antibiotic-resistant microbes that are major threats to human health. Drug-resistant *Campylobacter* was listed as a pathogen "with a threat level of serious", with approximately one quarter of the *Campylobacter* infections reported each year in the US being attributed to drug-resistant strains. Human *Campylobacter* infections are typically self-limited and resolve without the need for antibiotic treatment. However, antibiotic treatment may be indicated for certain patients, e.g. those with invasive illness and high risk individuals such as infants, elderly and HIV patients. In such cases, fluoroquinolones (e.g. ciprofloxacin) and macrolides (e.g. azithromycin or erythromycin) are drugs of choice. However, ciprofloxacin resistance has become frequently encountered in *Campylobacter*[7]. Fortunately, incidence of macrolide resistance in *C. jejuni* has remained low, ensuring continued therapeutic effectiveness of these antibiotics.

The emergence of ciprofloxacin resistance in *Campylobacter* from human cases in the US temporally coincided with the approval of fluoroquinolones (specifically enrofloxacin, closely related to ciprofloxacin) to control poultry diseases. A review article by Nelson et al. (see suggested readings) highlights two studies that found increasing *Campylobacter* isolates with resistance to fluoroquinolones. From 1990 to 1999, the prevalence of fluoroquinolone resistant *Campylobacter* increased from 0 to 18%. The other

study found no resistant *Campylobacter* isolates during the years of 1982-1992 but after the introduction of the fluoroquinolones in the poultry industry in the early 1990's there was a jump up to 41% in 2001[8]. Such findings led to an eventual government-mandated ban in 2005 of fluoroquinolone use in poultry pre-harvest.

What are the public health and food safety concerns related to drug resistance in *Campylobacter*?

Drug resistance in *Campylobacter* can confer enhanced food safety hazards, disease burden and economic costs in several distinct ways:

Resistance to drugs of choice for treatment of human campylobacteriosis can compromise treatment outcomes for those patients for whom treatment may be indicated.

Drug resistance may be associated with higher disease burden. For instance, drug-resistant strains may result in longer-lasting illness than strains lacking resistance[7]. It is also possible (but not yet demonstrated) that additional pathogenicity attributes are impacted in drug-resistant strains. Possible outcomes might include lower numbers required for human infection and more severe symptoms.

Drug resistance may also confer enhanced ability to colonize birds, thus increasing prevalence of *Campylobacter* in the food supply. In certain strains of *C. jejuni*, ciprofloxacin resistance was accompanied with higher ability to colonize the intestine of chickens[9]. On the contrary, and as a fortuitous public health boon, in *C. jejuni* acquisition of macrolide resistance results in impaired overall fitness and bird colonization potential[10], thus

contributing to the continuing low prevalence of such resistance in *C. jejuni* and ensuring effectiveness of macrolides as drugs of choice when treatment of human illness is indicated. Drug-resistant strains may be more able to survive in vectors and environments relevant to transmission and foodborne disease risks, including excreted feces, water, insects and protozoa. Survival may be also impacted on raw poultry, in water and milk, and on utensils, surfaces and equipment in the kitchen and in food processing and food service environments. Appropriately designed studies are needed to characterize fitness impacts such as these and those listed in 5 below.

Drug-resistant strains may have enhanced ability to tolerate abiotic stresses such as ambient oxygen (*Campylobacter* is an obligate microaerophile), sub-optimal temperature, dehydration, exposure to disinfectants or other treatments.

In addition to ciprofloxacin, resistance is commonly encountered to several other antibiotics[11]. Figure 3 shows 15 turkey-derived *C. jejuni* and *C. coli* isolates grown with and without specific antibiotics. It is evident that different isolates exhibit different antibiotic susceptibility profiles.

How does *Campylobacter* acquire resistance to antibiotics?

There are numerous methods by which *Campylobacter* can acquire resistance to an antibiotic. An innate system to pump out antibiotics (the multidrug resistance efflux pump CmeABC) has been extensively characterized in *Campylobacter* for its multiple roles not only in drug resistance but numerous other adaptations as well. This intrinsic ability of *Campylobacter* to pump harmful compounds out of the cell provides it with baseline

resistance to a number of antibiotics and other toxic compounds. At higher doses, a drug can overcome the pump's ability to get rid of the compound and additional dedicated mechanisms will be required for resistance[11,12]. However, in *Campylobacter* the simple presence of dedicated resistance determinants may not be sufficient for high levels of resistance to the corresponding antibiotics, unless a functional, intact CmeABC efflux system is also present.

Resistance mechanisms vary depending on the antibiotic. For instance, resistance to macrolides (e.g. erythromycin) and fluoroquinolones is typically conferred by simple single-base mutations in the sequence of pre-existing chromosomal genes (the 23S rRNA gene and the gene encoding DNA gyrase, respectively). Poultry can carry extremely high levels of *Campylobacter*. This means each bird can carry billions of cells and there are generally thousands of birds in each flock. Each one of those bacterial cells has the potential to undergo a mutation that would lead to macrolide or fluoroquinolone resistance. Once established in one strain, such mutations can be disseminated to others via natural transformation: as cells harboring the mutation propagate and die they release DNA which becomes taken up by other *Campylobacter* cells in the vicinity, with the potential of the mutated sequence to replace the previous wild type gene. *Campylobacter* is well known for its propensity to acquire DNA via such natural transformation.

Such mutation and gene replacement events take place at various frequencies, but the presence of the relevant antibiotic can constitute a powerful selection pressure, with the resistant version of the gene eventually predominating in the population. Potential impacts on animal colonization can constitute alternative selection pressures, as may be the case with

ciprofloxacin resistance in certain *C. jejuni* strains: if resistant strains with the mutant version of the DNA gyrase gene have enhanced capacity to colonize the chicken intestine they will eventually out-compete their wild-type parental counterparts.

Resistance can also involve acquisition of new genes, frequently from other bacteria. For instance, tetracycline resistance is typically mediated by a specific gene, *tet(O)*, which appears to have been originally acquired from other bacteria (likely *Enterococcus*), but now seems to have infiltrated the genome of most strains of *C. jejuni* and *C. coli* from agricultural source[13]. This gene is frequently harbored on autonomously replicating genetic elements (plasmids), though integration into the chromosome has also been documented. Again, stable integration of the resistance gene in the chromosome opens the way for subsequent dissemination to other campylobacters via natural transformation. In contrast, genes on plasmids become disseminated not by transformation, but instead primarily via transfer of the plasmid during conjugation: direct cell-to-cell contact between a live donor cell (which harbors the plasmid) and a live recipient cell (which will acquire the plasmid).

Mutations in pre-existing genes and acquisitions of new resistance genes are expected to take place all the time, with frequencies dependent on environmental conditions, the genetic attributes of the strains involved, and the composition of the microbial community in which *Campylobacter* finds itself. Selective pressure from the use of antibiotics is not a requirement for emergence of the resistance attributes. If, however antibiotics are used, the resistance attributes will confer a powerful selective pressure in favor of the cells harboring the resistance determinant. As discussed above, similar selective pressures can accrue from fitness impacts of the resistance (e.g. to colonization or environmental persistence).

Concluding remarks

The issue of antibiotic resistance in *Campylobacter* and other zoonotic foodborne pathogens is complex, with diverse sources for resistance and multiple factors determining the persistence of resistant strains. Agricultural and human uses of antibiotics are two major sources of selective pressures for resistance, with great potential for interventions involving reduced and judicious antibiotic use both in the farm and in the clinic. However, selection acts at the level of not only the entire microbial cell but also the entire population and the entire microbial communities of which *Campylobacter* and other pathogens are but members. In the face of reduced antibiotic exposure or even complete absence of drug use, the fate of established or newly emerging (e.g. via mutation and gene acquisition events) populations of resistant strains will be shaped by those strains' fitness trajectories, to which resistance itself may make important contributions. The case of the 2005 fluoroquinolone ban in poultry is a poignant example: no marked reductions are yet seen in prevalence of fluoroquinolone resistance in poultry-derived campylobacters. However, would this not be expected, if the resistant strains had higher ability to colonize birds and to survive, persist and disseminate in the farm ecosystem? Research that further characterizes these fitness trajectories of resistant strains is clearly needed for better estimates and predictions of the organisms' persistence and ecological success in agriculture and in human disease.

Suggested Reading

Nelson JM, Chiller TM, Powers JH, Angulo FJ. Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. 2007. Clin Infect Dis. 44(7):977-80.

http://www.fda.gov/food/foodborneillnesscontaminants/causesofillnessbadbugbook/ucm070024.htm

http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf#page=49

REFERENCES

Centers for Disease Control and Prevention (CDC). 2014. National Center for Emerging and Zoonotic Infectious Diseases. *Campylobacter*. [Accessed: December 20, 2014.] http://www.cdc.gov/nczved/divisions/dfbmd/diseases/campylobacter/#what.

Centers for Disease Control and Prevention (CDC). 2012. Trends in Foodborne Illness in the United States, 2012. [Accessed: December 23, 2014.] http://www.cdc.gov/features/dsfoodnet2012/.

Hermans D, Pasmans F, Messens W, Martel A, Van Immerseel F, Rasschaert G, Heyndrickx M, Van Deun K, Haesebrouck F. 2012. Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*. Vector Borne Zoonotic Dis. 12(2):89-98. doi: 10.1089/vbz.2011.0676. Epub 2011

Humphrey S, Chaloner G, Kemmett K, Davidson N, Williams N, Kipar A, Humphrey T, Wigley P. (2014) *Campylobacter jejuni* is not merely a commensal in commercial broiler chickens and affects bird welfare. MBio. 5(4): doi: 10.1128/mBio.01364-14

Food and Drug Administration. (2012). *Campylobacter Jejuni*. In Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins (pp. 14-17).

Adkin A, Hartnett E, Jordan L, Newell D, Davison H. 2006. Use of a systematic review to assist the development of *Campylobacter* control strategies in broilers. J Appl Microbiol. 100(2):306-15.

Centers for Disease Control and Prevention (CDC). 2013. Antibiotic Resistance Threats in The United States. [Accessed 20, 2014] http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf#page=49

Nelson JM, Chiller TM, Powers JH, Angulo FJ. Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. 2007. Clin Infect Dis. 44(7):977-80.

Luo N1, Pereira S, Sahin O, Lin J, Huang S, Michel L, Zhang Q. 2005. Enhanced in vivo fitness of fluoroquinolone-resistant *Campylobacter jejuni* in the absence of antibiotic selection pressure. Proc Natl Acad Sci USA. 102(3):541-6.

Luangtongkum T, Shen Z, Seng VW, Sahin O, Jeon B, Liu P, Zhang Q. (2012) Impaired fitness and transmission of macrolide-resistant *Campylobacter jejuni* in its natural host. Antimicrob Agents Chemother.56(3):1300-8. doi: 10.1128/AAC.05516-11.

Ge B, Wang F, Sjölund-Karlsson M, McDermott PF. 2013. Antimicrobial resistance in *Campylobacter*: susceptibility testing methods and resistance trends. J Microbiol Methods. 2013. Oct;95(1):57-67. doi: 10.1016/j.mimet.2013.06.021. Epub 2013 Jul 1

Guo B, Lin J, Reynolds DL, Zhang Q. 2010. Contribution of the multidrug efflux transporter CmeABC to antibiotic resistance in different *Campylobacter* species. Foodborne Pathog Dis. Jan;7(1):77-83. doi: 10.1089/fpd.2009.0354

Taylor DE, Hiratsuka K, Ray H, Manavathu EK. 1987. Characterization and expression of a cloned tetracycline resistance determinant from *Campylobacter jejuni* plasmid pUA466. J Bacteriol. 169(7):2984-9.

Photos:

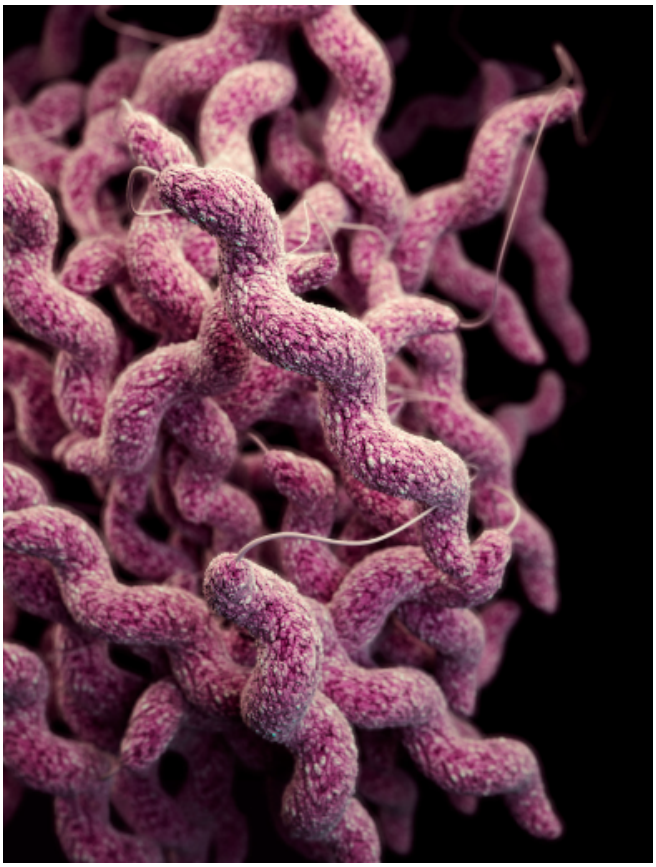


Figure 1: Artistic rendition of *Campylobacter* based on scanning electron microscopy. Courtesy of the Public Health Image Library, Centers for Disease Control and Prevention, http://www.cdc.gov/drugresistance/threat-report-2013/images/cover.jpg

Figure 2: Turkey house showing the close proximity of the birds which serves to facilitate transmission of *Campylobacter* through a flock.

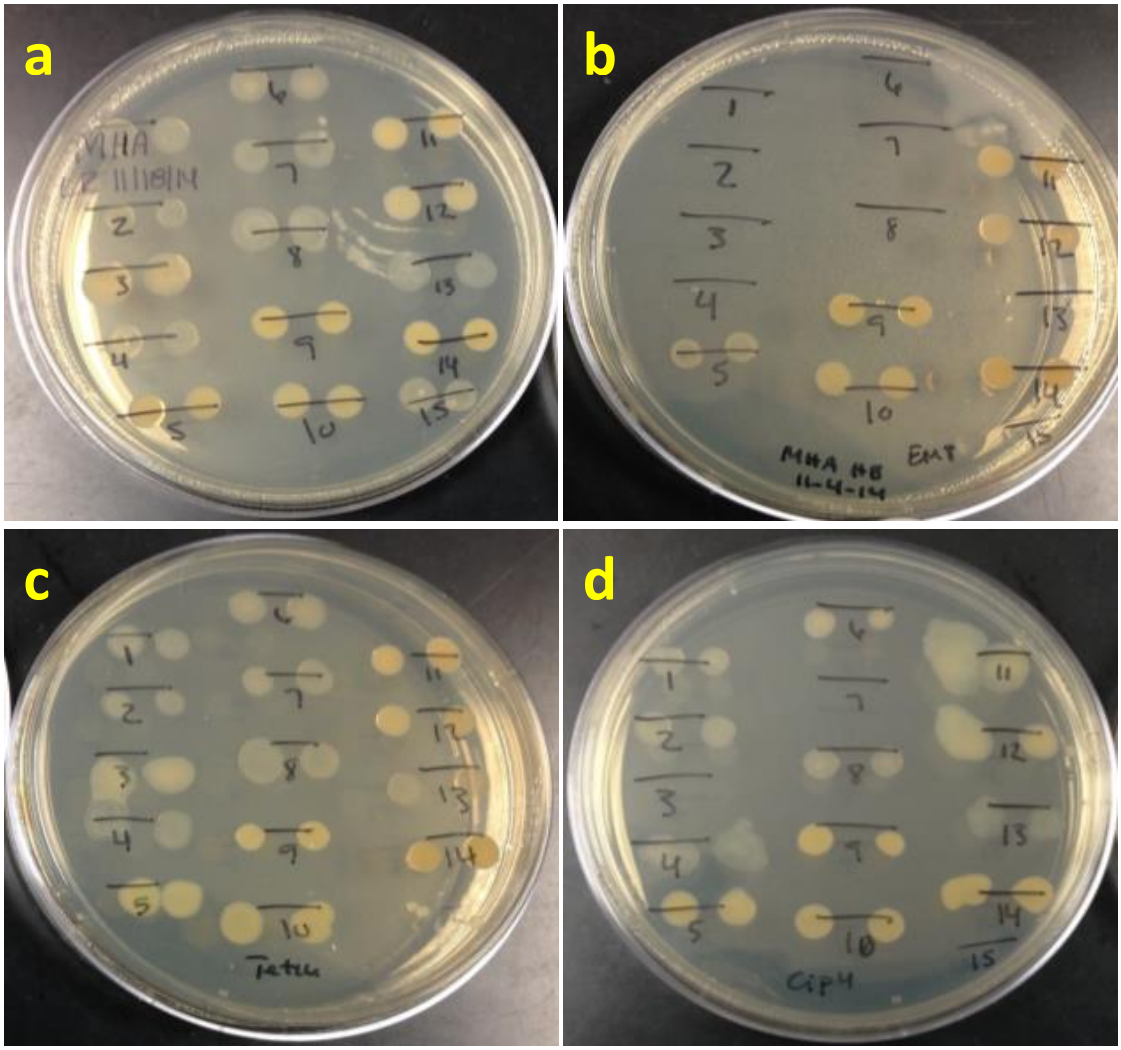


Figure 3: *Campylobacter spp.* on Mueller-Hinton agar plates with a) no antibiotic, b) 8 µg/ml erythromycin, c) 16 µg/ml tetracycline, and d) 4 µg/ml ciprofloxacin.

Credit: S. Kathariou lab