ABSTRACT

JORDAN, JOHARI SHENEE'. Diversity of *Campylobacter jejuni* and *Campylobacter coli* in the Ceca of Individual Turkeys. (Under the direction of Dr. Sophia Kathariou).

Campylobacter is a leading cause of diarrheal illness in the United States and other industrialized countries, with most cases attributed to *Campylobacter jejuni* and *C. coli* (85-90% and 5-10%, respectively). It is estimated that *Campylobacter* is annually responsible for 0.8 million (9%) of the reported foodborne illness cases in the United States. *Campylobacter* spp. colonizes numerous animals, and poultry contaminated with *Campylobacter* is major vehicle for human disease. In spite of extensive research on poultry colonization with *Campylobacter*, limited data are available concerning the diversity of *Campylobacter* within individual animals. To examine this diversity we applied phenotypic along with genotypic methods such as antimicrobial susceptibility testing and pulsed-field gel electrophoresis (PFGE) for determination of species and strain type. Although these techniques are employed during times of outbreak investigations and general laboratory practices, in most cases only one colony is chosen when determining species and strain types. In this study we assessed diversity of *Campylobacter* within the intestine (cecum) of 23 individual turkeys grown conventionally in North Carolina. For each cecum, five cultures, each originating from a single colony on the selective medium *Campylobacter* Blood-Free Selective Media (CCDA), were purified and characterized to determine species (*C. jejuni* or *C. coli*) and antimicrobial susceptibility profiles for six antibiotics (tetracycline, streptomycin, erythromycin, kanamycin, nalidixic acid and ciprofloxacin). To detect additional populations of *C. coli* we also analyzed five cultures from CCDA supplemented with erythromycin (CCDA-E), an antibiotic to which *C. coli* (but not *C. jejuni*) can be resistant. Diverse

Campylobacter strains were recovered from the cecum of 15 of the 23 birds. Of these 15 ceca that yielded diverse strains, 8 harbored diverse strains of *C. coli* while 7 harbored both *C. jejuni* and *C. coli*. Three samples yielded exclusively *C. jejuni* on CCDA but erythromycin-resistant *C. coli* on CCDA-E. PFGE analysis of isolates from the same bird revealed that isolates of the same species and the same antimicrobial susceptibility profile had indistinguishable or closely related PFGE profiles. The findings suggest that individual turkeys frequently harbor diverse strains of *C. jejuni* or *C. coli,* and that estimates based on a single colony per sample may underestimate the diversity of this pathogen in turkeys.

Diversity of *Campylobacter jejuni* and *Campylobacter coli* in the Ceca of Individual Turkeys

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DEDICATION

I would like to dedicate this thesis to my family whom always provided love and support and in the memory of my dear brother and friend Dr. Henri M. Parker and my grandfather Everett 'Papa' Jordan.

BIOGRAPHY

Born in N. Charleston, SC, Johari Jordan moved around because of her father's duties in the Navy, but finally settled down in Goose Creek, SC. She attended high school in Goose Creek, SC and went on to obtain a BS in Biology from Claflin University. Receiving a fellowship, she attended Delaware State University where she completed her MS in Agriculture focusing on Food Safety Science. Through this fellowship she had the opportunity to work at the USDA in Wyndmoor, PA under the direction of Dr. Joshua Gurtler. It was during time spent at the USDA that North Carolina State University was suggested to continue her education. After taking one year off, Ms. Jordan began her studies at NCSU in the Food, Bioprocessing and Nutrition Sciences Department.

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CHAPTER 1: Comprehensive Literature Review

1.1 Introduction

Belonging to the Campylobacteraceae family, *Campylobacter* is a gram negative, microaerophilic, non-spore forming bacterium. *Campylobacter* and other members of the Campylobacteraceae family are typically motile with a characteristic cork screw-like motion, caused by a single polar unsheathed flagellum at one or both ends of their cells (Vandamme, 2000). To obtain their energy, *Campylobacter* use amino acids or tri-carboxylic acid cycle intermediates instead of fermenting or oxidizing carbohydrates (Snelling *et al.*, 2005). Although prevalent now, it was not until the 1970's that *Campylobacter* enteritis was discovered by the collaboration of veterinarians and physicians (Butzler, 2004).

Over the past three decades, *Campylobacter* species have emerged as a significant clinical pathogen, specific to human health concerns, because of the frequent isolations from humans, animals, food and water. While there are at least 15 species of *Campylobacter*, eight of them are classified as thermophile campylobacters and are associated with foodborne illness causing mostly diarrhea. The two species of *Campylobacter* in particular that are known for their involvement in foodborne illness are *C. jejuni* and *C. coli* (Moore *et al.*, 2005; Butzler, 2004), with *C. jejuni* being implicated in more foodborne illness cases than *C. coli*.

1.1.2 *Campylobacter jejuni*

C. jejuni causes most foodborne infections (approximately 85-90% of the cases) (Altekruse *et al.*, 1999; Ahn *et al.*, 2012). However, knowing which strains of *C. jejuni* cause the most infections is important too. *C. jejuni* has two subspecies, *C. jejuni* ssp. *doylei* and *C. jejuni*

ssp. *jejuni*. To distinguish between the two, nitrate reduction and cephalothin susceptibility is used according to a study performed by Allos (2001). Because the pathogenicity of *C. jejuni* ssp. *doylei* is unknown, most research is geared towards *C. jejuni* ssp. *jejuni*. *C. jejuni* has the standard characteristics of *Campylobacter* species, however, to distinguish between *C. jejuni* and *C. coli*, the hippuricase gene is typically used which is found only in *C. jejuni*. It is has been suggested that because of its small genome, *C. jejuni* requires growth media of a complex nature, however, when pH conditions exceed 4.9 *C. jejuni* is not able to grow despite being in the presence of the necessary complex media such as CCDA (Collins and Lyne, 1985; Ketley, 1997; Vandamme, 2000; Snelling *et al.*, 2005). While it is the most frequently implicated in foodborne illnesses, *C. jejuni* is not the only *Campylobacter* species pathogenic to humans. Although there are many other *Campylobacter* species that may be associated with foodborne disease, the next more prevalent is *Campylobacter coli*.

1.1.3 *Campylobacter coli*

Although implicated in 5-10% of foodborne illness cases (Gillespie *et al.*, 2002; Tam *et al.*, 2003), much less research is done on *Campylobacter coli* than *C. jejuni*. While *C. coli* is responsible for less foodborne illnesses than *C. jejuni*, there has been an increase in its recognition as a human pathogen leading to the development of two multilocus sequence typing (MLST) systems to better understand the epidemiology of the bacteria (Dingle *et al.*, 2005; Miller *et al.*, 2005; Miller *et al.*, 2006). Unlike *C. jejuni*, *C. coli* is often resistant to the antibiotic erythromycin and other macrolides. Transformation has the ability to mediate the acquisition of high-level resistance to erythromycin in strains obtained from animal sources (Kim et al., 2006). While *C. coli* does not cause as many foodborne illnesses in

industrialized countries as *C. jejuni*, investigators in the US are noticing a high prevalence of *C. coli* in turkeys pre-harvest and at slaughter compared to the predominant presence of *C. jejuni* in Europe (Luangtongkum *et al.*, 2006; Wright *et al.*, 2008).

Campylobacter infections, while self limiting have been known to cause other complications that occur after infection such as arthritis, Reiter syndrome and the more known post-infection complication, Guillain-Barré syndrome (Altekruse *et al.*, 1998; Thomas *et al.*, 1999; Hong *et al.*, 2004). It is because of these self-limiting characteristics, that patients need nothing other than electrolyte balancing and rehydration (Peterson 1994; Koenraad *et al.*, 1997; Allos 2001; Snelling *et al.*, 2005). In those, however that may have a combination of illnesses or those who have deficient immune systems (Snelling *et. al.*, 2005). However with the ongoing use of antibiotics during animal production, there has been an increase and concern for those *Campylobacter* strains that have become resistant to some of the antibiotics used for both the treatment of humans and animals.

1.2 Historical Perspective of Campylobacter

The first documentation of *Campylobacter* did not occur until the 1970s. However, articles published by Escherich, in 1886 described a spiral-shape bacterium found in the colons of children with diarrhea. From this observation he called the illness cholera infantum; however, when trying to culture these bacteria on agar they were non-culturable (Butzler, 2004; Escherich, 1886). It was in 1985, during the Third International *Campylobacter* Workshop held in Ottawa, that Kist reported the findings of Escherich's allowing them to be fully recognized (Kist, 1985). This however, did not immediately begin

the study of *Campylobacter*, but it did allow scientists to begin to look at other sources and compare these bacteria with others found in different sources such as animals.

1.2.1 *Campylobacter* in the Veterinary World

Campylobacter has been recognized to be associated with veterinary disease for more than 40 years but because of its appearance, it was originally considered a *Vibrio*. The beginnings of *Campylobacter* in animals began with McFadyean and Stockman, two veterinarians that began observing epizootic abortions in ewes (1913). The results obtained from this research is what led to the assumption that the unknown bacterium whose morphology resembled *Vibrio,* was indeed a member of the *Vibrio* family.

Further studies included the infectious abortions in bovines that occurred in the United States as observed by Smith (1919), who described the unknown bacterium's shape as a spirillum. Upon the completion of his work, Smith noticed the bacterium present in the aborted fetuses of the bovines in his study had the same characteristics as the bacterium found in the work of McFadyean and Stockman. From this research, Smith assumed that it was indeed the same bacterium which Smith and Taylor named *Vibrio fetus* (Smith and Taylor, 1919). *Vibrio fetus* was then found in similar studies that were continually being conducted to observe the abortions that were occurring in various animals. Now having an identity, scientists were now looking into other infections, caused by *V. fetus*, which was later changed to *V. jejuni*, as well as its pathogenicity, as investigated by Stegenga and Terpstra in 1949. Prior to the study of Stegenga and Terpstra, Doyle (1944) also described a similar organism that they found commonly associated with swine dysentery (Butzler, 2004). With the breakthroughs that were being made to identify unknown bacteria in animals, there still was not an official name

for *Campylobacter*, nor had it been isolated. Until infections were noticed in humans, *Campylobacter* was still known as a vibrio or an 'unknown organism.'

1.2.2 *Campylobacter* and Humans

The first well documented case of *Campylobacter* infection occurred in the US in 1938 involving contaminated milk causing an outbreak of diarrhea, affecting 355 inmates from two state institutions that were adjacent to one another. Of the 355 effected inmates, 73 were tested negative as to having any type of bacteria present that would cause the diarrheal illness. Using microscopic methods, 31 of those same victims were indeed positive with *V. fetus* (Levy, 1946). Months later *V. fetus* was isolated from blood samples obtained from three pregnant women admitted to the hospital for fevers caused by unknown sources lasting for approximately four weeks. Of the three women, two aborted their fetuses. Vinzent *et al*. (1947) observed the blood and examined the placenta to which they found necrotic and inflammatory areas. The characteristics of *Vibrio* were being compared by King (1957) at the time, to an actual *Vibrio* spp. previously described by Vinzent. It was King's methods that showed that although this 'unknown bacterium' has certain characteristics in common with Vinzent's bacterium, their biochemical and antigenic characteristics were different (King, 1957, King, 1962, Butzler, 2004). Because of these differences, King referred to it as "related *Vibrio"* because of its difficulty to culture allowing the bacterium to be renamed later by Sebald and Véron (1963) as *Campylobacter*. Although now newly named, scientists, especially King, noticed the difficulty to culture *Campylobacter* in which classified it as viable but non-culturable. King insisted that the infections that were occurring were not rare, but difficult to detect because there were no methods devised to assist in the study of

Campylobacter. Methods for isolation unfortunately were not developed until later after her death (Butzler, 2004).

Isolation of *Campylobacter* did not occur until 1968 by Dekeyser, a scientist in Brussels, Belgium for the National Institute for Veterinary Research. In conjunction with Butzler and his team, *Campylobacter* was isolated from the feces of a 20-year-old female who had severe diarrhea and fever (Dekeyser *et al.*, 1972; Butzler, 2004). Through a special filtration method, fecal samples were resuspended in solution and passed through filters that were approximately 0.65 μm in size. Using the method should have allowed only *Campylobacter* organisms to pass through, allowing them to isolate *C. jejuni* found in the feces of this patient. The filtrate was then inoculated using a selective medium thus allowing no other enteric pathogens, which were isolated from the stools of this patient to grow. To confirm the presence of *Campylobacter*, blood was drawn from this patient for isolation purposes. The relationship between isolates from humans and those from animals such as poultry, sheep and pigs were demonstrated by using an antigenic typing method to show the invasive ability *C. jejuni* was capable of as demonstrated in poultry by agglutination and fixation with antisera raised from *C. jejuni* and *C. coli* strains referenced by Butzler (1974).

1.2.3 Clinical indications of *Campylobacter* infections

Campylobacter jejuni infections are the one of the main causes of self-limiting gastrointestinal illness in developed countries. *C. jejuni* infections can be characterized as causing diarrhea and fever with abdominal cramping occurring some of the time. However, these symptoms are also caused by other foodborne pathogens making it somewhat difficult to distinguish (Blaser *et al.*, 1979; Butzler, 2004). The incubation period for *Campylobacter*

is typically 2-5 days, and as long as 10 days. After the diarrhea occurs, some patients are known to have a period of malaise, myalgia, abdominal pain and fever with fresh blood possibly appearing in the stools by the third day of symptoms. Although diarrhea typically last for approximately 2-3 days, the abdominal cramping is known to persist well afterwards. If there are signs of fresh blood, pus or mucus present, this is a sign of colorectal inflammation, which is not uncommon with patients that have had *Campylobacter* infections. Other post *Campylobacter* infections that can occur range from mucosal oedema and hyperemia to mucosal friability. Teenage or young adult patients have more severe abdominal pain that has often mimicked acute appendicitis, however most patients have inflammation of the ileum and jejunum (Crushell *et al.*, 2004; Butzler, 2004). Although diseases such as cholecystitis, pancreatitis and peritonitis occur rarely, these immunoproliferative small intestinal diseases have been associated with *C. jejuni* infections as well (Crushell *et al.*, 2004; Lecuit *et al.*, 2004). Reactive arthritis has also been associated with *Campylobacter* infections and the more commonly known Guillain-Barré syndrome (GBS) are also rare, but are the more serious conditions of all those associated with *Campylobacter* infections. It is stated that typically 1 in three patients whom suffered from GBS have had a *Campylobacter* infection (Butzler, 2004).

1.3 Prevalence of Campylobacter in animals

Campylobacter has been known to not only effect poultry but also other animals such as swine, horses, cattle and dogs to name a few. In 1985, Manser and Dalziel, investigated the prevalence of *Campylobacter* in other animals besides poultry through fecal sampling. *Campylobacter* was found in cattle, sheep, pigs, goats, horses, antelopes, dogs and other

animals, with cattle having the highest number of positive *Campylobacter* samples while dogs had the least. Of all the campylobacters that were isolated, 95% belonged to the thermophilic group of *Campylobacter* with the most common species, *C. jejuni*, being found in cattle and sheep. Manser and Dalziel (1985) showed that outside of poultry, *Campylobacter* is commonly present in other animals, with more prevalence in cattle, sheep and pigs, with pigs having the highest carriage rate of the three. This was also noted in other countries such as Germany and Finland (Manser and Dalziel, 1985). It was also noted that while the isolation of *Campylobacter* from the goats tested were infrequent, if goat's milk is consumed unpasteurized, there would be a risk of a person becoming infected with *Campylobacter*. In addition to the previously mentioned animals, other sources of *Campylobacter* infection can come from shellfish that have been in contaminated water sources (Moore *et al.*, 2005; Wilson and Moore, 1996) and household pets, especially younger children that may come in contact with puppies that have symptoms of Campylobacter infection (Moore *et al.*, 2005; Sopwith *et al*., 2003). While it is important to detect the presence of *Campylobacter* in all animals, the prevalence of *Campylobacter* in animals used for human consumption causes for a greater understanding. Turkeys have long been associated with *Campylobacter*, specifically *C. jejuni* and recently *C. coli*.

1.4 Prevalence of Campylobacter in turkeys

Poultry, specifically turkeys typically serve as a host for the bacteria where *Campylobacter* resides as a commensal organism. While there have been extensive studies showing the prevalence of *Campylobacter* in poultry, typically broiler flocks have been most frequently studied (Newell and Fearnley, 2001). Zhao *et al.* (2001) conducted a study on the

prevalence of *Campylobacter* spp. in chicken, turkey, pork and beef used for retail purposes and found 14% of the 172 turkey samples were positive for *Campylobacter* with *C. coli* being more prevalent than *C. jejuni*. This study also showed that there were multiple serotypes or genotypes of the same species found in one sample, excluding ground turkey. The close proximity with which turkeys are grown results in high prevalence of thermophilic *Campylobacter* in organic and conventional turkey production systems (Luangtongkum *et al.*, 2006). According to Sahin *et al.* (2002) poultry used for commercial means is a major reservoir for *C. jejuni* thereby increasing the incidence of human infections through the mishandling of raw poultry or the consumption of raw and/or undercooked poultry.

With the human population becoming more health-conscious, consumers prefer more lean meat, thereby causing the consumption of turkey to rise on a steady basis. Subsequently, the consumption of turkey is parallel with that of the high prevalence of *Campylobacter* especially during processing which causes increases in the amount of occurrences of human enteritis (Shandera *et al.*, 1992). In a study conducted by Logue *et al.* (2003), *Campylobacter* was detected on the carcasses of turkeys in two processing facilities in the US. Because *Campylobacter* is found at such high rates while turkeys are alive, it can only be expected that we find *Campylobacter* present during processing and possibly even in packaged products.

1.5 Antibiotics and *Campylobacter* resistance

1.5.1 Introduction

Although *Campylobacter* is ranked fourth among diarrheal causing agents in the US, infections are self-limiting, thereby patients do not have a need for antibiotics as a treatment

option. However, for those patients with more severe cases, particularly those with autoimmune diseases, the use of antibiotics may be required. Antibiotics such as naladixic acid and ciprofloxacin (fluoroquinolones) and macrolides have been given to treat acute gastroenteritis. However, the resistance that the bacteria may develop to these antibiotics will have an impact on the how effective they will be.

In the afore mentioned research conducted by Butzler and Dekeyser (1973), stool samples were taken from not only patients but also from individuals that showed no signs of illness to search for a specific antibody serum to develop a therapeutic scheme at the time. They found antibodies with specific complement fixing capabilities to strains of *C. jejuni* when isolated from the stools of children with diarrhea. It was in this study that they also noticed that *C. jejuni* was highly susceptible to erythromycin, which allowed this antibiotic to be used in therapeutic treatments (Butzler, 1973; Butzler *et al.*, 1974; Butzler, 2004). Researchers and physicians were able to conclude with the use of erythromycin in clinical diarrhea cases that *C. jejuni* was the cause because erythromycin does not have any effect on other pathogens that affect the intestinal tract (Butzler, 2004).

1.5.2 Antibiotic Use in Turkey Production

Various antibiotics are used in the conventional protection of turkeys. This causes concern because the *Campylobacter* strains found in these poults and adult turkeys may become resistant to the antibiotics that are used during turkey production (Endtz *et al.*, 1991; Smith *et al.*, 1999; McDermott *et al.*, 2002). Antibiotics of the quinolones family are often used as a treatment option when *Campylobacter* infections are more severe and require the use of antibiotics (Butzler, 2004). Because of *Campylobacter*'s increased resistance that has

occurred over the years, the Food and Drug Administration (FDA), Center for Veterinary Medicine (CVM) in the US banned the use of fluoroquinolones in 2005 (Parsons, 2012).

1.5.3 Tetracycline

Tetracycline is an antibiotic that is used to treat against both aerobic and anaerobic bacteria that cause infections. This antibiotic has been used as a therapeutic treatment and growth promoter on commercial farms in food animals. Tetracycline's resistance mechanism consists of preventing protein synthesis by binding to a ribosome acceptor site preventing the attachment of aminoacyl-tRNA (Chopra and Roberts, 2001). Without this, the elongation process involved with making proteins ceases (Taylor and Tracz, 2005).

Resistance to tetracycline in *Campylobacter* is because of the production of the Tet(O) protein that is capable of binding to ribosomes thereby inhibiting tetracycline's function when inside the bacterial cell. However, unlike other antibiotics, when Tet(O) binds to post-translocation ribosome, protein synthesis still occurs in the presence of tetracycline (Taylor and Courvalin, 1988; Noah *et al.*, 1999; Chopra and Roberts, 2001;Connell *et al.*, 2003). As with other antibiotics, which will be discussed later, it is thought that the increased use of tetracycline for therapeutic and growth promotion purposes in animals produced for commercial use has caused an increase in resistance to tetracycline, especially in the case of *Campylobacter* (Chopra and Roberts, 2001; Gibreel *et al.*, 2004).

1.5.4 Streptomycin and Kanamycin: the Aminoglycosides

Aminoglycosides like streptomycin and kanamycin are known to act against aerobic bacteria such as staphylococci and a few mycobacteria. The properties of aminoglycoside antibiotics allow them to bind to the 30S ribosomal subunit thus interfering with protein

synthesis interrupting the elongation process and interfering with proofreading activity of the ribosome. Because of this proteins are not translated correctly. (Jana and Deb, 2006; Zhang and Plummer, 2008).

1.5.5 Erythromycin

Belonging to the macrolide class of antibiotics, erythromycin is the antibiotic of choice when *Campylobacter* infections occur in humans with immunocompromised systems. When used to fight off bacterial infections, erythromycin, like tetracycline, prevents the synthesis of proteins by inhibiting translocation, which is needed for elongation of the peptide chain causing interference in the production of proteins (Gibreel and Taylor, 2006). The resistance that some bacteria have towards erythromycin is associated with mechanisms such as target modification, enzymatic inactivation or enhanced efflux of drugs (Gibreel and Taylor, 2006). It has also been observed that this could also prevent erythromycin from attaching to its specific target (Nakajima, 1999; Poehlsgaard and Douthwaite, 2005).

1.5.6 Fluoroquinolones: Nalidixic acid and Ciprofloxacin

Fluoroquinolones, a bactericidal drug that inhibits DNA gyrase, were first introduced in 1986 to the US treating typical infections that occurred in humans. Topoisomers are important to the supercoiling of chromosomal DNA and without it, replication of DNA is interrupted. However, a point mutation in the fluoroquinolones binding site can lead to resistance to fluoroquinolones (Hooper *et al.*, 1987; Altekruse and Tollefson, 2003; Nelson *et al.*, 2007).

Before fluoroquinolones were introduced for human use in the US, fluoroquinolone-resistant *Campylobacter* strains from human infections were uncommon. These strains

increased drastically when fluoroquinolones were approved for use in poultry (Friedman *et al.*, 2000; Altekruse and Tollefson, 2003). Fluoroquinolone resistant *Campylobacter* strains that caused infections have the possibility to increase the duration of symptoms and hospitalization. Using fluoroquinolones in poultry farming may have contributed to the compromise of the use of fluoroquinolones in humans (Nelson *et al.*, 2007). Because of these issues the FDA prohibited the use of fluoroquinolones in 2005 (Nelson *et al.*, 2007; Smith and Fratamico, 2010). Multidrug resistance has been indicated in studies (Ge *et al.*, 2003; Luangtongkum *et al.*, 2006) observing turkeys that were raised in a conventional manner.

1.6 Phenotypic and Genotypic methods of typing Campylobacter (Detection methods)

1.6.1 Introduction

The genus *Campylobacter* consists of related Gram-negative bacteria that are typically found in the gastrointestinal tracts of a various animal species (Wassenaar and Newell, 2000). To characterize and improve the epidemiology of *Campylobacter*, genotyping techniques can be used. The use of molecular subtyping is useful when investigating sources of infection occurrences and possible routes of transmission in animals and humans (Wassenaar and Newell, 2000). Antibiotic susceptibility testing, *fla*-typing, Multilocus Sequence Typing (MLST) and pulsed-field gel electrophoresis (PFGE) along with many other methods can be used to further characterize foodborne agents such as *Campylobacter* on a phenotypic and genotypic level. However the use of genotyping methods can be more highly effective than the use of phenotypic methods alone. The use of molecular subtyping has become a staple in outbreak investigations (Gissendorf *et al.*, 1995).

Typically two methods are necessary to obtain reliable data, especially for *Campylobacter* because of its high diversity and weakly clonal population (Wassenaar and Newell, 2000)

1.6.2 Antibiotic susceptibility testing

There are various methods that can be used when performing antimicrobial susceptibility testing when trying to determine the resistance level of a particular species of bacteria or when trying to determine the similarities and differences amongst genus and species of bacteria. These methods include disc diffusion, broth microdilution, agar dilution and the Epsilometer-test (E-test). These tests have been used when trying to determine the susceptibility profiles of *Campylobacter* spp. to a series of antibiotics that are typically used in laboratory settings and some that may be used in production. However, of these methods, the only approved method by the National Committee for Clinical laboratory Standards (NCCLS) is an agar dilution protocol (Moore *et al.*, 2005). The use of antibiotics has been around for years in the use of human and animal treatment. Now, however, as the use of various antibiotics increases, so does the resistance of particular bacterial strains. For this reason, the use of antibiotic susceptibility testing is used for strain typing before moving on to a more expensive subtyping method such as PFGE.

1.6.3 Pulsed-Field Gel Electrophoresis (PFGE)

To differentiate *C. jejuni* from *C. coli*, hippurate hydrolysis test can used because *C. jejuni* is positive Skirrow and Benjamin, 1980). However, a more updated method to differentiate between *C. jejuni* and *C. coli* is the use of multi-plex PCR by the use of species-specific primer, *hip* (*C. jejuni*) and *ceuE* (*C. coli*) (Marshall *et al.*, 1999; Houng *et al.*, 2001). Strains can be further analyzed by use of antimicrobial susceptibility testing and by

genotypic methods, such as Pulsed-Field Gel Electrophoresis (PFGE).

Developed in 1984 by Schwartz and Cantor (1984) PFGE is a highly discriminatory method used for genotyping bacteria (On *et al.*, 2008). A standardized protocol for PFGE has been established in which bacterial cells are combined with melted agarose and poured into molds specifically for PFGE plugs resulting in plugs of agarose containing bacterial cells. The bacteria (depending on type) are then typically washed in a lysis buffer and digested with restriction enzymes that rarely cut. The DNA fragments are separated by the use of an electrophoresis machine where the polarity of the current is changing intermittently running in three different directions. This allows for complete separation of large DNA fragments that would not be easily detected using the standard agarose and electrophoresis methods. The gel is stained with ethidium bromide and patterns are visualized using a high-resolution camera. The enzymes typically used for PFGE include *Sma*I, *Sal*I, *Kpn*I, *Apa*I and *Bss*HII. Most studies genotyping *Campylobacter* have used the *SmaI* enzyme while *KpnI* is typically chosen next (Ribot *et al.*, 2001, Michaud *et al.*, 2000; Fitzgerald *et al.*, 2001). While PFGE is often used, Wassenaar and Newell (2000) have noted that it is not only time consuming, but it is also tedious and requires specialized equipment but is still considered a 'gold standard' when performing molecular epidemiology studies for outbreaks and surveillance studies.

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CHAPTER 2: Diversity of Campylobacter jejuni and C. coli in the Ceca of Individual Turkeys

2.1 Abstract

Campylobacter, primarily *C*ampylobacter *jejuni* and *C. coli,* is a leading cause of human diarrheal illness in the United States and contaminated poultry are a major vehicle. Pre-harvest surveillance of *Campylobacter* typically involves isolation of a single culture per sample, which fails to yield information on potential diversity of the pathogen within individual samples. In this study we assessed diversity of *Campylobacter* within the cecum of 23 individual turkeys grown conventionally in North Carolina. For each cecum, five cultures, each originating from a single colony on *Campylobacter* blood-free selective medium (CCDA), were purified and characterized to determine species (*C. jejuni* or *C. coli*) and antimicrobial susceptibility profiles for six antibiotics (tetracycline), streptomycin, erythromycin, kanamycin, nalidixic acid and ciprofloxacin). To detect additional populations of *C. coli* we also analyzed five cultures from CCDA supplemented with erythromycin (CCDA-E), an antibiotic to which *C. coli* (but not *C. jejuni*) can be resistant. Diverse *Campylobacter* strains were recovered from the cecum of 15 of the 23 birds. Of these 15 ceca that yielded diverse strains, 8 harbored diverse strains of *C. coli* while 7 harbored both *C. jejuni* and *C. coli*. Three samples yielded exclusively *C. jejuni* on CCDA but erythromycin-resistant *C. coli* on CCDA-E. Pulsed-field gel electrophoresis (PFGE) analysis of isolates from the same bird revealed that isolates of the same species and the same antimicrobial susceptibility profile had indistinguishable or closely related PFGE profiles. The findings suggest that individual turkeys frequently harbor diverse *Campylobacter* strains,

and that estimates based on a single colony per sample may underestimate the diversity of this pathogen in turkeys.

2.2 Introduction

Campylobacter is a leading cause of human diarrheal illness in the United States and other industrialized countries. *C. jejuni* causes 85% of all cases of campylobacteriosis and *C. coli* follows, causing 5-10% of the cases (Allos, 2001). Of the 31 major pathogens that cause 9.4 million episodes of foodborne illness in the United States, *Campylobacter* is responsible for 0.8 million (9%) of the cases and 15% of the hospitalizations (Scallan *et al.*, 2011). The pathogen is found within the gastrointestinal tract of animals and human infection is caused by the consumption of those animals, especially poultry. Symptoms of campylobacteriosis typically include diarrhea, fever and abdominal cramps and at times cannot be distinguished from those caused by other pathogens such as *Salmonella*, *Shigella* and *Yersinia*. Campylobacteriosis can also be accompanied by long-term sequelea such as Guillain-Barré syndrome (GBS), an acute demyelination of the peripheral nervous system, and Miller Fisher syndrome. GBS typically affects 2 persons per 100,000 in the United States each year and is especially likely after infection with *C. jejuni* strains of particular serotypes (Skirrow and Blaser, 2000; Allos, 2001). The occurrence of diseases such as GBS show that *Campylobacter* infections have a serious impact on the safety of foods provided for human consumption. Antibiotics are not typically used in campylobacteriosis. When antibiotic treatment is indicated, the antibiotics of choice are ciprofloxacin and erythromycin (Skirrow and Blaser, 2000; Allos, 2001).

Antibiotics are used on farms when animals become ill or to prevent illness from occurring, particularly in younger animals. The use of antibiotics however, can cause complications because the *Campylobacter* that is typically found in these animals may

become resistant, with the potential for human infections with antibiotic-resistant strains (Engberg et al., 2001). Because *C. jejuni* and *C. coli* are predominately implicated in human disease it is important to understand the antimicrobial susceptibility attributes of these species.

Pre-harvest surveillance of antimicrobial susceptibility in *Campylobacter* from animals typically involves the characterization of strains obtained from feces or intestinal contents. In a longitudinal study conducted by Quintana-Hayashi and Thakur (2012) we find evidence of such practices. In this study the authors investigated the persistence of antimicrobial resistant *Campylobacter* strains in swine production. They obtained isolates from fecal samples of swine as well as surrounding areas, however upon isolation of presumptive positive *Campylobacter* isolates, only one colony was chosen for further characterization. Thakur and Gebreyes (2010) took a different approach and chose three presumptive *Campylobacter* colonies from each fecal/carcass sample. While studies such as these are being conducted and the use of more than one presumptive *Campylobacter* colony is being chosen in other animals, we see no evidence of these same practices occurring in poultry, specifically turkeys. Thus there is need to assess the diversity of *Campylobacter* in individual birds and to determine if it is indeed necessary to chose more than one presumptive *Campylobacter* colony in future investigations.

2.3 Materials and Methods

Bacterial strains and growth conditions

Campylobacter isolates characterized in this study are listed in Table 1. The isolates were derived from the cecum of 23 turkeys. Each bird was from a different flock grown conventionally in North Carolina, and, in most cases, a different farm (Table 1). Ceca were kindly provided to us by Dr. Stephen Clarke, who obtained them from turkey flocks participating in the Turkey Intestinal Health Survey Program for conventionally grown turkeys. Each cecum was placed in a sterile zip-lock bag and transported to the laboratory on ice.

Cecal contents (approx. 0.1 g) were directly plated on plates of *Campylobacter* Blood-Free Selective Media (CCDA) (Oxoid, Basingstoke, England) and on CCDA supplemented with erythromycin (10 μg/ml; Sigma, St. Louis, MO) (CCDA-E). Plates were incubated for 48 h at 42°C in anaerobic jars containing a CampyPak Plus microaerobic system (Becton Dickinson, Sparks, MD). Single colonies (for each sample, up to 5 each from CCDA and CCDA-E) were further purified on Mueller-Hinton agar (MHA; Difco, Becton Dickinson) or sheep blood agar (SBA; Remel, Lenexa, Kansas) under microaerobic conditions and pure cultures were preserved at -80°C (Smith *et al.*, 2004). To determine species (*C. jejuni* or *C. coli*), multiplex polymerase chain reaction (PCR) was used with *hip* and *ceu* primers, as described (Gu *et al*., 2008). Bacteria were preserved at -80°C in cryovials containing brain heart infusion (BHI) medium (Difco, Sparks, MD) with 20% glycerol.

Antimicrobial susceptibility profile determination

Antimicrobial susceptibility profile determination was performed as previously described (Gu *et al.*, 2008) except that the following antibiotics were used: tetracycline (10 µg/ml), streptomycin (15 µg/ml) and erythromycin (10 µg/ml). Antibiotics were purchased from Sigma Chemical Co. (St. Louis, MO) except kanamycin (25 µg/ml), nalidixic acid (20 µg/ml) and ciprofloxacin (4 µg/ml), which were purchased from Fisher Biotech (Fair Lawn, NJ). *C. jejuni* ATCC 33560 (purchased from the American Type Culture Collection) was used as quality assurance strain for antibiotic susceptibility testing.

***Pulsed-field gel electrophoresis* (PFGE)**

Selected isolates were cultured on MHA for 48 h at 42°C. PFGE was performed using *Sma*I (New England Biolabs) according to the PulseNet protocol (http://www.cdc.gov/PULSENET/protocols.htm) and as performed by D'lima *et al.* (2007). Tagged image file format (tiff) images of banding patterns from PFGE were analyzed using the BioNumerics (version 4.6; Applied Maths, Saint-Marten-Latem, Belgium) software.

2.4 Results

In this study, cecal contents from 23 *Campylobacter*-positive birds were examined in terms of the diversity of isolates in the same bird. Multiple isolates from each bird were obtained by direct plating on CCDA and on CCDA supplemented with erythromycin (CCDA-E) and were characterized in terms of species (*C. jejuni* or *C. coli*), antimicrobial susceptibility profile and PFGE. Table 1 provides a summary of all isolates obtained from the 23 individual birds.

The majority (15/23, 65%) of the ceca were found to harbor more than one *Campylobacter* strain. Diversity was suggested by the detection of both *C. jejuni* and *C. coli* in several of these samples, as well as by the fact that isolates from the same bird exhibited diverse antimicrobial susceptibility profiles, even when they were the same species.

C. jejuni was identified among the CCDA-derived isolates from seven birds, either exclusively (birds 12-14) or together with *C. coli* (birds 8-11) (Table 2). When multiple isolates of *C. jejuni* were obtained from the same cecal sample, all were found to have the same antimicrobial susceptibility profile with the exception of sample 13, from which one of the five *C. jejuni* isolates had profile TSQ, while the other four were TKQ (Table 2). *C. coli* was obtained when the cecal contents of these birds were plated on CCDA-E, even from the three samples which yielded exclusively *C. jejuni* on CCDA (Table 1). There were no cases where *C. jejuni* was detected on CCDA-E.

Several (n=17) ceca yielded exclusively C. coli, but eight of these (birds 1-7 and bird 15) yielded strains with two or, less commonly, three different antimicrobial susceptibility profiles (Table 2). For most of these samples, the isolates obtained on CCDA-E were

Table 1. Antimicrobial susceptibility profiles of *Campylobacter* isolated on CCDA and CCDA-E.

Bird	Farm	Company	Age	CCDA[1]	CCDA-E
1	1	A	35	TQ (n=4), TEQ (n=1)	TEQ (n=5)
2	2	A	28	TSEKQ (n=4), TSEK (n=1)	TSEKQ (n=5)
3	2a	A	28	TQ (n=2), TSEKQ (n=2), TK (n=1)	TSEKQ (n=5)
4	3	A	20	TSEKQ (n=2), TQ (n=3)	TSEKQ (n=5)
5	4	A	14	TSEKQ (n=4), TQ (n=1)	TSEKQ (n=5)
6	5	A	34	TQ (n=2), TEQ (n=1), TSEKQ (n=2)	TSEKQ (n=3), TEQ (n=2)
7	6	B	10	TSEKQ (n=4), TE (n=1)	TSEKQ (n=3), TEKQ (n=2)
8	7	A	24	TSEKQ (n=4), **TSK (n=1)**	TSEKQ (n=5)
9	8	B	43	TSEKQ (n=4), **TSKQ (n=1)**	TSEKQ (n=5)
10	9	C	26	TSEKQ (n=4), **TSKQ (n=1)**	TSEKQ (n=5)
11	10	C	42	TSEKQ (n=2), **TSQ (n=3)**	TSEKQ (n=5)
12	11	A	13	**TSQ (n=5)**	TSEKQ (n=5)
13	12	C	50	**TKQ (n=4), TSQ (n=1)**	TSEKQ (n=5)
14	13	C	42	**TSQ (n=5)**	TSEKQ (n=5)
15	7a	A	24	TSEKQ (n=5)	TSEKQ (n=4), TEKQ (n=1)
16	14	A	11	TSEKQ (n=5)	TSEKQ (n=5)
17	15	A	21	TSEKQ (n=5)	TSEKQ (n=5)
18	16	A	39	TSEKQ (n=5)	TSEKQ (n=5)
19	17	A	24	SEQ (n=5)	SEQ (n=5)
20	18	A	32	TSEKQ (n=5)	TSEKQ (n=5)
21	19	B	21	TSEK (n=5)	TSEK (n=5)
22	20	C	63	TSEKQ (n=5)	TSEKQ (n=5)
23	21	C	42	TSEKQ (n=5)	TSEKQ (n=5)

T=Tetracycline, S=Streptomycin, E=Erythromycin, K=Kanamycin, Q= Quinolones (Naladixic Acid and Ciprofloxacin). Isolates with antimicrobial susceptibility profiles in bold are *C. jejuni*.

erythromycin-resistant *C. coli* of antimicrobial susceptibility profiles (primarily TSEKQ) also identified among CCDA-derived isolates. However, occasionally (birds 7 and 15) erythromycin-resistant *C. coli* obtained on CCDA-E exhibited profiles not encountered among CCDA-derived *C. coli* (Table 1).

It could be noted that when two different antimicrobial susceptibility profiles were detected among isolates from the same cecum, one frequently predominated (e.g. found in 4 of the 5 isolates of the same species) (Table 2). Similarly, in three of the four ceca that yielded both *C. jejuni* and *C. coli* on CCDA, one species (*C. coli*, in all three cases) predominated (4 of the five isolates obtained on CCDA (Table 1).

No evidence for diversity could be obtained for isolates from eight of the 23 samples (birds 16-23) (Table 1): All ten isolates from these samples were of the same species (*C. coli* in all cases), and had the same antimicrobial susceptibility profile regardless of whether they were obtained on CCDA or CCDA-E. Of the eight samples, six (16-18, 20, 22, 23) yielded *C. coli* resistant to all the antibiotics in the panel (profile TSEKQ), while isolates from the remaining two samples (19 and 21) had profiles TSE and TSEK, respectively (Table 1).
For five samples, the inclusion of CCDA-E allowed us to detect diversity that might have been missed if only CCDA had been employed. This was especially notable for the three samples (birds 12-14) from which all CCDA-derived isolates were *C. jejuni*, while *C. coli* was isolated on CCDA-E (Table 2). For the other two samples (birds 7 and 15) some of the erythromycin-resistant C. coli isolates obtained on CCDA-E exhibited an antimicrobial susceptibility profile (TEKQ) not encountered among those obtained on CCDA (Table 2), as

Table 2. Antibiotic profile of isolates that were *C. jejuni* positive cecal contents on CCDA.

Strain	Farm[1]	T	S	E	K	Q
SC1825-5	9-C	+	+	-	+	+
SC1830	12-C	+	-	-	+	+
SC1830-2	12-C	+	-	-	+	+
SC1830-3	12-C	+	-	-	+	+
SC1830-4	12-C	+	+	-	-	+
SC1830-5	12-C	+	-	-	+	+
SC1834-2	10-C	+	+	-	-	+
SC1834-3	10-C	+	+	-	-	+
SC1834-4	10-C	+	+	-	-	+
SC1836	13-C	+	+	-	-	+
SC1836-2	13-C	+	+	-	-	+
SC1836-3	13-C	+	+	-	-	+
SC1836-4	13-C	+	+	-	-	+
SC1836-5	13-C	+	+	-	-	+
SC1789-3	8-B	+	+	-	+	+
SC1748-5	7a-A	+	+	-	+	-
SC1774	11-A	+	+	-	-	+
SC1774-2	11-A	+	+	-	-	+
SC1774-3	11-A	+	+	-	-	+
SC1774-4	11-A	+	+	-	-	+
SC1774-5	11-A	+	+	-	-	+

Lower case letter indicates different turkey house on same farm.
Capital letter indicates company.

described above. Figure 1 provides a graphical representation of the diversity and or lack of diversity detected in each bird.

PFGE-based analysis

PFGE was used to further analyze a subset of the isolates to determine whether strains from the same sample could be genotypically different although they were of the same species and showed the same antimicrobial susceptibility profile. .

Multiple isolates from several birds were analyzed by PFGE. In most cases, isolates from the same bird, and of the same species with the same antimicrobial susceptibility profile, had indistinguishable PFGE profiles (Figures 2 and 3). For instance, multiple *C. coli* isolates with the TSEKQ antimicrobial susceptibility profile and all derived from birds 20 and 23 exhibited the same PFGE pattern (see Table 1 and Figure 3). We only noted one instance of isolates from the same bird and with the same antimicrobial susceptibility exhibiting certain differences in PFGE profiles. Specifically, analysis of five C. coli TSEKQ isolates from bird 4 revealed that one exhibited certain PFGE band differences from the other four, even though the PFGE profiles were related (Figure 3). Our analysis also revealed that C. coli isolates from the same bird but with different antimicrobial susceptibility profiles also had clearly distinct PFGE patterns (Figure 3). In figure 4 we see the same instance with bird 19. These isolates were also *C. coli* strains with the same antimicrobial susceptibility profile, however unlike its counterparts, these isolates profile were SEQ.

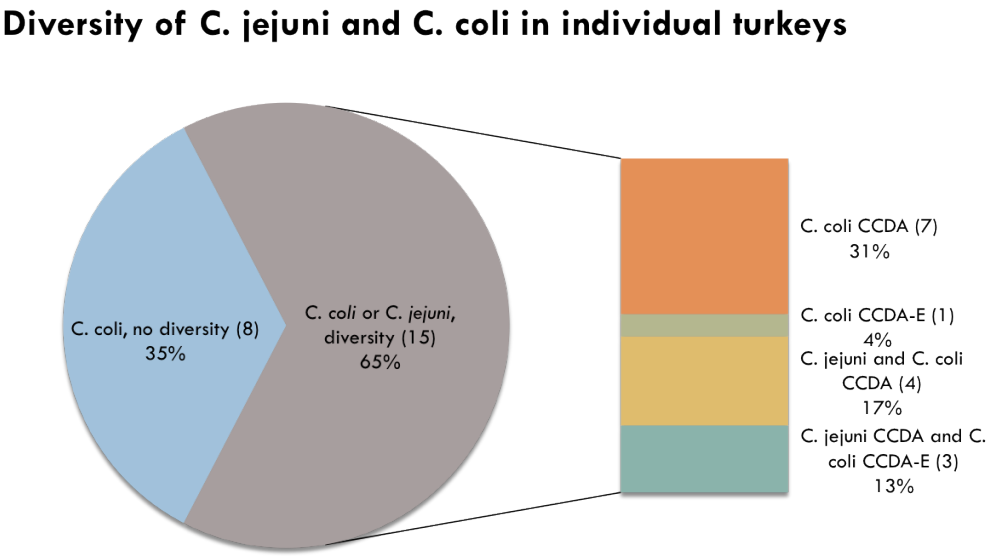

Figure 1. Categorization of birds based on diversity of isolates. No diversity represents birds for which all isolates were *C. coli* with the same antimicrobial profile. Diversity shows a mixture of *C. coli* and *C. jejuni*. Extended bar represents further analysis of diversity shown in the birds.

Figure 3 provides a visual of *C. coli* isolates that have the same and varying antimicrobial susceptibility profile. As expected, those with varying profiles (i.e. TSEKQ vs. TQ) showed different PFGE profiles. However, isolates from bird 4 showed a difference in banding patterns when comparing lanes 2, 6 and 7 with lane 5. While their antimicrobial susceptibility profiles are the same and they are all *C. coli* isolates, the addition and absence of three bands indicates two different strains of *C. coli* with the same antibiotic susceptibility profile. Lanes 3 and 4 having TQ profiles adds an additional strain suggesting three different strains of *C. coli* in bird 4. In bird 6 we see a similar pattern in that varying antibiotic profiles give different strain types. Other instances of the presence of more than one strain found in a bird can be observed in the figure 4 with bird 15. In this instance, bird 15 isolates were all *C. coli*, however the isolates antimicrobial susceptibility profiles were varying in that there was an isolate whose profile indicated there may have been more than one strain present. As we can see in figure 4, bird 15 indeed has two different strains of *C. coli* with different profiles.

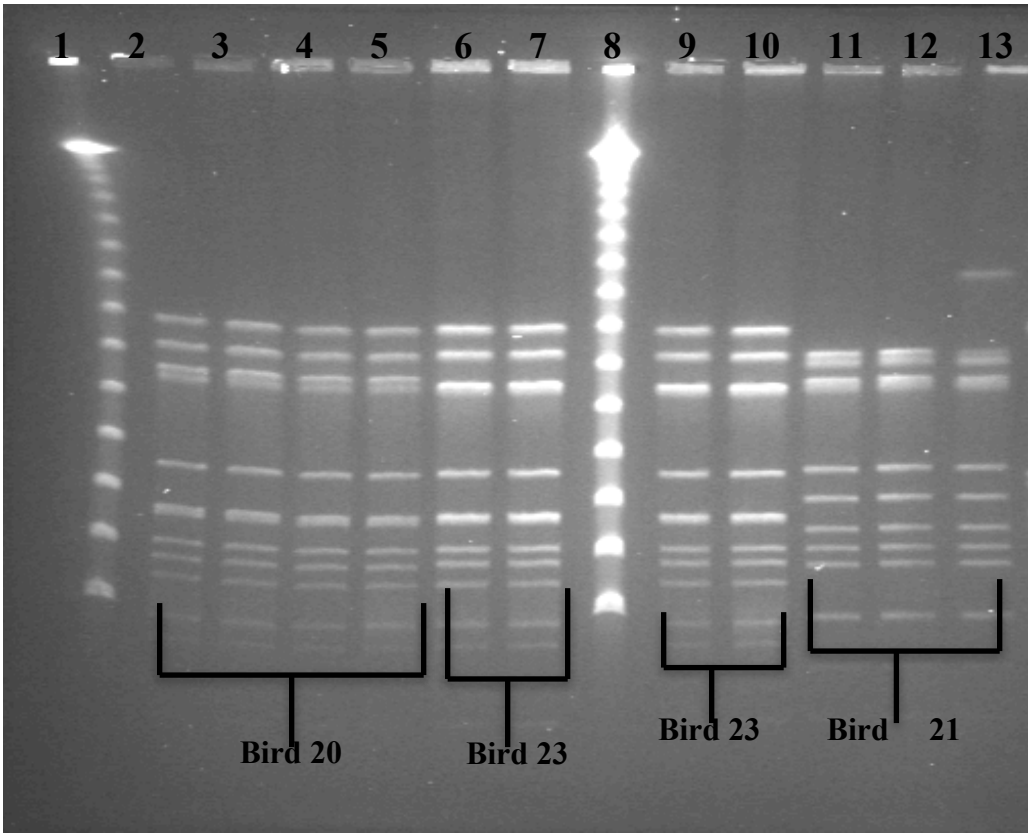

Figure 2. Pulsed-field gel electrophoresis of *C. coli* isolates from the same bird and having the same antimicrobial susceptible profile. Lanes: 1 and 8, Lambda ladder PFGE marker; 2-5, (2-5: isolates SC1821-3, SC1821E, SC1821E-3, and SC1821E-4 (all TSEKQ *C. coli* from bird 20); 6-7, 9-10: isolates SC1835-3, SC1835-4, SC1835E-3 and SC1835E-4 (all TSEKQ *C. coli* from bird 23); 11-13, isolates SC1817-4, SC1817E-3 and SC1817E-4 (all TSEK *C. coli* from bird 21).

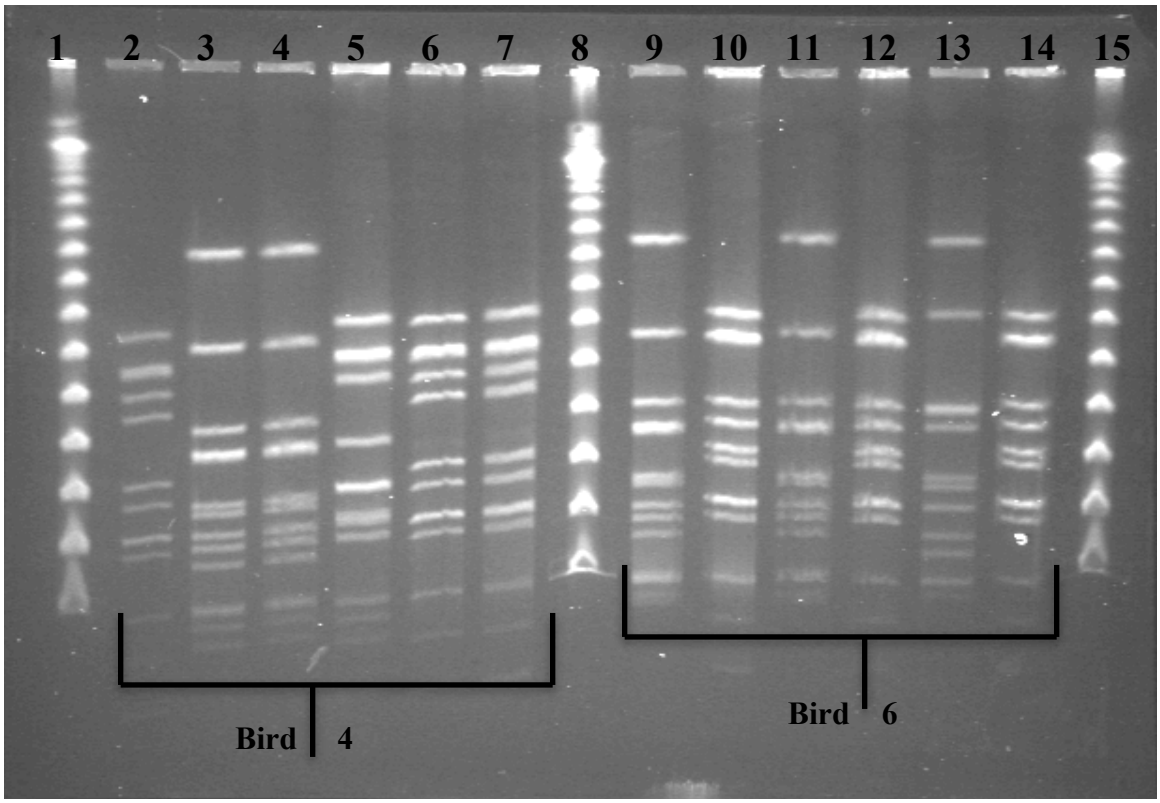

Figure 3. Pulsed-field gel electrophoresis of *C. coli* isolates from individual birds with varying antimicrobial profiles. Lanes 1 8 and 15, Lambda ladder PFGE marker.; lanes 2-7: isolates SC1769, SC1769-3, SC1769-5, SC1769E-2, SC1769E-4, SC1769E-5; lanes 2 and 5-7, TSEKQ *C. coli* from bird 4; 3 and 4, TQ *C. coli* from bird 4; 9 and 11, TQ *C. coli* from bird 6; 10, 12 and 14, TSEKQ *C. coli* from bird 6; 13, TEQ *C. coli* from bird 6.

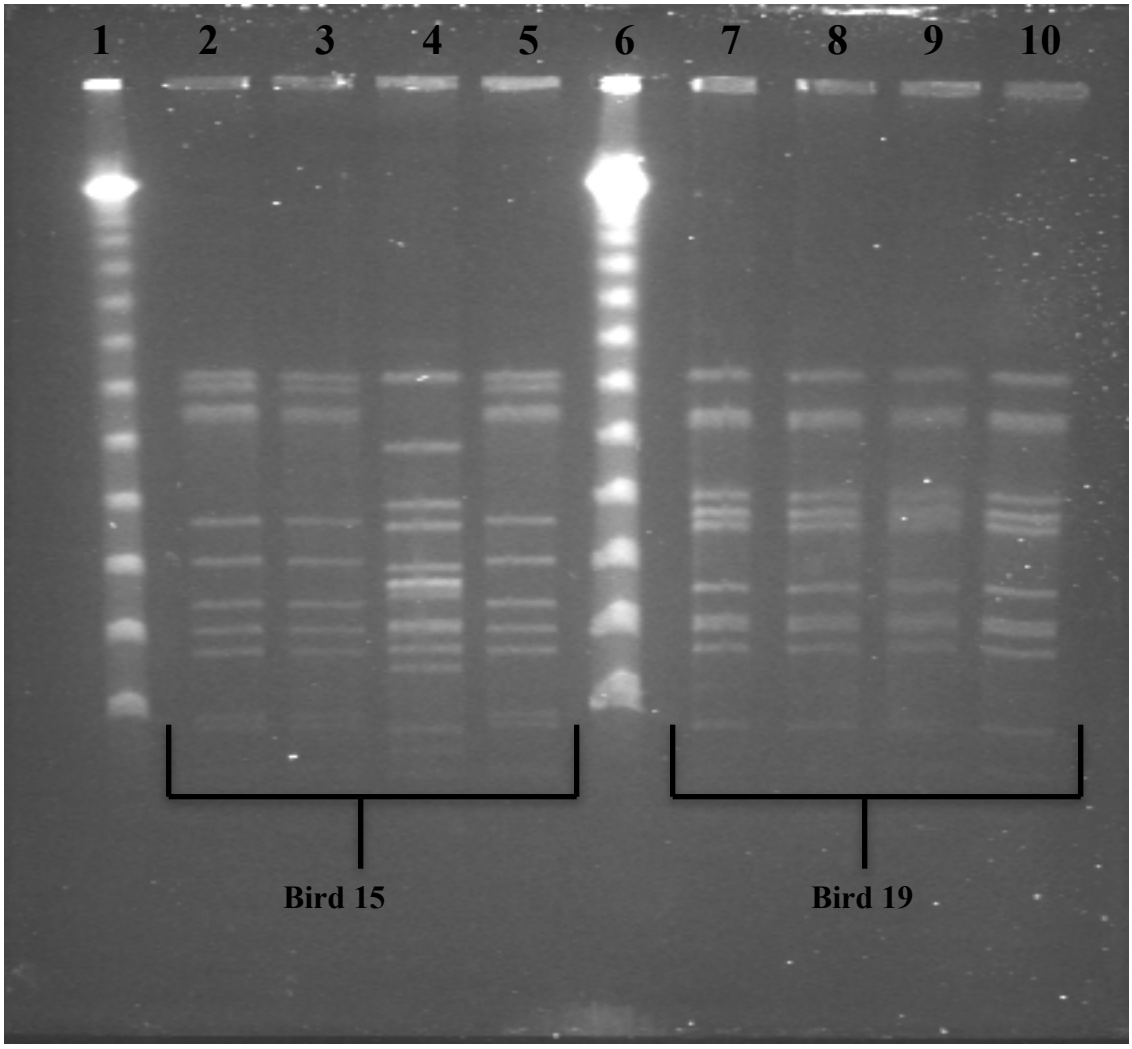

Figure 4.PFGE of *C. coli* isolates from individual birds. Lanes 1 and 6 contain the Lambda Ladder PFGE marker. Lanes 2-5 isolates SC1745, SC1745-2, SC1745E and SC1745E-2; lanes 2, 3, 5 TSEKQ; lane 4 TEKQ. Lanes 7-10 isolates (Farm 17) SC1764-2, SC1764-3, SC1764E-2, and SC1764E-3 (all SEQ).

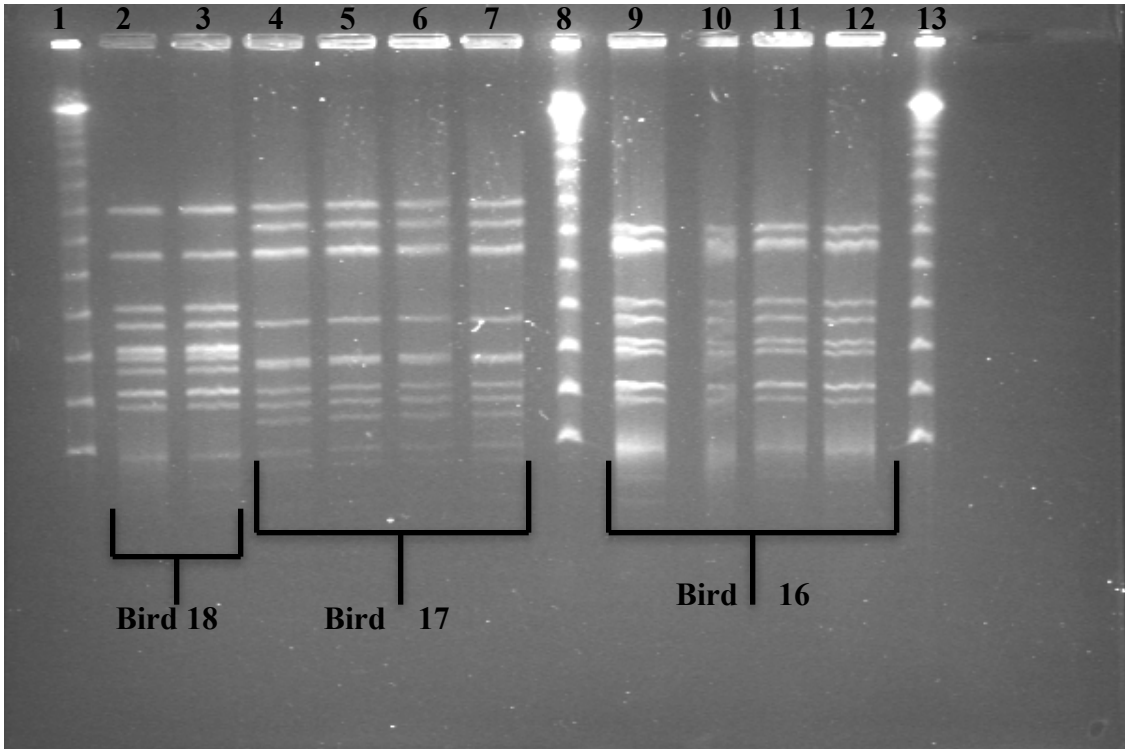

Figure 5. PFGE of *C. coli* isolates from individual birds. Lanes 2-3 isolates SC1742 and SC 1742-2 (all TSEKQ). Lanes 4-7 isolates SC1737, SC1737-2, SC1737E and SC1737E-2 (all TSEKQ). Lanes 9-12 isolates SC1727, SC1727-2, SC1727E and SC1727E-2 (all TSEKQ). Lanes 1, 8 and 13 contain the Lambda Ladder PFGE marker for measuring banding size.

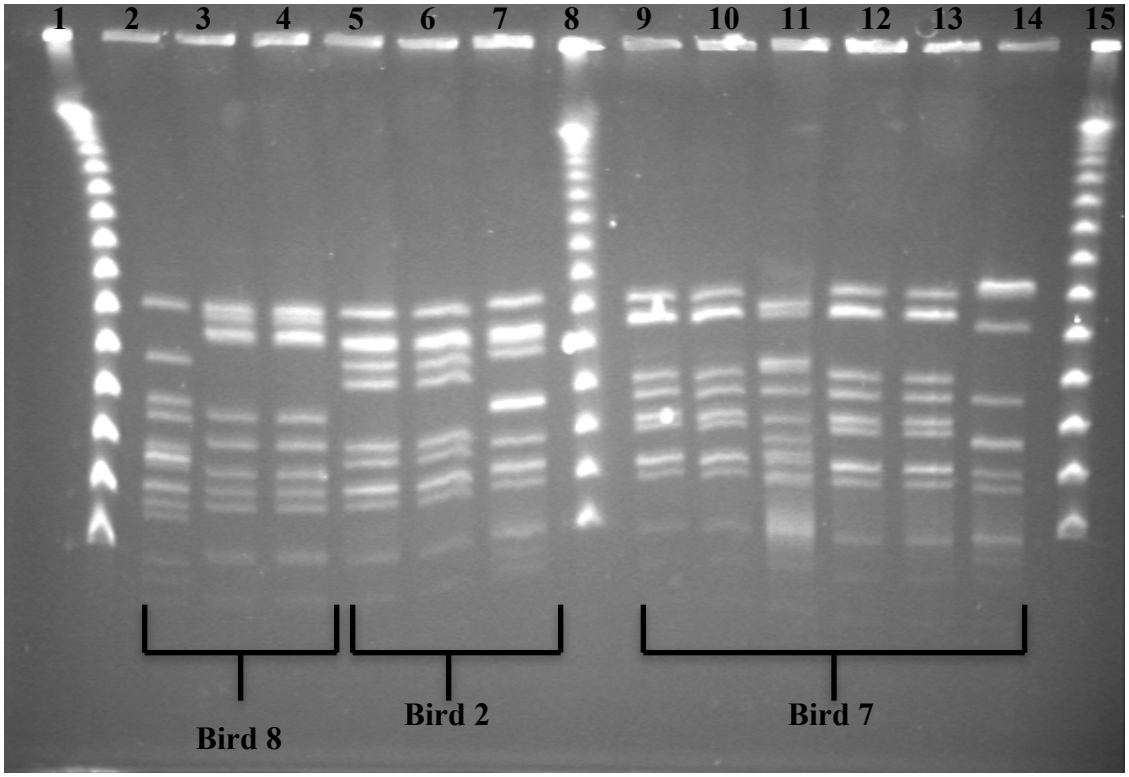

Figure 6. PFGE of *C. coli* isolates from individual birds. Lanes 2-4 isolates SC1748-5 (TSK; *C. jejuni*), SC1745E-2 and SC1745E-3 (TSEKQ). Lanes 5-7 isolates SC1754 (TSEKQ), SC1754-2 (TSEKQ) and SC1754-3 (TSEK). Lanes 9-14 isolates SC1799-2 (TSEKQ), SC1799-3 (TE), SC1799-4 (TSEKQ), SC1799E-3 (TSEKQ) and SC1799E-4 (TEKQ). Lanes 1, 8 and 15 contain the Lambda Ladder PFGE marker for measuring banding size.

2.6 Discussion

Our findings indicate that while isolates obtained from individual turkeys can appear to be the same strain phenotypically, when genotypic detection methods are used differences in banding pattern can provide evidence that isolates may not be identical but related in some manner. We observed instances of such evidence in birds that showed a resistance to all antibiotics used as well as those that showed resistance to just a select few (i.e. SEQ). While typically we observed the same strain when isolates antibiotic profiles were the same, in one case we noticed a difference in banding pattern between two *C. coli* isolates with the same TSEKQ profile (bird 4). The absence of bands in the PFGE allowed confirmation of two different strains of *C. coli*. This trend was only found once in this situation, however in cases where the antibiotic profiles were different, we noticed a difference in banding pattern as expected indicating the presence of more than one strain type of the *C. coli* isolate. The variety of species types and strains detected in the current study suggests the usefulness of choosing more than one isolate per positive sample. Although this would not give a definite answer as to how many different species or strains of *Campylobacter* may be present in individual turkeys, it would allow us to gauge the likelihood that there is more than one type of *Campylobacter* present.

Little is currently known about the diversity of *Campylobacter* spp. within individual birds, especially turkeys. As mentioned previously, in some cases there were indications of both *C. jejuni* and *C. coli* present in a given bird while in other cases *C. coli* alone was present and in some case multiple strains. Even in cases were *Campylobacter* was observed in swine and other food, only one isolate per positive sample was typically chosen. In a study

conducted by Quintana-Hayashi and Thakur (2012), 2,908 *Campylobacter* isolates were isolated with there being a mixture of *C. coli* and *C. jejuni*. While in this study, there were more *C. coli* isolates present than *C. jejuni*, the authors still chose to obtain only one presumptive *Campylobacter* colony per sample instead of multiples. While it appears as though the results from these findings are similar to ours in that there is still proof that diversity is present, however, if there are numerous colonies present and only one is chosen, there is a chance that the colony next to is different whether it be a difference in species type or even a different but related strain type. As was suggested by our birds, when isolates are introduced to certain environments such as media containing an antibiotics, in our case erythromycin, there can be strains that are able to flourish in the presence of inhibiting agents to other strains. These findings confirm that while we may observe similarities in antimicrobial susceptibility profiling such as *C. coli* isolates that are resistant to all antibiotics, further analysis using detection methods such as PFGE should also be used to confirm genotypic similarity among the isolates.

Currently the significance of choosing more than one isolate per positive sample remains poorly understood. While knowing the exact number of positive colonies to choose is unknown, our data suggest that choosing more than one colony per positive sample has the potential to reveal diversity of *Campylobacter* species and strains within individual turkeys that would not be evident if only a single isolate were to be characterized.

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APPENDIX

Appendix A

Table 3. Birds with *C. coli* isolates that show no diversity.

ID	Farm	T	S	E	K	Q
SC1821	20-C	+	+	+	+	+
SC1821-2	20-C	+	+	+	+	+
SC1821-3	20-C	+	+	+	+	+
SC1821-4	20-C	+	+	+	+	+
SC1821-5	20-C	+	+	+	+	+
SC1821E	20-C	+	+	+	+	+
SC1821E-2	20-C	+	+	+	+	+
SC1821E-3	20-C	+	+	+	+	+
SC1821E-4	20-C	+	+	+	+	+
SC1821E-5	20-C	+	+	+	+	+
SC1835	21-C	+	+	+	+	+
SC1835-2	21-C	+	+	+	+	+
SC1835-3	21-C	+	+	+	+	+
SC1835-4	21-C	+	+	+	+	+
SC1835-5	21-C	+	+	+	+	+
SC1835E	21-C	+	+	+	+	+
SC1835E-2	21-C	+	+	+	+	+
SC1835E-3	21-C	+	+	+	+	+
SC1835E-4	21-C	+	+	+	+	+
SC1835E-5	21-C	+	+	+	+	+
SC1727	14-A	+	+	+	+	+
SC1727-2	14-A	+	+	+	+	+
SC1727-3	14-A	+	+	+	+	+
SC1727-4	14-A	+	+	+	+	+
SC1727-5	14-A	+	+	+	+	+
SC1727E	14-A	+	+	+	+	+
SC1727E-2	14-A	+	+	+	+	+
SC1727E-3	14-A	+	+	+	+	+
SC1727E-4	14-A	+	+	+	+	+
SC1727E-5	14-A	+	+	+	+	+

Table 3 (continued).

ID	Farm	T	S	E	K	Q
SC1737	15-A	+	+	+	+	+
SC1737-2	15-A	+	+	+	+	+
SC1737-3	15-A	+	+	+	+	+
SC1737-4	15-A	+	+	+	+	+
SC1737-5	15-A	+	+	+	+	+
SC1737E	15-A	+	+	+	+	+
SC1737E-2	15-A	+	+	+	+	+
SC1737E-3	15-A	+	+	+	+	+
SC1737E-4	15-A	+	+	+	+	+
SC1737E-5	15-A	+	+	+	+	+
SC1742	16-A	+	+	+	+	+
SC1742-2	16-A	+	+	+	+	+
SC1742-3	16-A	+	+	+	+	+
SC1742-4	16-A	+	+	+	+	+
SC1742-5	16-A	+	+	+	+	+
SC1742E	16-A	+	+	+	+	+
SC1742E-2	16-A	+	+	+	+	+
SC1742E-3	16-A	+	+	+	+	+
SC1742E-4	16-A	+	+	+	+	+
SC1742E-5	16-A	+	+	+	+	+
SC1783	18-A	+	+	+	+	+
SC1783-2	18-A	+	+	+	+	+
SC1783-3	18-A	+	+	+	+	+
SC1783-4	18-A	+	+	+	+	+
SC1783-5	18-A	+	+	+	+	+
SC1783E	18-A	+	+	+	+	+
SC1783E-2	18-A	+	+	+	+	+
SC1783E-3	18-A	+	+	+	+	+
SC1783E-4	18-A	+	+	+	+	+
SC1783E-5	18-A	+	+	+	+	+

Table 3 (continued).

ID	Farm	T	S	E	K	Q
SC1764	17-A	+	+	+	+	+
SC1764-2	17-A	+	+	+	+	+
SC1764-3	17-A	+	+	+	+	+
SC1764-4	17-A	+	+	+	+	+
SC1764-5	17-A	+	+	+	+	+
SC1764E	17-A	+	+	+	+	+
SC1764E-2	17-A	+	+	+	+	+
SC1764E-3	17-A	+	+	+	+	+
SC1764E-4	17-A	+	+	+	+	+
SC1764E-5	17-A	+	+	+	+	+
SC1817	19-B	+	+	+	+	+
SC1817-2	19-B	+	+	+	+	+
SC1817-3	19-B	+	+	+	+	+
SC1817-4	19-B	+	+	+	+	+
SC1817-5	19-B	+	+	+	+	+
SC1817E	19-B	+	+	+	+	+
SC1817E-2	19-B	+	+	+	+	+
SC1817E-3	19-B	+	+	+	+	+
SC1817E-4	19-B	+	+	+	+	+
SC1817E-5	19-B	+	+	+	+	+

Table 4. Analysis of multiple isolates from15 isolates with varying antimicrobial profiles.

ID	Farm	Hip	ceu	T	S	E	K	Q
SC1825	9-C	-	+	+	+	+	+	+
SC1825-2	9-C	-	+	+	+	+	+	+
SC1825-3	9-C	-	+	+	+	+	+	+
SC1825-4	9-C	-	+	+	+	+	+	+
SC1825-5	9-C	+	-	+	+	-	+	+
SC1825E	9-C	-	+	+	+	+	+	+
SC1825E-2	9-C	-	+	+	+	+	+	+
SC1825E-3	9-C	-	+	+	+	+	+	+
SC1825E-4	9-C	-	+	+	+	+	+	+
SC1825E-5	9-C	-	+	+	+	+	+	+
SC1830	12-C	+	-	+	-	-	+	+
SC1830-2	12-C	+	-	+	-	-	+	+
SC1830-3	12-C	+	-	+	-	-	+	+
SC1830-4	12-C	+	-	+	+	-	-	+
SC1830-5	12-C	+	-	+	-	-	+	+
SC1830E	12-C	-	+	+	+	+	+	+
SC1830E-2	12-C	-	+	+	+	+	+	+
SC1830E-3	12-C	-	+	+	+	+	+	+
SC1830E-4	12-C	-	+	+	+	+	+	+
SC1830E-5	12-C	-	+	+	+	+	+	+
SC1834	10-C	-	+	+	+	+	+	+
SC1834-2	10-C	+	-	+	+	-	-	+
SC1834-3	10-C	+	-	+	+	-	-	+
SC1834-4	10-C	+	-	+	+	-	-	+
SC1834-5	10-C	-	+	+	+	+	+	+
SC1834E	10-C	-	+	+	+	+	+	+
SC1834E-2	10-C	-	+	+	+	+	+	+
SC1834E-3	10-C	-	+	+	+	+	+	+
SC1834E-4	10-C	-	+	+	+	+	+	+
SC1834E-5	10-C	-	+	+	+	+	+	+

Table 4 (continued).

ID	Farm	Hip	ceu	T	S	E	K	Q
SC1836	13-C	+	-	+	+	-	-	+
SC1836-2	13-C	+	-	+	+	-	-	+
SC1836-3	13-C	+	-	+	+	-	-	+
SC1836-4	13-C	+	-	+	+	-	-	+
SC1836-5	13-C	+	-	+	+	-	-	+
SC1836E	13-C	-	+	+	+	+	+	+
SC1836E-2	13-C	-	+	+	+	+	+	+
SC1836E-3	13-C	-	+	+	+	+	+	+
SC1836E-4	13-C	-	+	+	+	+	+	+
SC1836E-5	13-C	-	+	+	+	+	+	+
SC1789	8-B	-	+	+	+	+	+	+
SC1789-2	8-B	-	+	+	+	+	+	+
SC1789-3	8-B	+	-	+	+	-	+	+
SC1789-4	8-B	-	+	+	+	+	+	+
SC1789-5	8-B	-	+	+	+	+	+	+
SC1789E	8-B	-	+	+	+	+	+	+
SC1789E-2	8-B	-	+	+	+	+	+	+
SC1789E-3	8-B	-	+	+	+	+	+	+
SC1789E-4	8-B	-	+	+	+	+	+	+
SC1789E-5	8-B	-	+	+	+	+	+	+
SC1799	6-B	-	+	+	+	+	+	+
SC1799-2	6-B	-	+	+	+	+	+	+
SC1799-3	6-B	-	+	+	-	+	-	-
SC1799-4	6-B	-	+	+	+	+	+	+
SC1799-5	6-B	-	+	+	+	+	+	+
SC1799E	6-B	-	+	+	+	+	+	+
SC1799E-2	6-B	-	+	+	-	+	+	+
SC1799E-3	6-B	-	+	+	+	+	+	+
SC1799E-4	6-B	-	+	+	-	+	+	+
SC1799E-5	6-B	-	+	+	+	+	+	+

Table 4 (continued).

ID	Farm	Hip	ceu	T	S	E	K	Q
SC1732	1-A	-	+	+	-	+	-	+
SC1732-2	1-A	-	+	+	-	-	-	+
SC1732-3	1-A	-	+	+	-	-	-	+
SC1732-4	1-A	-	+	+	-	-	-	+
SC1732-5	1-A	-	+	+	-	-	-	+
SC1732E	1-A	-	+	+	-	+	-	+
SC1732E-2	1-A	-	+	+	-	+	-	+
SC1732E-3	1-A	-	+	+	-	+	-	+
SC1732E-4	1-A	-	+	+	-	+	-	+
SC1732E-5	1-A	-	+	+	-	+	-	+
SC1748	7-A	-	+	+	+	+	+	+
SC1748-2	7-A	-	+	+	+	+	+	+
SC1748-3	7-A	-	+	+	+	+	+	+
SC1748-4	7-A	-	+	+	+	+	+	+
SC1748-5	7-A	+	-	+	+	-	+	-
SC1748E	7-A	-	+	+	+	+	+	+
SC1748E-2	7-A	-	+	+	+	+	+	+
SC1748E-3	7-A	-	+	+	+	+	+	+
SC1748E-4	7-A	-	+	+	+	+	+	+
SC1748E-5	7-A	-	+	+	+	+	+	+
SC1754	2-A	-	+	+	+	+	+	+
SC1754-2	2-A	-	+	+	+	+	+	+
SC1754-3	2-A	-	+	+	+	+	+	-
SC1754-4	2-A	-	+	+	+	+	+	+
SC1754-5	2-A	-	+	+	+	+	+	+
SC1754E	2-A	-	+	+	+	+	+	+
SC1754E-2	2-A	-	+	+	+	+	+	+
SC1754E-3	2-A	-	+	+	+	+	+	+
SC1754E-4	2-A	-	+	+	+	+	+	+
SC1754E-5	2-A	-	+	+	+	+	+	+

Table 4 (continued).

ID	Farm	Hip	ceu	T	S	E	K	Q
SC1759	2a-A	-	+	+	-	-	-	+
SC1759-2	2a-A	-	+	+	+	+	+	+
SC1759-3	2a-A	-	+	+	-	-	+	-
SC1759-4	2a-A	-	+	+	+	+	+	+
SC1759-5	2a-A	-	+	+	-	-	-	+
SC1759E	2a-A	-	+	+	+	+	+	+
SC1759E-2	2a-A	-	+	+	+	+	+	+
SC1759E-3	2a-A	-	+	+	+	+	+	+
SC1759E-4	2a-A	-	+	+	+	+	+	+
SC1759E-5	2a-A	-	+	+	+	+	+	+
SC1764	17-A	-	+	-	+	+	-	+
SC1764-2	17-A	-	+	-	+	+	-	+
SC1764-3	17-A	-	+	-	+	+	-	+
SC1764-4	17-A	-	+	-	+	+	-	+
SC1764-5	17-A	-	+	-	+	+	-	+
SC1764E	17-A	-	+	-	+	+	-	+
SC1764E-2	17-A	-	+	-	+	+	-	+
SC1764E-3	17-A	-	+	-	+	+	-	+
SC1764E-4	17-A	-	+	-	+	+	-	+
SC1764E-5	17-A	-	+	-	+	+	-	+
SC1769	3-A	-	+	+	+	+	+	+
SC1769-2	3-A	-	+	+	+	+	+	+
SC1769-3	3-A	-	+	+	-	-	-	+
SC1769-4	3-A	-	+	+	-	-	-	+
SC1769-5	3-A	-	+	+	-	-	-	+
SC1769E	3-A	-	+	+	+	+	+	+
SC1769E-2	3-A	-	+	+	+	+	+	+
SC1769E-3	3-A	-	+	+	+	+	+	+
SC1769E-4	3-A	-	+	+	+	+	+	+
SC1769E-5	3-A	-	+	+	+	+	+	+

Table 4 (continued).

ID	Farm	Hip	ceu	T	S	E	K	Q
SC1774	11-A	+	-	+	+	-	-	+
SC1774-2	11-A	+	-	+	+	-	-	+
SC1774-3	11-A	+	-	+	+	-	-	+
SC1774-4	11-A	+	-	+	+	-	-	+
SC1774-5	11-A	+	-	+	+	-	-	+
SC1774E	11-A	-	+	+	+	+	+	+
SC1774E-2	11-A	-	+	+	+	+	+	+
SC1774E-3	11-A	-	+	+	+	+	+	+
SC1774E-4	11-A	-	+	+	+	+	+	+
SC1774E-5	11-A	-	+	+	+	+	+	+
SC1778	4-A	-	+	+	+	+	+	+
SC1778-2	4-A	-	+	+	+	+	+	+
SC1778-3	4-A	-	+	+	+	+	+	+
SC1778-4	4-A	-	+	+	-	-	-	+
SC1778-5	4-A	-	+	+	+	+	+	+
SC1778E	4-A	-	+	+	+	+	+	+
SC1778E-2	4-A	-	+	+	+	+	+	+
SC1778E-3	4-A	-	+	+	+	+	+	+
SC1778E-4	4-A	-	+	+	+	+	+	+
SC1778E-5	4-A	-	+	+	+	+	+	+
SC1786	5-A	-	+	+	-	-	-	+
SC1786-2	5-A	-	+	+	-	+	-	+
SC1786-3	5-A	-	+	+	+	+	+	+
SC1786-4	5-A	-	+	+	+	+	+	+
SC1786-5	5-A	-	+	+	-	-	-	+
SC1786E	5-A	-	+	+	+	+	+	+
SC1786E-2	5-A	-	+	+	+	+	+	+
SC1786E-3	5-A	-	+	+	+	+	+	+
SC1786E-4	5-A	-	+	+	-	+	-	+
SC1786E-5	5-A	-	+	+	-	+	-	+

Table 5. Isolates chosen for PFGE analysis.

ID	Farm	Hip	ceu	T	S	E	K	Q
SC1727	14-A	-	+	+	+	+	+	+
SC1727-2	14-A	-	+	+	+	+	+	+
SC1727E	14-A	-	+	+	+	+	+	+
SC1727E-2	14-A	-	+	+	+	+	+	+
SC1732	1-A	-	+	+	-	+	-	+
SC1732-2	1-A	-	+	+	-	-	-	+
SC1732E	1-A	-	+	+	-	+	-	+
SC1732E-2	1-A	-	+	+	-	+	-	+
SC1732E-4	1-A	-	+	+	-	+	-	+
SC1732E-5	1-A	-	+	+	-	+	-	+
SC1737	15-A	-	+	+	+	+	+	+
SC1737-2	15-A	-	+	+	+	+	+	+
SC1737E	15-A	-	+	+	+	+	+	+
SC1737E-3	15-A	-	+	+	+	+	+	+
SC1742	16-A	-	+	+	+	+	+	+
SC1742-2	16-A	-	+	+	+	+	+	+
SC1745	7b-A	-	+	+	+	+	+	+
SC1745-2	7b-A	-	+	+	+	+	+	+
SC1745E	7b-A	-	+	+	-	+	+	+
SC1745E-2	7b-A	-	+	+	+	+	+	+
SC1754	2a-A	-	+	+	+	+	+	+
SC1754-2	2a-A	-	+	+	+	+	+	+
SC1754-3	2a-A	-	+	+	+	+	+	-
SC1759	2b-A	-	+	+	-	-	-	+
SC1759-4	2b-A	-	+	+	+	+	+	+
SC1759-5	2b-A	-	+	+	-	-	-	+
SC1759E-3	2b-A	-	+	+	+	+	+	+
SC1764	17-A	-	+	-	+	+	-	+
SC1764-2	17-A	-	+	-	+	+	-	+
SC1764-3	17-A	-	+	-	+	+	-	+
SC1764-4	17-A	-	+	-	+	+	-	+
SC1764E	17-A	-	+	-	+	+	-	+

Table 5 (continued).

ID	Farm	Hip	ceu	T	S	E	K	Q
SC1769	3-A	-	+	+	+	+	+	+
SC1769-3	3-A	-	+	+	-	-	-	+
SC1769-5	3-A	-	+	+	-	-	-	+
SC1769E-2	3-A	-	+	+	+	+	+	+
SC1769E-5	3-A	-	+	+	+	+	+	+
SC1778-2	4-A	-	+	+	+	+	+	+
SC1778-4	4-A	-	+	-	-	-	-	+
SC1778E-2	4-A	-	+	+	+	+	+	+
SC1778E-4	4-A	-	+	+	+	+	+	+
SC1786	5-A	-	+	+	-	-	-	+
SC1786-4	5-A	-	+	+	+	+	+	+
SC1786-5	5-A	-	+	+	-	-	-	+
SC1786E	5-A	-	+	+	+	+	+	+
SC1786E-2	5-A	-	+	+	+	+	+	+
SC1786E-4	5-A	-	+	+	-	+	-	+
SC1786E-5	5-A	-	+	+	-	+	-	+
SC1799-2	6-B	-	+	+	+	+	+	+
SC1799-3	6-B	-	+	+	-	+	-	-
SC1799-4	6-B	-	+	+	+	+	+	+
SC1799E-3	6-B	-	+	+	+	+	+	+
SC1799E-4	6-B	-	+	+	-	+	+	+
SC1817-4	19-B	-	+	+	+	+	+	-
SC1817E-3	19-B	-	+	+	+	+	+	-
SC1817E-4	19-B	-	+	+	+	+	+	-
SC1821-3	20-C	-	+	+	+	+	+	+
SC1821-4	20-C	-	+	+	+	+	+	+
SC1821E-3	20-C	-	+	+	+	+	+	+
SC1821E-4	20-C	-	+	+	+	+	+	+
SC1830	12-C	+	-	-	-	-	-	-
SC1830-4	12-C	+	-	-	-	-	-	-
SC1830E-2	12-C	-	+	+	+	+	+	+
SC1830E-5	12-C	-	+	+	+	+	+	+

Table 5 (continued).

ID	Farm	Hip	ceu	T	S	E	K	Q
SC1835-3	21-C	-	+	+	+	+	+	+
SC1835-4	21-C	-	+	+	+	+	+	+
SC1835E-3	21-C	-	+	+	+	+	+	+
SC1835E-4	21-C	-	+	+	+	+	+	+