Abstract

Smith, Katie Sullivan. *Campylobacter* Colonization in Turkey Flocks Reared in North Carolina. (Under the direction of Dr. Sophia Kathariou)

Campylobacter spp are currently the leading cause of foodborne acute bacterial gastroenteritis in people in industrialized countries. It is estimated by the Centers for Disease Control and Prevention that *Campylobacter* accounts for 2.4 million human cases of gastroenteritis annually in the United States. The main risk for acquiring *Campylobacter* infection is handling raw poultry and/or consumption of undercooked meat of animal origin, particularly poultry. Research concerning *Campylobacter* in poultry has been focused on broilers; little information is available on the colonization of turkeys. Consumption of turkey products has increased considerably in recent years; therefore, there is a need to focus attention on this potential vehicle of campylobacteriosis.

First, a longitudinal study was conducted on two pairs of sibling turkey flocks obtained from the same hatch that shared a common breeder source. One flock from each pair was raised commercially whereas the other was raised under contract at the Teaching Animal Unit (TAU) at the North Carolina State University Veterinary School. Time of placement, feed formulations, and bird density were the same among the members of each pair. At the completion of the production cycle, birds of each flock were processed in commercial processing plants, following standard feed withdrawal and transport protocols. Both commercial flocks became colonized at 2 to 3 weeks of age and remained colonized through processing while the flocks raised at the TAU remained free of *Campylobacter* until processing.

In addition to prevalence of *Campylobacter* spp. other epidemiological factors of interest in this study were antibiotic resistance profiles, species colonizing the birds, and strain types present throughout the life of the birds. The turkeys were predominantly colonized by *C. coli* (84-88%) with *C. jejuni* accounting for the remainder of the isolates. Both commercial flocks were colonized by a limited number of strains with one dominant strain being isolated throughout the life of the bird. *C. coli* isolates were resistant to a variety of antibiotics including erythromycin and fluoroquinolones. *C. jejuni* isolates were also resistant, but were more likely to be sensitive to erythromycin and fluoroquinolones.

These results indicate that vertical transmission, if occurring, was not sufficient for colonization of these turkey flocks by *Campylobacter* and points towards the important role of flock management in preventing colonization. In addition, the turkeys were colonized by one main strain type which could be due to selective pressures related to antibiotic treatments. The level of antibiotic resistance in both the *C. coli* and *C. jejuni* isolates is of definite food safety concern.

The high prevalence of *C. coli* in the turkeys was unexpected and developed an interest in evaluating broilers raised in the same geographical region to determine if they also were predominantly colonized by *C. coli*. A cross-sectional survey was conducted on 32 farms from 2 broiler integrators in the same region. Sixteen of 32 farms had flocks that were colonized with *Campylobacter* at 4 weeks of age. Of these 16 flocks, 10 were primarily colonized by *C. jejuni* whereas 5 flocks were predominantly colonized by *C. coli.* No samples could be purified from one of the *Campylobacter*-positive flocks. There was a high level of resistance in both the *C. jejuni* and *C. coli* isolates to several

antibiotics including those that are used to treat human illnesses. Resistance to multiple antibiotics was more common in the turkey isolates. However, fluoroquinolone resistance was more prevalent in the broiler isolates. These findings indicate that broilers in eastern North Carolina were primarily colonized by *C. jejuni* and that the high prevalence of *C. coli* colonization that we observed in the commercial turkey flocks that we studied was likely not related to the specific geographic region. A possible explanation for the prevalence of *C. coli* in the turkeys is that integrators in North Carolina often raise turkeys as well as hogs, which are typically colonized by *C. coli*. This is not a common practice for broiler integrators. Thus, the prevalence of *C. coli* in turkeys is more likely to be dependent upon practices within the turkey industry in N. Carolina rather than geographic region.

Campylobacter Colonization in Turkey Flocks Reared in North Carolina

by

Katie Sullivan Smith

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Approved by:

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Chair of Advisory Committee

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Dedication

I would like to dedicate this Masters thesis in loving memory of Gretchen Budd Smith for her generous giving and support. She had a wonderful way of always making me feel like I could conquer anything no matter how big or how small. Thank you grandma, I love you and miss you. God Bless.

Biography

Katie Sullivan Smith was born in Durham, North Carolina on April 1, 1977. She graduated from Jesse O. Sanderson High School in Raleigh, North Carolina in June, 1995. She then attended North Carolina State University and obtained a B.S. in Food Science in December, 2000. After working for a year on a research project under the direction of Dr. Brian Sheldon and Dr. Lee-Ann Jaykus she decided to apply to graduate school. She started her M.S. degree in August of 2001 under the guidance of Dr. Sophia Kathariou in the department of Food Science at North Carolina State University.

She was awarded a Master's of Science in July 2003 and was off to Seattle, Washington to start her professional career.

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Comprehensive Literature Review of *Campylobacter* in Poultry Production

Literature Review: *Campylobacter* in Poultry Production

Introduction

Campylobacter spp. are the leading cause of acute bacterial gastroenteritis in people in industrialized countries (Friedman et al., 2000). According to the United States Centers for Disease Control and Prevention, (CDC), *Campylobacter* is estimated to cause about 2.4 million cases of human gastroenteritis every year in the United States. It is well-known that the main risk factor for *Campylobacter* infections is the consumption of foods of animal origin, in particular poultry (Deming et al., 1987, Linton et al., 1996). *Campylobacter* is considered to be a commensal organism in poultry. It is estimated that as many as 90% of broilers and turkeys may harbor *Campylobacter* while showing little or no clinical signs of illness (Shane, 1991, Wallace et al., 1997). Though significant effort has been invested to monitor *Campylobacter* colonization in broilers, the factors that determine the rate of colonization and the prevalence of *Campylobacter* as well as the transmission routes remains largely unknown. Little information is available on the colonization of turkeys by this pathogen. The best way to produce food products that are free of *Campylobacter* is to prevent *Campylobacter* at the farm. However, in order to achieve this goal we need a better understanding of factors that influence colonization at the farm level. The aim of this review is to summarize several aspects of the epidemiology of *Campylobacter* in poultry production.

Campylobacter and Human Infection

Campylobacter has been recognized as an important etiological agent of gastroenteritis since the 1970s (Tauxe, 1992; Nachamkin, 2000). Currently, *Campylobacter* spp are the leading cause of bacterial acute gastroenteritis in industrialized countries (Friedman, 2000). There are several species of *Campylobacter* which have been implicated in causing human illness (C. *jejuni, C. coli, C. lari,* and *C. uppsaliensi*s) (Friedman et al., 2000). More than 90% of human infections are caused by *C. jejuni*, with *C. coli* accounting for most of the remainder (Van Looveren et al., 2001). Generally, cases of campylobacteriosis are sporadic, which makes them hard to track. However, there have been outbreaks associated with raw milk and water (Kapperud et al., 1992; Altekruse et al., 1999). Several studies have indicated the main risk of *Campylobacter* infections is handling raw poultry and/or consumption of undercooked meat of animal origin, particularly poultry (Deming et al., 1987; Linton, 1996; Skirrow, 1982; Kapperud et al., 1992; Alketruse et al., 1999).

The clinical symptoms of campylobacteriosis typically include acute, self limiting gastroenteritis characterized by diarrhea, fever, and abdominal cramping within 3 to 5 days following exposure (Robinson et al., 1981; Black et al., 1988; Allos, 2001). *Campylobacter* infections have also been associated with severe secondary autoimmune sequelae such as Guillian-Barre Syndrome (GBS) which is characterized by temporary paralysis (Park et al., 1991). As few as 500 cells have been shown to cause illness (Robinson et al., 1981; Black et al., 1988). Although the illness is usually self-limiting, young children (less than 1 year) and immunosuppressed individuals are at risk for a more serious infection which can result in death (Friedman, 2000). Deaths attributed to

Campylobacter infection in the United States are estimated at 680-730 annually (Saleha, 1998). Severe or prolonged cases of campylobacteriosis often require antibiotic treatment usually in the form of erythromycin or fluoroquinolones (Nachamkin et al., 2000; Engberg et al., 2001). For this reason, the emergence of antibiotic resistance in *Campylobacter* isolated from both human and animal isolates is of public health concern.

Campylobacter **in Poultry**

Prevalence in Broilers

Campylobacter spp., specifically *C. jejuni* and *C. coli,* are thought to be commensal inhabitants of broilers. As many as 90% of chickens may harbor this organism and even with high colonization rates, colonized animals show little or no clinical signs of illness (Luechtfeld and Wang, 1982; Shane et al., 1991; Stern et al., 1988; Kapperud et al., 1993). The primary species of *Campylobacter* that colonizes chickens is *C. jejuni*, accounting for 80-90% of the isolates while *C. coli* accounts for 10-20% of the isolates (Van Looveren et al., 2001; Wedderkopp et al., 2001). Campylobacters in living birds are isolated in the highest numbers from the large intestine, ceacum, and cloaca (Corry and Atabay, 2001). The numbers range from 10^5 to 10^9 cfu per g of intestinal contents (Berndston et al., 1992; Stern et al., 1999). High levels of contamination at processing and subsequently at retail are indicative of the high levels of colonization during poultry production. Thus, control measures must be implemented at the farm.

Modes of Transmission and Control

The efforts to develop appropriate control at the farm are currently hindered by inadequate knowledge of the sources and modes of transmission of *Campylobacter* to

poultry flocks (Stern et al., 2001). Several sources have been considered such as vertical transmission from parent to progeny via the egg (Cox et al., 1999; Jacobs- Reitsma et al., 1995), contaminated drinking water (Kapperud et al., 1993), horizontal transmission from one flock to the next via contaminated litter (Montrose et al., 1985), and horizontal transmission from the environment through rodents or pests, other food animals, farm personnel via boots, and transport trucks (Stern et al., 1992). Most studies have concluded that the best way to approach control during production is through biosecurity measures. Specifically, the most important methods appear to be; treatment of drinking water and cleaning of the drinking systems between flocks, although studies with chlorinated water and non-chlorinated water have shown little effect; hygiene measures for workers or visitors such as disinfection or change of boots and clothing before entering the rearing environment; and finally control of rodents, wild birds, and flies on the farm (Corry and Atabay, 2001).

Epidemiological Aspects

Campylobacter can be isolated from the broiler flock at 3 to 4 week of age and is rapidly transmitted through an entire flock. Isolation rates remain at 100% until slaughter (Jacobs Reitsma, 1995, Pokamuanski et al., 1986). Although humans are usually infected with a single serotype, poultry may be infected with as many as 5 different serotypes of *Campylobacter* (Jacobs-Reitsma, 1995). This number can change depending on the type of isolation method used. This diversity can also be seen in antibiotic resistance patterns from isolates within the same flock (Jacobs-Reitsma, 1994). The change in serotypes throughout the production cycle may indicate the continuous flow of campylobacters

entering the broiler house and dominance of a newly introduced serotype (Jacobs-Reitsma et al., 1995).

Antibiotic Use in Production

Studies have linked the emergence of antibiotic resistant *Campylobacter* with the use of antimicrobial agents in veterinary medicine (Endtz et al., 1991; Smith et al., 1999; McDermott et al., 2002). This appears to be the case specifically with the increase in the resistance to fluoroquinolones (such as enrofloxacin) following the approval of the use of enrofloxacin in poultry production. Enrofloxacin is not however used to treat *Campylobacter* in poultry, but rather other diseases such as respiratory or *E. coli* infections (Barrow et al., 1998). This increase in resistance has been documented in several countries, including the United States, Canada, Netherlands, and Spain (Gaudreau et al., 1998; Endtz et al., 1991; Smith et al., 1999; Sanchez et al., 1994). The rapid increase in resistance in the United States prompted the FDA, Center for Veterinary Medicine (CVM) to announce a proposal to withdraw the approval for the use of fluoroquinolones in poultry in 2000 (White et al., 2002).Evidence supporting this regulatory shift came from surveillance programs, published literature, risk assessment, and other sources indicating that the increase in resistance was a serious public health concern (White et al., 2002).

Antibiotic Resistance in Poultry Isolates

Campylobacter resistance to fluoroquinolones used in food animal production was first described in Europe but has since become a major concern in other developed and developing countries. Overall the resistance to several antibiotics is higher in animal isolates than in human isolates (Saenz et al., 2000). In addition, the prevalence of

resistance to the macrolides and fluroquinolones is higher in *C. coli* than *C. jejuni* from both human and animal origin (Saenz et al., 2000; Prats et al., 2000; Jenson and Aarestrup, 2001). Although the antibiotic resistance profiles of animal isolates is not always the same as clinical isolates, a study done in Denmark by Aarestrup et al. (2001) indicated that resistance in clinical isolates to avoparicin, tylosin, erythromycin, virginiamycin, and avilamycin were reduced after these agents were banned from animal agriculture.

Campylobacter in Turkeys

The majority of research involving *Campylobacter* has been focused on broilers. Limited information is available on the colonization of turkeys. The importance of turkeys in the transmission of campylobacters was described in a study by Shandera et al. (1992) in which there was an outbreak of campylobacteriosis in Los Angeles associated with the consumption of processed turkey meat. In the United States the consumption of turkey has increased 220 percent since 1970 as consumption of turkey is no longer seasonal. With the availability of a variety of products, turkey meat has become a year round staple (http://www.eatturkey.com/press/conspr/industry.html).

More recent retail studies have also implicated turkey as a potential vehicle in the transmission of campylobacters. A study was conducted in the Washington, D.C. area to assess the prevalence of *Campylobacter* spp. in retail chicken, turkey, pork and beef (Zhao et al., 2001). Fourteen percent of the 172 turkey samples yielded *Campylobacter*. *C. coli* was more prevalent than *C. jejuni* in the turkey samples (19 samples were positive for *C. coli* and 4 samples were positive for *C. jejuni*). The prevalence did seem to vary over the 14-month sampling period; however there appeared to be no correlation with

seasonality; *Campylobacter* was isolated in both the cold and warm months. In addition, this study concluded that multiple serotypes or genotypes of the same species can be found in one meat sample which compounds the challenges to molecular sub-typing methods used for epidemiological or outbreak investigations.

Information focused on *Campylobacter* colonization during the production period is more limited. The colonization of turkeys by thermophilic *Campylobacter* has been described in one study by Wallace et al. (1998) in which surveillance of two flocks, reared at one site, revealed that the birds became colonized by *C. jejuni* between 3 and 4 weeks of age, and suggested a succession of biotypes during the lifetime of the flock (21 weeks). As in studies with broilers, the source of *Campylobacter* appeared to be from horizontal transmission and once *Campylobacter* appeared it rapidly spread through the flock. The contamination level of *Campylobacter* was also higher in the cecum than in any other area in the digestive tract.

Effect of *Campylobacter* on Turkey Production

Though *Camyplobacter* is considered a commensal organism in broilers there is some evidence that *Campylobacter* may have a negative effect in turkey production. During studies of poult enteritis, Lam et al. (1992) frequently encountered *C. jejuni* in both the affected and normal poults. This observation led to a study to determine the pathogenicity of *C. jejuni* of turkey origin on turkey embryos and poults. Lam et al. (1992) showed that *C. jejuni* of turkey origin caused a transient diarrhea in poults and significantly reduced weight gain during a 3 week observation period. Though the pathogenic mechanism of *C. jejuni* was unclear, the *C. jejuni* visibly had a deleterious effect on the turkey embryos and poults. Inoculation of 10-day old embryos by the yolk-

sac route resulted in death, and upon histological examination, the embryos exhibited generalized hemorrhages throughout the skin and skeletal muscles. Some embryos showed hemorrhages in the liver and the heart (Lam et al., 1992).

In preliminary studies at the North Carolina State University Veterinary School, it was also found that turkey flocks that were highly colonized with *Campylobacter* showed lower performance when compared with sibling flocks that were *Campylobacter*-negative (N. Reimers , and J. Barnes, unpublished). In addition, recent work by Barnes et al. (2000) has shown that *Campylobacter* spp. may play a role in Poult Enteritis Syndrome (PEMS), a clinical syndrome in which turkey flocks have mortality of 9% during days 7-28 or mortality of 1% per day over three consecutive days. Subclinical forms of the syndrome are responsible for growth depressions of 10-15% (Barnes et al., 2000). These findings are extremely important in states such as North Carolina, where following a PEMS outbreak turkey production dropped from 53.3 million to 42 million birds per year and caused an economic loss estimated at 133 to 136 million dollars (Barnes et al., 2000). These economic losses continue to plague the turkey industry.

Antibiotic Use in Turkey Production

With the large increase in turkey consumption, there will undoubtedly be more human foodborne illness attributed to turkey products, thus the use of antibiotics in turkey production is a relevant food safety concern. Poults are extremely fragile in the first few weeks of production and for this reason; antibiotics such as fluoroquinolones are often administered as principle agents in the prophylaxis and treatment of enteric infections (Van Looveren et al., 2001).

A study by Van Looveren et al. (2001) was done in Belgium to compare the antibiotic susceptibilities of *Campylobacter* isolates obtained from several food animals (pigs, broilers, layers, and turkeys). Samples were taken from the neck skin of turkeys in slaughterhouses and cutting rooms. One hundred isolates were obtained; 94 of the isolates were *C. jejuni* and the 6 remaining isolates were *C. coli*. The *C. jejuni* isolates had a high level of resistance to ampicillin (33%). This level of resistance was found to be significantly higher ($p < 0.05$) than the levels found in *Campylobacter* isolated from broilers or layers sampled in the study. Resistance to nalidixic acid was also high (44.7%) and resistance to ciprofloxacin was 35.1%. Tetracycline resistance was also highest in the turkey isolates (37.2%).

Recent studies in our laboratory, (Smith et al., unpublished), showed that two turkey flocks raised in North Carolina were primarily colonized with *C. coli* (84-88%) while *C. jejuni* accounted for the remainder of the isolates. Both flocks had a dominant strain type isolated in the first 5 weeks of production that was resistant to multiple antibiotics. At processing 43% of the *C. coli* and *C. jejuni* isolates obtained from one of the flocks were resistant to fluoroquinolones and 8% were resistant to all antibiotics tested. In the other flock, a multiresistant strain type was dominant and was isolated throughout the production cycle of the flock. These studies indicate a high prevalence of antibiotic resistance in *Campylobacter* spp. isolated from turkeys. This finding is of food safety relevance and indicates that antibiotic use in turkey production should merit the same amount of attention as antibiotic use in the broiler production system.

Summary

Campylobacter has become the leading cause of acute bacterial gastroenteritis in people in industrialized countries. In addition, there are serious secondary diseases that may occur following campylobacteriosis. Thus, *Campylobacter* contamination is undeniably a major food safety issue. Control strategies have improved both at the farm and in the processing environment; however *Campylobacter* remains a food safety concern. The majority of research on campylobacters thus far has focused on broilers. Little is known about the colonization of turkeys by *Campylobacter*. Consumption of turkey products has increased by a large margin in recent years; therefore, there is a need to focus attention on this potential vehicle of campylobacteriosis.

A relatively recent concern is the emergence of antibiotic resistance in *Campylobacter* spp., in particular to those antibiotics used to treat human illness. Studies have indicated that campylobacters isolated from broilers have a high level of antibiotic resistance to antibiotics of human clinical and veterinary relevance. It is well known that antibiotics are used in rearing turkeys, particularly at a young age. More studies should be done to evaluate the epidemiology of *Campylobacter* spp. in the turkey production.

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Chapter II

A Longitudinal Analysis of *Campylobacter* Colonization in Two Sibling Turkey Flocks Reared Under Instructional and Commercial Conditions

ABSTRACT

Campylobacter spp. are currently the leading bacterial cause of acute gastroenteritis in people in industrialized countries. *Campylobacter* is highly prevalent in flocks of commercial poultry (broilers and turkeys); however significant ambiguity exists concerning the key factors contributing to colonization of birds, especially in regard to the impact of vertical transmission of *Campylobacter* from breeder hens to young birds.

The focus of this study was to conduct a longitudinal analysis of *Campylobacter* colonization in two pairs of sibling turkey flocks (4 flocks total). Flocks of each pair shared breeder hen populations, and were obtained from the same hatch. One member of each pair was grown on a commercial farm, whereas the other was grown in an instructional demonstration unit within a 60 mile radius. Time of placement, feed formulations, and bird density were the same among the members of each pair. At the completion of the production cycle, the birds of each flock were processed in commercial processing plants, following standard feed withdrawal and transport protocols. Both flocks grown at the commercial farms became colonized by *Campylobacter* by 2 to 3 weeks of age and remained colonized until processing. In contrast, *Campylobacter* could not be isolated from either of the instructional unit flocks at any time during the production cycle. The results indicate that vertical transmission, if occurring, was not sufficient to render these flocks *Campylobacter*-positive. Evaluation of the operating procedures in each farm pair suggested that husbandry features such as proper litter maintenance, use of footbaths, and lack human traffic from other commercial turkey farms to the instructional unit farms could be among the leading factors responsible for the absence of *Campylobacter* in the *Campylobacter*-free flocks.

Introduction

Campylobacter spp. (primarily C. *jejuni* and C. *coli*) are currently the leading bacterial cause of acute foodborne gastroenteritis in industrialized countries (Friedman et al., 2000). *Campylobacter* infections have also been associated with severe autoimmune sequelae such as Guillain Barre Syndrome, which is characterized by temporary paralysis (Park et al., 1991). The main risk for human *Campylobacter* infections is the consumption of contaminated foods of animal origin, particularly poultry (Deming et al., 1987; Linton, 1996). As many as 90% of broiler chickens and turkeys may harbor *Campylobacter* (Luechtefeld and Wang, 1982; Wallace et al., 1997) and colonized animals commonly have no clinical signs of illness (Shane et al., 1991).

Considerable effort has been invested to monitor *Campylobacter* colonization in poultry, however the majority of studies have involved broilers, and limited information is available on the colonization of turkeys. A longitudinal study of *Campylobacter* colonization of turkey flocks was described in detail in only one study. Surveillance of two flocks, reared at one site, revealed that the birds became colonized by *C. jejuni* between 3 and 4 weeks of age and suggested a succession of biotypes during the lifetime of the flock (21 weeks) (Wallace et al., 1998).

The factors that determine the rate of colonization and prevalence of *Campylobacter* in poultry pre-harvest are still poorly understood. The literature contains conflicting reports on the role of vertical transmission of *Campylobacter* in poultry (Cox et al., 2002; Jacobs-Reitsma et al., 1995). Although the impact of vertical transmission is still being evaluated, several studies have indicated that hygiene and biosecurity measures could have an important role in preventing *Campylobacter* infection of poultry (Gibbens

et al., 2001; Humphrey et al., 1993). *Campylobacter* contamination is widespread and difficult to control in the poultry slaughter and processing environment (Berndtson et al., 1996; Humphrey et al., 1993), therefore the production of birds that are free of *Campylobacter* infection at the time of slaughter is an important way to reduce *Campylobacter* contamination of poultry products (Gibbens et al., 2001; Jacobs-Reitsma, 1997).

Studies describing the possible impact of vertical transmission and other factors of colonization of turkeys are lacking. The focus of this study was to determine the prevalence, species, and antibiotic resistance profiles of *Campylobacter* isolates in sibling turkey flocks reared in North Carolina and in addition to evaluate the possibility for vertical transmission. A longitudinal study of colonization in turkey flocks obtained from the same hatch and breeder populations, but grown on different farms was investigated in two different years. *Campylobacter* was isolated and purified from the ceca of five randomly selected birds from each farm on a weekly basis. Antibiotic resistance profiles were determined by the disk diffusion method and PCR employed the hippuracase and ceurolin primers specific for *C. jejuni* and *C. coli* respectively.

Materials and Methods

Turkey flocks involved in this study.

Four turkey flocks (T_1, C_1, T_2, and C_2) were involved in this study. Flocks $T_1$and C_1 were initiated August 2001, with the day-old poults originating from the same commercial hatch with shared breeder hen populations. Flock T_1 consisted of 2100 hens raised under contract at the Teaching Animal Unit (TAU) of the College of Veterinary

Medicine at North Carolina State University. The comparison flock (flock C_1) consisted of female hatch-mates of the TAU birds, grown at a commercial farm. Flocks T_2 and C_2 were initiated the last week of September 2002 as day-old poults obtained from the same hatch and with shared breeder hen populations. Flock T_2 (~ 2100 hens) was reared at the TAU and Flock C_2 (toms) was reared commercially. The TAU flock and the commercial flock, in both pairs, were in the same climatic zone, within a 60-mile radius of each other, and were raised on common feed. Bird density (birds / ft^2) and vaccination regimens for all four flocks were within the guidelines routinely followed by commercial turkey growers, except that the Hemorrhagic Enteritis Virus vaccination normally given to poults at 5 weeks of age was not administered to the TAU grown flocks. Identical procedures for transport and feed withdrawal (24 hours prior to slaughter) were followed for all sampled flocks. Birds from flocks T_1 and C_1 were commercially processed at week 14 at the same processing plant, following standard practices, and sold at retail. Birds from Flock C_2 were commercially processed at week 11 at the same processing plant as flocks T_1 and C_1. In the turkey industry hens are commonly processed at week 13, whereas toms at week 21. Therefore, we were unable to obtain plant samples from Flock C_2 (toms) because they were processed significantly later than the counterpart TAU flock (hens), and at a plant outside of N. Carolina.

Sample collection.

Flocks T_1 and C_1 were sampled during weeks 1-13. Ceca (one cecum per bird) were obtained from five clinically healthy freshly killed birds from each flock randomly chosen from different areas of the turkey house. Fresh fecal droppings, when sampled, were from different birds, and were also collected from different areas of the turkey

house. The same turkey house was surveyed each week. The birds were killed by cervical dislocation following the guidelines of the approved Institutional Use and Animal Care Committee's protocol at North Carolina State University. Finishing samples from week 14 of flocks T_1 and C_1 were obtained from the processing plant, immediately following evisceration. A total of 50 randomly chosen carcasses were sampled from flock T_1 (1 cecum per bird). The schedule at the plant processing flock C_1 changed without our knowledge, but the plant manager randomly collected and made available to us 9 carcasses to be examined bacteriologically. A segment of the gastrointestinal tract including ceca, duodenal loop, ileum, and Meckle's Diverticulum was removed from the viscera, placed in a large whirl-pak, and transported to the laboratory on ice, where the cecum, ileum, and Meckle's Diverticulum were dissected out aseptically and placed in separate sterile petri dishes. Samples were processed within 24 hours after collection.

Flock T_2 reached marketing weight faster than expected, and was processed at 11 weeks. A segment of the gastrointestinal tract including ceca, duodenal loop, ileum, and Meckle's Diverticulum was removed from the viscera of 15 randomly chosen carcasses, and processed as described above except samples were also taken from the duodenal loop.

Cecal samples were collected from weeks 1-8, and fresh fecal samples were examined during weeks 5-15 from flock C_2. Both cecal and fresh fecal samples were collected for weeks 5-8, to ensure that *Campylobacter* recovery from the two types of samples was comparable. The screening of only fecal droppings during weeks 9, 13, and 15 allowed us to avoid killing the birds, and thus minimized the economic impact of the study on the farmer. Fecal samples were collected in sterile polypropylene tubes

(Corning), and transported to the laboratory on ice. This flock was processed at 21 weeks in a processing plant outside of N. Carolina, and it was not possible to collect plant samples. Hence, samples were collected from flock C_2 through 15 weeks of age.

Samples from breeder hens

In efforts to try to identify the issue of vertical transmission or lack thereof, 11 fecal samples were collected from the breeder flock that was the main source for commercial flock C_2. These samples were collected in the last week of September which was approximately 10 days after commercial flock C_2 had hatched. All samples were plated directly and by enrichment. Eleven samples were positive when plated directly and 7 samples were positive following enrichments. Fourteen of these 18 samples were purified (11 direct and 3 enriched). There were 11 *C.coli* samples (8 direct and 3 enriched) and 3 *C.jejuni* samples found upon speciation (all from direct platings).

***Campylobacter* Isolation.**

In the laboratory, the same basic procedure for *Campylobacter* isolation and purification was used for both cecal and fecal samples. Each cecum was placed into a sterile petri dish and two incisions were made with a flame-sterilized scalpel, one in the mid-section and one at the end nearest the ileo-cecal junction. Approximately 0.1g of cecal contents from each incision site was streaked directly onto modified Charcoal Cefaperazone Deoxycolate Agar (CCDA; Oxoid, Hampshire, England) with the corresponding supplement (SE 155, Oxoid), which contained 16 mg l^{-1} of cefoperazone and 5 mg l^{-1} of amphotericin B (Oxoid, Hampshire, England). The same protocol was used for the fecal samples approximately 0.1 g from the interior of the sample was streaked directly onto modified CCDA. Plates were incubated in anaerobic jars

containing a CampyPak Plus microaerobic system (Beckton Dickinson, MD) at 42°C for 48 hours. Purifications were done on Mueller Hinton Agar (MHA) (Difco) or Sheep Blood Agar (SBA) (Remel, Lenexa, KS), under microaerobic conditions at 42°C for 36h. Bacteria from suspected *Campylobacter* colonies were observed with phase contrast microscopy for typical shape and motility.

Growth curves were determined with two *C. coli* strains and two *C. jejuni* strains. All four strains were isolated from processing plant samples. The two *C. coli* strains were isolated from the cecum of one bird, whereas 1 *C. jejuni* strain was isolated from the Meckle's Diverticulum of the same bird and the other *C. jejuni* strain was isolated from Meckle's Diverticulum of a different bird. Each culture was grown on SBA microaerobically at 42°C for 48h. One loopful of each culture was transferred into 10ml of MHB and grown for 36h. One ml of each culture was then transferred into 10ml of fresh MHB in duplicate. The cultures were incubated microaerobically at 42°C and the absorbency (600nm) was read at 4, 12, and 24h. The absorbency readings from the two *C. coli* cultures and two *C. jejuni* cultures were averaged at each time point.
(see appendix A)

DNA extraction.

DNA was extracted from SBA-grown cells (ca. ½ of a confluent 15 mm-diameter plate) using the DNeasy Tissue Kit (Qiagen, Valencia, CA), following the procedures supplied by the vendor. DNA was resuspended in 200µl of buffer provided with the kit**.**

Polymerase Chain Reaction (PCR).

PCR employed the C. *jejuni* specific *hip* primers (5′ ATG ATG GCT TCT TCG GAT AG3' and 5′ GCT CCT ATG CTT ACA ACT GC 3' (Marshall et al., 1999) and the

C. coli specific *ceu* primers CC1 (5'GAT TTT ATT ATT TGT AGC AGC G3' and CC2 5' TCC ATG CCC TAA GAC TTA ACG 3') (Houng et al., 2001). Reactions were carried out in 25µl total volume containing 0.5µl of each primer, 2.5µl of 10 x buffer (TaKaRa, Fisher), 2.0µl of DNTP mix, 0.125µl of X-Taq (TaKaRa, Fisher) and 0.5µl of genomic DNA as template. PCR amplification used a Progene thermocycler. The conditions were 95°C for 5 min, followed by 30 cycles each consisting of 95°C for 1 min, 50°C for 1min and 72°C for 2 min, with a final extension at 72°C for 5 min. PCR fragments were run on a 1.5% Tris Borate-EDTA gel for approximately 30 minutes at 95 volts. For *fla* PCR, the primers were (5' ATG GGA TTT CGT ATT AAC AC 3' and 5' CAA AAT GTT TTA AGA TTA CTA CAG 3') (Nachamkin et al., 1993) and the PCR conditions were as described above.

Molecular Subtyping of Strains.

Subtyping of strains was conducted by PCR-restriction fragment length polymorphism (RFLP) of the *flaA* gene, as described by Hald et al. (2001). PCR of *flaA* (*fla* PCR) was as described above, and 20µl of the PCR product (1.7 kb) was cut using the enzyme *Dde*I (New England Biolabs) following the conditions supplied by the vendor. The restriction fragments were separated on 3% Tris Borate-EDTA gel (150 min at 65 volts) and the band patterns were photographed and scanned.

RESULTS

Colonization by *Campylobacter* was efficient in the commercial flocks C_1 and C_2, but not detected flocks T_1 and T_2.

The four flocks were negative when screened on the day of placement. However, between 2 to 3 weeks of age at least 60%of the birds from commercial flocks C_1 and C_2

were colonized by *Campylobacter*. By the third week, both commercial flocks were 100% *Campylobacter*- positive and remained positive with the exception of the samples from week 13 of flock C_2 (Tables1 and 2, results are out of total isolates that could be purified. Some weeks there were multiple isolates purified/ bird. Other weeks some isolates could not be purified). The *Campylobacter* spp. colonizing flocks C_1 and C_2 was primarily *C. coli* (88% and 84%, respectively), all remaining isolates were *C. jejuni*. Although flock T_2 (hens) was processed at 11 weeks, its sibling commercial flock (flock C_2, toms) was grown longer. Toms in the turkey industry are usually marketed at 21 weeks of age. However, at 12 to 13 weeks of age flock C_2 experienced a major episode of coronavirus infection and was treated with copper sulfate (treatment records are provided in appendix B). The infection and / or treatment may have been responsible for the failure to isolate *Campylobacter* from any of the samples from week 13. In contrast, in the following sampling point at week 15, the majority of the samples from this flock were *Campylobacter*-positive (Table 1). The copper sulfate had been removed from the diet for approximately one week.

In contrast with the results obtained with the commercial flocks C_1 and C_2, no *Campylobacter* could be detected from the samples obtained from the birds in flocks T_1 and T_2 at any time during the production cycle. However, at processing some of the samples from flock T_1 and T_2 yielded colonies typical of *Campylobacter* on the CCDA plates. Samples from the ceca of 22 of 50 birds from flock T_1 yielded growth typical of *Campylobacter* on the CCDA. Fifteen of these samples had only 1-3 putative *Campylobacter* colonies per CCDA plate, whereas 7 cecal samples yielded moderate to large areas of confluent *Campylobacter*-like growth on the CCDA plates.

Colony growth from flock T_1 samples was typical of *Campylobacter* on the CCDA plates however upon further investigation the organisms appeared rather atypical. No growth could be obtained on MHA or SBA, as well as in Mueller Hinton Broth, after incubation at 42°C for 48h in a microaerobic environment. Repeated attempts to subculture the organisms on media other than CCDA, including motility agar using Mueller Hinton Broth with 0.4% agar were unsuccessful. However, the bacteria could be readily subcultured on CCDA, with or without the antibiotic supplement. Three putative *Campylobacter* isolates from flock T_1 were chosen for further characterization. DNA was isolated from CCDA-grown cells and used as template in PCR reactions with *hip* and *ceu* primers, for species determinations. However, no amplicon could be obtained from such PCR reactions, even when the annealing temperature was reduced to 46°C. When the *fla* primers were used, amplicons could not be obtained at the 50°C annealing temperature, but were readily obtained when a 46°C annealing temperature was used. *Campylobacter* was isolated from the gastrointestinal tract of three of the 15 birds from flock T_2 screened at the slaughter plant. One isolate was recovered from the duodenal loop of one bird and two isolates were recovered from Meckle's Diverticulum of two other birds. Growth on the CCDA plates was low to moderate from all three birds. No positive isolates could be obtained from any other areas of the gastrointestinal tract even after repeated attempts at isolation. The failure to detect *Campylobacter* from the ceca of these same birds, even following repeated sampling indicates that the organisms recovered from the duodenal loop and Meckle's Diverticulum were likely acquired by ingestion during transport of the birds from the farm to the processing plant since these areas are located higher in the digestive tract than the ceca .

DISCUSSION

Cecal samples were used to determine *Campylobacter* prevalence in flocks T_1, C_1 and T_2, as previous studies indicated that in turkeys the cecum was the site with the largest numbers of *Campylobacter* (Wallace et al., 1998). Samples from both the cecum and from fresh fecal droppings of flock C_2 in weeks 5 through 8 produced comparable results in terms of *Campylobacter* recovery. Only fresh fecal droppings were sampled in weeks 13 and 15, to avoid unnecessary killing and wastage of the birds from this commercial farm. Direct plating of cecal samples or fecal droppings yielded better recovery of *Campylobacter* than enrichments (data not shown), in agreement with results from others (Musgrove et al., 2001). Hence direct plating was used routinely for the isolation of *Campylobacter* in this study.

Transmission routes of *Campylobacter* in the poultry production system remain poorly understood. The literature contains conflicting reports on the role of vertical transmission of *Campylobacter* in broilers (Chuma et al., 1997; Cox et al., 2002; Jacobs-Reitsma et al., 2001; Jacobs-Reitsma et al., 1995; Petersen et al., 2001) and vertical transmission investigations with turkeys have not been reported.

The commercial and non-commercial turkey flocks investigated in this study shared common breeders for the supply of the eggs and day-old poults. Thus, our results indicate that vertical transmission alone, if it operates, was not sufficient for effective colonization of the flocks. In this study, the commercial flocks became rapidly colonized, whereas the instructional flocks appeared to remain free of *Campylobacter*. Furthermore, analysis of fecal samples from the breeder flock which provided day-old poults for flocks T_2 and C_2 indicated that the breeders were colonized with

Campylobacter, but *fla* typing revealed that these strains did not match those isolated from flock C_2, which provides further evidence that, if vertical transmission occurs it is rare, and colonization cannot be completely attributed to this phenomenon (data not shown).

We cannot exclude the possibility that flocks T_1 and T_2 were colonized at levels below those that could be detected with our methods. However, the direct plating methods that we used is capable of detecting 10 CFU of the organism / g cecal content, and since two separate isolations were pursued from each cecum it is unlikely that substantial colonization existed but was undetected. The few isolates obtained from the plant samples in flocks T_1 and T_2 could be attributed to contamination during transport or cross- contamination at the plant. Studies have shown that transport crates are frequently contaminated, and *Campylobacter*-negative flocks can become rapidly contaminated by strains found in the transport crates and trucks as well as in the processing environment (Newell et al., 2001; Stern et al., 1995). This type of contamination seems the most likely, especially when positive plant samples were obtained from flock T_2 which was negative the night before being loaded onto the truck. *Campylobacter* was only isolated from the upper areas of the digestive tract of three birds (of 15 screened), and was not isolated from the ileum or the ceca, even after repeated isolation attempts, indicating that the bacteria had recently colonized the birds, and had not become established in the lower portions of the GI tract.

The observed timing of colonization of the commercial flocks (2 weeks, with 100% colonization by 3 weeks) was in agreement with the report by Wallace et al. (1998) in which the two flocks that were studied were colonized between 3 and 4 weeks of age.

Longitudinal studies of colonization of more than 40 other commercial flocks also indicate that colonization is commonly seen at 2 weeks, and is frequently 100% by 3 weeks of age (S.Kathariou, J. Barnes and D. Carver, unpublished results). These findings highlight the extent to which the apparent absence of colonization of flocks T_1 and T_2 by *Campylobacter* was unusual. In our longitudinal studies with more than 40 commercial turkey flocks no flocks were identified that were apparently *Campylobacter*-free throughout their pre-harvest growth (S.Kathariou, J. Barnes and D. Carver, unpublished results).

The factors that prevented, or at least markedly reduced, *Campylobacter* colonization of the flocks T_1 and T_2 remain unidentified. Obvious factors such as the incoming day-old birds, feed, and bird density (Numbers of birds per ft^2) can be excluded, as these were not different between flocks C_1 and C_2 and flocks T_1 and T_2. Suggestive evidence, however, points to the likely importance of implemented biosecurity measures. Unlike the commercial flocks, the Teaching Animal Unit (flocks T_1 and T_2) routinely used well-kept footbaths for everyone entering the turkey house. This may have minimized or prevented possible transmission from the numerous students, interns and support staff that enter the facility, even though the turkey house is in proximity to teaching units rearing other food animals that can serve as hosts for *Campylobacter* (swine, cattle). The possible impact of footbaths in reduction of *Campylobacter* transmission has been suggested in studies with broilers (Humphrey et al., 1993). Another plausible explanation could be although the TAU was in proximity to other animals that harbor *Campylobacter*, there were no other turkey farms nearby and interns and staff do not enter the turkey house after being on a commercial farm without

showering. This is typically not the case on a commercial farm where one service technician may visit several in one day. In addition to enhanced biosecurity, proper litter management may have played an important role in the prevention or reduction of *Campylobacter* in the TAU birds. The litter in the TAU farm (flocks T_1 and T_2) was meticulously tilled daily, and accumulations of wet litter were avoided. In contrast, wet litter (especially under the drinkers) was frequently seen in the commercial turkey houses.

Although the facilities for flocks T_1 and T_2 had a prolonged downtime between flocks, we do not think that this had a significant impact on the lack of colonization. The literature reports that newly established broiler farms become readily contaminated by *Campylobacter* similarly to existing operations (Gregory et al., 1997), and this is supported by our experience with turkey farms in North Carolina (S. Kathariou and D. Carver, unpublished findings). Another factor that did not appear to have significant impact was the degree of human traffic entering the turkey houses. Numerous individuals (students, interns, support staff) entered the TAU turkey house daily. Although in other studies human traffic has been associated with colonization of the flocks (Refregier-Petton et al., 2001; Shreeve et al., 2000), its impact in the TAU facility may have been minimized. There were no turkey farms in close proximity to the TAU and no one was permitted in the TAU after being on another turkey farm without showering. The isolation of the TAU in combination with the use of footbaths may have been responsible for minimizing the impact of human traffic.

In conclusion, we show that colonization by *Campylobacter*, while rapid and extensive in the commercial turkey flocks were maintained at levels undetectable by

culture-based procedures in two sibling flocks from a shared breeder population. These data indicate that vertical transmission alone could not account for the differences in levels of colonization between the flocks. Our study also indicates that biosecurity measures, specifically the use of footbaths and meticulous litter management practices in combination with isolation of the TAU are likely, albeit still not rigorously proven, factors contributing to prevention of turkey colonization by *Campylobacter*.

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Table 1

Colonization of Flock C_1 by *Campylobacter*

Week[1]	*Campylobacter*-positive birds (%)	*C. jejuni*/ *C. coli*[4]
1	0/5 (0%)	NA
2	3/5 (60%)	0/3
3-9	35/35 (100%)	0/29
10	5/5 (100%)	2/4
11	5/5 (100%)	ND[2]
12	5/5 (100%)	0/8
13	5/5 (100%)	1/8
14[3]	9/9 (100%)	0/21

[1]Two cecal samples collected per bird and analyzed as described in Materials and Methods. Data was combined for the two sites as there was no difference between isolates.

[2]ND, Not Determined. Cultures from these samples were not preserved

[3]Cecal samples from birds in the processing plant (n=27) three cecal collections/ bird

[4] Ratio of *C. jejuni* to *C. coli* out of the total isolates purified from each week. Some weeks multiple isolates could be obtained that appeared different and all were speciated. Other week some isolates could not be purified. Thus, number of positive birds and isolate number may not match up.

Table 2

Commercial Colonization of Flock C_2 by *Campylobacter*

Week[1]	*Campylobacter*- positive birds (%)	*C.jejuni* /*C. coli*[3]
1-2	0/10	NA
3	5/5 (100%)	2/5
4	5/5 (100%)	0/6
5		
Cecal	5/5 (100%)	2/8
Fecal	9/9 (100%	4/5
6-8		
Cecal	15/15 (100%)	0/14
Fecal	17/20 (85%)	0/5
13[2]	0/10	NA
15	14/16 (88%)	0/4

[1]Cecal samples for weeks 1-8, fecal samples for weeks 5-8, 13 and 15. Two cecal samples were collected per bird and analyzed as described in Materials and Methods. Data was combined for the two sites as there was no difference between the isolates.

[2] Flock was diagnosed with coronavirus infection

[3] Ratio of *C. jejuni* to *C. coli* out of the total isolates purified from each week. Some weeks multiple isolates could be obtained that appeared different and all were speciated. Other weeks some isolates could not be purified. Thus, number of positive birds and isolate number may not match up.

Chapter III

A longitudinal Analysis of Strain Types and Antibiotic Resistance Profiles of *Campylobacter* in Two Turkey Flocks Reared in North Carolina

Abstract

Campylobacter spp. are currently the leading cause of foodborne acute bacterial gastroenteritis in industrialized countries. Handling and consumption of poultry products is considered to be the primary source of human infection. In addition, an issue of current interest is the dissemination of antibiotic resistance among *Campylobacter* spp. For these reasons, there is a growing need to identify the sources of *Campylobacter* contamination at production. Information on prevalence, strain types, and antibiotic resistance attributes may help to identify and monitor for possible routes of transmission and may also identify future strategies for eventual reduction of the incidence of antibiotic resistance.

The aim of this study was to conduct a longitudinal analysis of two commercial turkey flocks from the time of placement through processing. The key factors of interest were strain type and antibiotic resistance profiles of the *Campylobacter* isolates. Both commercial flocks rapidly became colonized by a limited number of strains, with one dominant strain being isolated throughout the production period. Antibiotic resistance among the *Campylobacter coli* isolates was widespread and was directed to a variety of antibiotics, including erythromycin and fluoroquinolones. *Campylobacter jejuni* isolates were also resistant to a wide range of antibiotics but were more likely to be sensitive to erythromycin and fluoroquinolones. Analysis of *Campylobacter* strains from the breeder hens indicated that the commercial flocks were colonized by sources other than the breeder flocks. The observed high level of antibiotic resistance in both *C. coli* and *C.*

jejuni is of food safety relevance. Antibiotic resistance can be disseminated from *Campylobacter* to other bacteria that cause human illness.

Introduction

Campylobacter spp. (primarily C. *jejuni* and C. *coli*) are currently the leading bacterial cause of acute gastroenteritis in people in industrialized countries (Friedman et al., 2000). *Campylobacter* infections have also been associated with severe autoimmune sequelae such as Guillain Barre Syndrome, which is characterized by temporary paralysis (Nachamkin et al., 1998). The main risk for *Campylobacter* infections is the consumption of contaminated foods of animal origin, particularly poultry (Deming et al., 1987, Linton, 1996). As many as 90% of broiler chickens and turkeys may harbor *Campylobacter* (Luechtefeld and Wang, 1982, Wallace et al., 1997), however, even with high colonization rates, affected animals may show little or no clinical signs of illness (Shane, 1991).

There are several factors that affect the rate and spread of *Campylobacter* colonization at the production level. Some of these factors include host susceptibility, different transmission routes or mechanisms of colonization, and the ubiquitous nature of *Campylobacter* spp. (Bailey, JS, 1993). These aspects of *Campylobacter* infection indicate a growing need for a multi-faceted approach to controlling *Campylobacter* colonization at the farm. The best way to markedly reduce the contamination of poultry products with *Campylobacter* would be to produce flocks that are free of *Campylobacter* upon arrival at the processing plant (Bailey, JS, 1993 and Jacobs-Reitsma, 1997). In order to achieve this goal, there is a need for better understanding of the factors that

influence changes in prevalence and in strain types of *Campylobacter* in poultry flocks. In addition, an issue of current interest is the dissemination of antibiotic resistance among *Campylobacter* spp. Information on prevalence, strain types, and antibiotic resistance attributes may help to indicate possible routes of transmission and may also identify future strategies for eventual reduction of the incidence of antibiotic resistance in *Campylobacter*.

Significant effort has been invested to monitor *Campylobacter* colonization in poultry, however the majority of studies have involved broilers, and limited information is available on the colonization of turkeys by this pathogen. Colonization of turkey flocks was described in detail in only one study. Surveillance of two flocks, reared at one site, revealed that the birds became colonized by *C. jejuni* between 3 and 4 weeks of age, and suggested a succession of biotypes during the lifetime of the flock (21 weeks) (Wallace et al., 1998).

The focus of this study was to conduct a longitudinal analysis of two commercial turkey flocks from the time of placement through processing. The strain types and antibiotic resistance profiles of the isolates were the key factors of interest. Isolates from the breeder flock that supplied the eggs for one of the commercial flocks were typed in order to investigate the possibility of vertical transmission.

Materials and Methods

Turkey flocks involved in this study.

Two commercial turkey flocks were involved in this study (flocks 1 and 2). Both flocks were initiated as day-old poults. Flock 1 consisted of ~8000 hens and was initiated August 2001. Flock 2 consisted of ~ 6000 toms and was initiated the last week of

September 2002. Flock 1 was processed at week 14 at a local processing plant. Flock 2 was monitored through week 15. No plant samples could be obtained from flock 2.

Antibiotic Resistance.

Resistance to tetracycline, erythromycin, kanamycin, nalidixic acid, and ciprofloxacin was determined by the disk diffusion method (BBL). Antibiotic concentrations were: tetracycline, 30µg; erythromycin, 15µg; kanamycin, 30µg; nalidixic acid, 30µg; and ciprofloxacin, 5µg. One *Campylobacter* colony was suspended in 1ml of Mueller Hinton Broth (MHB) and 200µl was plated on SBA. Disks were aseptically placed onto the inoculated SBA plates using a Becton Dickinson (BBL) disk dispenser. Plates were scored following microaerobic incubation at 42°C for 36 hours. Strains were considered resistant if there was no zone of inhibition around the disk after incubation at 42°C for 48 h. For ampicillin and streptomycin testing, bacteria were sub-cultured on MHA containing ampicillin (100µg $ml^{-1)}$ and streptomycin (15µg $ml^{-1)}$ and resistance was based on the presence of growth following microaerobic incubation at 42°C for 36 to 48h. For comparison, treatment records for the two flocks in this study are provided in appendix B.

Sample collection, *Campylobacter* isolations, DNA extractions, PCR, and PCR-RFLP were all performed according to the methods described in chapter II.

Results

Both commercial flocks were negative for *Campylobacter* on the day of placement but became colonized between 2-3 weeks of age and remained colonized throughout the production period. The predominant species of *Campylobacter* that

colonized these flocks was *C. coli* which accounted for 84% and 88% of the isolates respectively. The remaining isolates were all *C. jejuni*.

Antibiotic Resistance.

Overall, the incidence of antibiotic resistance was high for the *C. coli* isolates obtained from commercial flock 1, and remained high at processing (Table 1). The majority of the isolates that were obtained from the commercial flock 1 between week 2 and week 5 had a multi-resistant phenotype, showing resistance to all seven tested antibiotics (Table 1). The incidence of resistance to some of the antibiotics decreased as the birds aged. The decrease was especially noticeable for nalidixic acid and ciprofloxacin (100% strains were resistant in weeks 2-4, whereas 29 % were resistant in weeks 6-13, and 33% were resistant at processing). All isolates that were resistant to nalidixic acid were also resistant to ciprofloxacin.

The 8 *C. jejuni* isolates obtained from the processing plant samples of flock 1 were largely resistant to tetracycline, streptomycin, and ampicillin (> 80%), and 50% were resistant to kanamycin . Although all *C. jejuni* isolates were sensitive to erythromycin the resistance to the fluoroquinolones was 25%. At processing 34% of the *C. jejuni* and *C. coli* isolates combined were resistant to fluoroquinolones and at least one other antibiotic, and 8% were resistant to all antibiotics.

A preliminary study of the turkeys grown on the same farm as flock 2 in the previous growing season revealed that the birds were colonized by *C. coli* of a multi-resistant phenotype in weeks 2 through 4. At the end of the production cycle the multi-

resistant phenotype accounted for 20% of the isolates and 30% of the isolates were resistant to quinolones and at least one other antibiotic.

The *C. coli* isolates obtained from commercial flock 2 also had a large number of strains that were multi-resistant to all seven antibiotics in the first 5 weeks. Resistance to streptomycin, erythromycin, and the quinolones tended to decrease as the birds aged (table 2). However, the resistance to these antibiotics was still about 50% or higher.

There were only 8 *C. jejuni* isolates obtained from flock 2. Seven of these isolates were resistant to fluoroquinolones, whereas only 3 were resistant to streptomycin. None of the *C. jejuni* isolates were resistant to erythromycin.

There were 14 isolates collected from one of the breeder flocks for flock 2. Three of the isolates were *C. jejuni* and were resistant to tetracycline and ampicillin only. The remaining isolates were all *C. coli*. Eight of the 11 *C. coli* isolates were resistant to tetracycline, ampicillin, and the quinolones and 2 of the 11 were resistant to tetracycline, ampicillin, kanamycin, and the quinolones. None of the *C. coli* isolates from the breeders were erythromycin resistant.

Sub-typing

A total of 4 different strain types were detected by *fla* PCR-RFLP in the *C. coli* cecal isolates from flock 1 in weeks 2 through 13 (Fig. 1 lanes 1,2,3,5,). At processing, a total of 3 new *C. coli* strains types were detected (Fig 1, lanes 6, 8, 10). Lanes 3, 4, 7 and 9 mark the dominant strain, strain type III, which was isolated a total of 24 times throughout weeks 7-9, 12 and 13 as well as from the plant. Most (88%) of type III isolates shared the same resistance pattern (TSAEK), showing sensitivity only to the quinolones. Strain type I (lane 1) was isolated a total of 5 times and was a multi-resistant

strain type (TSAEKN) that was isolated between weeks 2 and 5. Strain type II (lane 2) was isolated from weeks 5 and 6 and showed sensitivity to streptomycin and erythromycin (TAKN). Subtype IV (lane 5) was isolated in weeks 12 and 13 and was multi-resistant (TSAEKN). The three *C. jejuni* strains isolated from the cecal samples at weeks 10 and 13 were of the same strain type (Fig. 1, lanes 11 and 12) (These data are summarized in Table 3).

Flock 2 had a total of 5 different *C. coli* and 4 different *C. jejuni* strain types. There was one dominant strain type (type I) that accounted for 77.8% of all the *C. coli* isolates and was isolated throughout the production cycle from week 3-15 (figure 2-lanes 10 and 11). This particular strain type also had a multi-resistant phenotype (TSAEKN). Strain type II (lane 12) was isolated in week 7 only and showed the same multi-resistant phenotype. Strain type III (lane 13) was also isolated in week 7 and was sensitive to streptomycin and erythromycin (TAKN). Strain type IV (lane 14) was isolated in week 7 only and was sensitive to streptomycin and erythromycin (TAKN). Strain type V (lane 15) was isolated once in week 9 and showed resistance to tetracycline, streptomycin, and kanamycin (TSK) (These data are summarized in Table 4).

There was 1 *C. jejuni* strain type (Fig 3 lanes 1-3) and 5 different *C. coli* strain types (Fig 2 lanes 1, 3, 6, 8, 9) isolated from the breeder flock samples. These strain types were unique to the breeder flock and were not isolated from the commercial flock. *C. coli* strain types I, II, III, IV (lanes 1, 3, 6, and 8) shared the same antibiotic resistance profile (TAN). Strain type II was the predominant strain type and accounted for 40% of the breeder flock *C. coli* isolates (Fig 2 lanes 3, 4, 5, and 7). All 3 C. *jejuni* strains had the

same *fla* type and shared the same resistance phenotype, resistance to tetracycline and ampicillin only (TA)

Discussion

There are highly conserved and variable regions present in the flagellin gene locus of *Campylobacter*. These attributes make this region a suitable region for PCR-RFLP. Several different restriction enzymes may be used to generate PCR product fragments and DdeI is the most discriminatory (Wassenaar and Newell, 2000). Although there are other methods used to type that may be more discriminatory such as AFLP, PCR-RFLP has proven to be useful, reliable, and relatively simple subtyping technique (Wassenaar and Newell, 2000). This technique was chosen for this study as in a study by Sammarco et al. PCR-RFLP proved to be a useful tool to compare isolates within a single outbreak against biochemical methods such as antimicrobial susceptibility testing (Sammarco et al., 2003)

The two turkey flocks involved in this study were colonized by at least 4 different strain types with a variety of antibiotic resistance profiles within the production cycle. This diversity in strain types as well as antibiotic resistance profiles are in accordance with findings in studies with broilers (Jacobs-Reitsma, 1995, Jacobs-Reitsma, 1997). According to Wallace et al. (1998) there is a succession of *Campylobacter* biotypes throughout the lifetime of turkey flocks.

Our results indicate colonization by a dominant strain type with succession of strains that were isolated less frequently. The occurrence of a dominant strain type could indicate one main source of contamination. Another reason for the dominant strain type could be selective pressure since strains of a given *fla* type tended to have a characteristic

antibiotic resistance profile (Tables 3, 4). A third explanation could be that the dominant strain type is a common strain type that frequently colonizes turkeys. Studies have shown that strains that have passed through a bird once can colonize more efficiently on the second passage (Ringoir and Korolik, 2003).

The occurrence of new strain types that were isolated less frequently may reflect the continuous flow of new campylobacters entering the flock through horizontal transmission routes (Jacobs-Reitsma, 1997). Due to the logistics of this study no samples were taken from water or other environmental sources. Future studies will need to investigate possible transmission routes through environmental reservoirs.

Several antibiotics used to treat turkeys are also used in human medicine. More importantly, there are no alternative antibiotics for some of these classes of drugs if resistance develops. According to the feed additive compendium fluoroquinolones, erythromycin and virginiamycin are approved for use in turkeys and there are no alternatives in human medicine. Other antibiotics that are used to treat humans and turkeys include penicillins, tetracyclines, gentamicin, chloramphenicol, lincomycin, and bacitracin. The number of antibiotics used to treat both humans and livestock highlight the concern for the emergence of antibiotic resistance.

A high level of resistance to multiple antibiotics was found in the *C. coli* isolates obtained from these flocks. *C. coli* isolates obtained from the first 5 weeks of the production cycle in both flocks were of a multi-resistant phenotype. Muli-resistant phenotypes, defined as resistance to 4 or more antibiotics, is more common in *C. coli* than *C. jejuni* isolates according to a study on poultry meat (Van Looveren et al., 2001). Antibiotics are frequently administered to poults in the first few weeks to prevent

secondary infections or to treat diarrheal disease. Although the resistance did decrease somewhat in both studies, the overall pattern is that the *C. coli* strains that colonized the birds in this study were resistant to a variety of antibiotics, such as erythromycin which is often used as a first line defense to treat gastrointestinal disease in humans. The dominant strain type (type III) in flock 1 accounted for 58% of all the isolates and was resistant to all antibiotics except the quinolones. This strain was isolated in weeks 7-13 and was primarily responsible for the observed trend in the antibiotic resistance data in which only the resistance to the quinolones seemed to decrease after the first 5 weeks. In flock 2, the dominant stain type accounted for 81% of the isolates and was resistant to all antibiotics. The decrease in resistance to streptomycin and erythromycin during weeks 7 through 9 in flock 2 corresponded to the introduction of strain types III and IV, which were sensitive to streptomycin and erythromycin and were isolated from the flock in week 7. Following week 7, resistance to streptomycin and erythromycin increased some with the disappearance of strain types III and IV and the prevalence of the multi-resistant strains.

Overall the *C. jejuni* isolates in both study flocks were much more sensitive to the macrolides, such as erythromycin and to the quinolones. Limited prevalence of macrolide resistance in *C. jejuni* has been reported in other studies (Aarestrup et al., 1997, Gaudreau and Gilbert, 1998). Prats et al (2000) and Van Looveren et al. (2001) also reported that quinolone resistance was much higher in *C. coli* than *C. jejuni* from poultry isolates.

According to our results it appears that the most plausible explanation for the occurrence of a dominant strain type is selection based on antibiotic pressure. Typically

turkeys will move to growout at 6 to 7 weeks which is in accordance with the antibiotic resistance and strain typing data in which new strains and antibiotic resistance profiles were isolated beginning in week 7. Flock 1 was moved to a different house on the same farm, whereas flock 2 was moved to a completely different farm. This move could also induce stress in the birds causing them to become colonized by additional strains. There are often new strains introduced at the plant or in transit to the plant (Newell et al., 2001). The isolation of 3 new strains from the processing plant samples of flock 2 is in accordance with the literature. These new strains could have come from a variety of sources such as the transport truck, crates, or cross-contamination from the plant. All of these sources should be considered in the design of future studies.

In conclusion, we show that both turkey flocks were colonized with at least four different strain types with one dominant strain type being isolated after the first few weeks and throughout the production period. The strain typing and antibiotic resistance data correlate indicating that strain types may have been selected on the basis of antibiotic resistance. The high level of antibiotic resistance, particularly in the dominant strain types in both flocks, is of relevant food safety concern. Antibiotics such as erythromycin are commonly used to treat severe gastroenteritis in humans. In addition, bacteria carrying antibiotic resistance often disseminate the resistance among other bacteria that can infect humans. On the basis of the strain typing data, we show that vertical transmission alone cannot account for colonization in flock 2. Future studies should be designed to identify and monitor possible horizontal routes of transmission and special attention should be given to determine whether the high prevalence of antibiotic resistant strains is in response to the use of antibiotics in the turkey flocks.

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Table 1. Incidence of antibiotic resistance in *C. coli* isolates obtained from commercial flock 1.

Flock 1

Antibiotic	Weeks 2-5 (n=7)	Weeks 6-13 (n=48)	Processing (n=28)
tet	100%	100%	95%
str	86%	94%	91%
amp	100%	100%	95%
em	86%	88%	64%
kan	100%	94%	77%
na	100%	29%	36%
cipro	100%	29%	36%

[1] tet- tetracycline; str-streptomycin; amp-ampicillin; em-erythromycin; kan-kanamycin; na-nalidixic acid; cipro-ciprofloxacin

Table 2. Incidence of antibiotic resistance of the *C. coli* isolates obtained from commercial flock 2.

Flock 2

Antibiotic	Weeks 3-6 (n=32)	Weeks 7-9 (n=15)	Week 15 (n=4)
Tet	100%	100%	100%
Str	100%	47%	80%
Amp	100%	100%	100%
Em	100%	47%	60%
Kan	100%	100%	100%
Na	100%	47%	60%
Cipro	100%	47%	60%

[1] tet-tetracycline; str-streptomycin; amp-ampicillin; em-erythromycin; kan-kanamycin; na-nalidixic acid; cipro-ciprofloxacin

Table 3

Summary of strain types in production cycle of flock 1.

C. coli				*C. jejuni*			
***fla* Types[1]**	**#**	**Week Isolated**	**Ab Res. Profile[3]**	**fla Types[2]**	**#**	**Week Isolated**	**Ab Res. Profile[3]**
CC I	5	2-5	TSAEKN	CJ I	3	10, 13	TSAKN, TKN
CC II	2	5,6	TAKN				
CC III	24	7-9, 12, 13	TSEK, TSAEKN				
CC IV	4	12, 13	TSAEKN				
Total	35				3		

[1] CC- *C. coli*
[2] CJ- *C. jejuni*
[3] T-tetracycline; S-streptomycin; A-ampicillin; E-erythromycin; K-kanamycin; N-naladixic acid and ciprofloxacin

Table 4

Summary of strain types in production cycle of flock 2.

C. coli				*C. jejuni*			
***fla* Types[1]**	**#**	**Week Isolated**	**Ab Res. Profile[3]**	***fla* Types[2]**	**#**	**Week Isolated**	**Ab Res. Profile[3]**
CC I	21	3-15	TSAEKN	CJ I	5	3-5	TAKN
CC II	2	7	TSAEKN	CJ II	1	5	TSAKN
CC III	1	7	TAKN	CJIII	1	5	TSAK
CC IV	1	7	TAKN	CJ IV	1	6	-
CC V	1	9	TSK	-	-		
Total	26			**Total**	8		

[1] CC- *C. coli*
[2] CJ- *C. jejuni*
[3] T-tetracycline; S-streptomycin; A-ampicillin; E-erythromycin; K-kanamycin; N-naladixic acid and ciprofloxacin

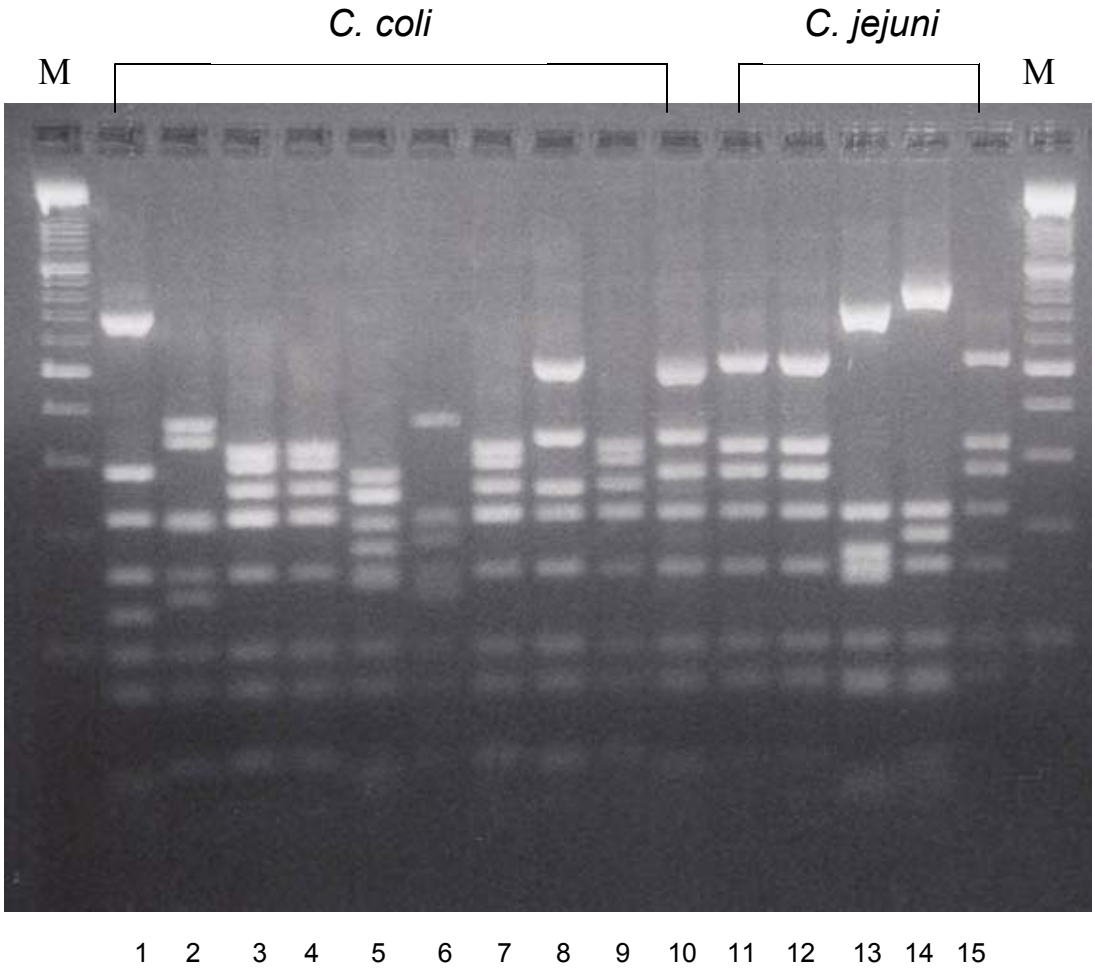

Fig 1. Representative *fla* types of *C. coli* and *C. jejuni* isolates obtained from flock 1**.**

Lanes 1-10, *C. coli* strain types: lane 1, week 2; lane 2, week 6; lane 3, week 7; lane 4, week 12; lane 5, week 12; lanes 6-10, plant samples. Lanes 1-15, *C. jejuni* strain types: lane 11, week 10; lane 12, week 13; lanes 13-15, plant samples. M- marker XIV

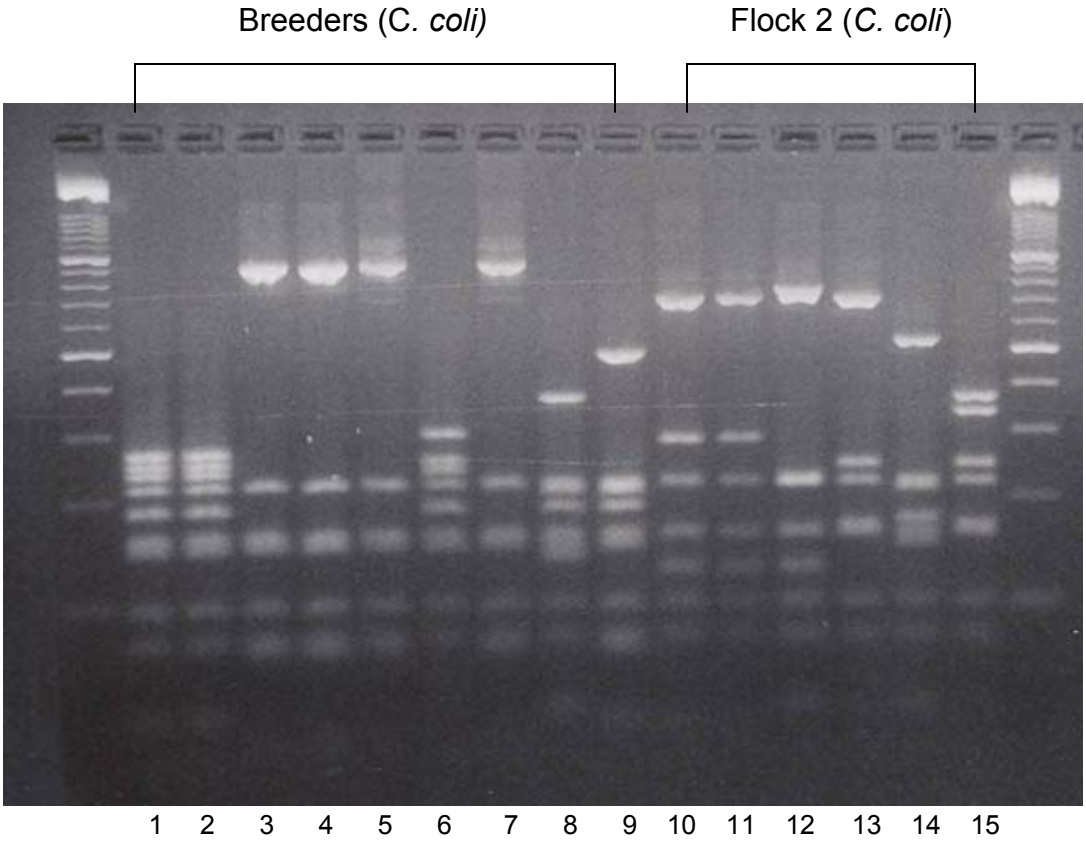

Fig 2. Representative *fla* types of *C. coli* isolates obtained from flock 2 in comparison to *C. coli* isolates obtained from their breeder flock

Lane 1-9 breeder flock isolates taken 10 days before flock 2 hatched. Lane 10, week 3; lane 11, week 6; lane 12, week 7; lane 13, week 7; lane 14, week 7; lane 15, week 9. M-marker XIV.

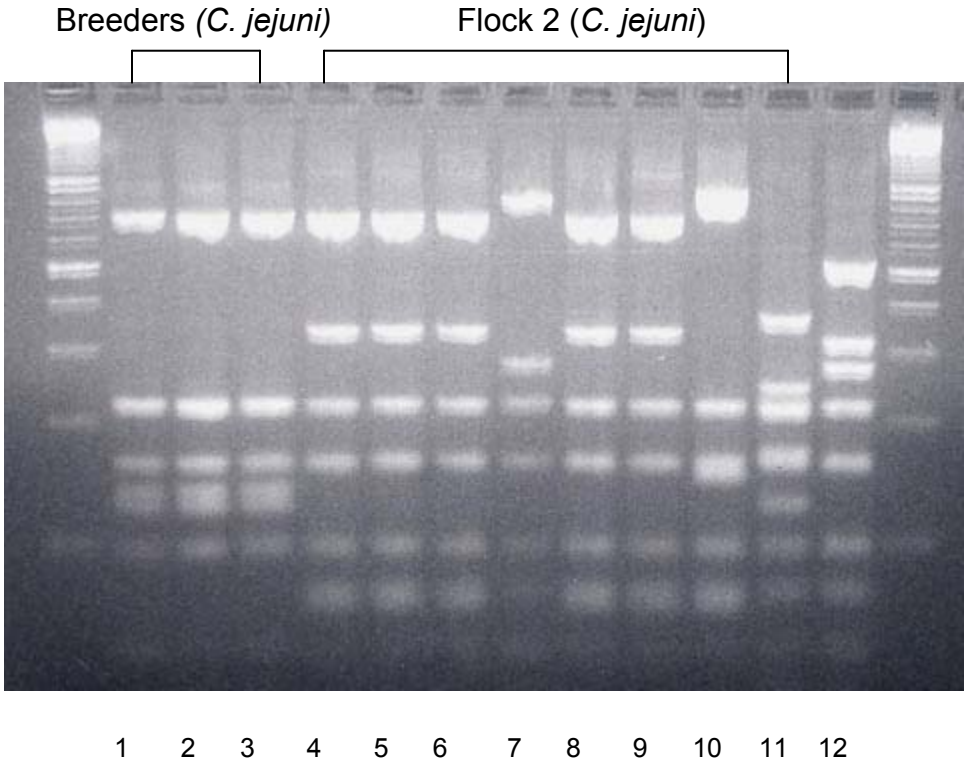

Fig 3. Representative *fla* types of *C. jejuni* isolates in flock 2 in comparison to *C. jejuni* isolates obtained from their breeder flock.

Lane 1-3 breeder flock isolates taken 10 days before flock 2 hatched. Lane 4-6, 8 and 9 represents the predominant *C. jejuni* strain type isolated in weeks 3 and 5. Lane 7, week 5; lane 10, week 5; lane 11, week 6; lane 12, control strain NCTC 11168.

Chapter IV

A Cross-sectional Survey of *Campylobacter* Colonization in Broilers Reared in Eastern North Carolina

Abstract

Campylobacter jejuni is the primary species that colonizes broiler chickens. In a longitudinal study of two turkey flocks raised in eastern North Carolina, the predominant species of *Campylobacter* that colonized turkeys was *C. coli*. A high level of antibiotic resistance was present among the isolates. To determine if this finding was also true for broilers chickens raised in the same geographic area, a cross-sectional survey was conducted on broilers. Four-week broiler chickens were sampled; on 32 farms from two broiler integrators in eastern North Carolina. Sixteen of 32 flocks were colonized with *Campylobacter*. The species colonizing the birds was determined in 15 of the 16 flocks; of these, 10 were colonized with *C. jejuni*, 5 were colonized by *C. coli*. Company A had 5 flocks that were colonized by *C. jejuni*, 4 flocks that were colonized by *C. coli*. Company B had 5 flocks that colonized by *C. jejuni*, 1 flock colonized by *C. coli*. There was a high prevalence of antibiotic resistance to several antibiotics in both the *C. jejuni* and *C. coli* isolates which is similar to what was found previously in the turkey isolates. Resistance was particularly high to fluoroquinolones. *C. jejuni* isolates were also highly resistant to tetracycline, whereas *C. coli* isolates were more resistant to kanamycin. Company A isolates were more resistant to tetracycline, erythromycin, kanamycin, and fluoroquinolones than company B. These findings indicate that the broiler chickens raised in eastern North Carolina are colonized by *C. jejuni* and isolates have a higher prevalence of antibiotic resistance to fluoroquinolones compared to turkey isolates obtained from the same region.

Introduction

Campylobacter has become the leading bacterial cause of acute gastroenteritis in people in industrialized countries. The main risk of *Campylobacter* infections is handling raw poultry and/or consumption of undercooked meat of animal origin, particularly poultry (Deming et al., 1987; Linton, 1996; Skirrow, 1982; Kapperud et al., 1992; Alketruse et al., 1999). *Campylobacter* spp., especially *jejuni* and *coli*, are considered to be commensal inhabitants of broilers. As many as 90% of chickens may harbor this organism however even with high colonization rates, affected animals often show little or no clinical signs of illness(Luechtfeld and Wang, 1982; Shane, 1991; Stern et al., 1988; Kapperud et al., 1993).

C. jejuni, accounts for 80-90% of the isolates, while *C. coli* only accounts for 10-20% of the isolates (Van Looveren et al., 2001; Wedderkopp et al., 2001). A longitudinal study of two turkey flocks in eastern North Carolina showed the primary species that colonized the turkeys was *C. coli* (84%-88%) and not *C. jejuni* (Smith et al., 2003). In addition, a high level of resistance to multiple antibiotics was discovered in the isolates. This finding developed an interest to compare the species of *Campylobacter* that colonize broilers raised in eastern North Carolina. A cross-sectional survey was conducted to determine the prevalence of *Campylobacter* species in broiler chickens raised in eastern North Carolina.

Materials and Methods

Sample Collection.

Flocks of 4- week old broiler chickens on 32 farms from two integrators in eastern North Carolina were cultured. Five randomly chosen clinically healthy birds were killed by cervical dislocation according to guidelines of the approved Institutional Use and Animal Care Committee's protocol at North Carolina State University. One cecum was collected from each of the five birds and examined bacteriologically to determine species and antibiotic resistance profiles. *Campylobacter* isolations and PCR were determined according to methods described in chapter II. Antibiotic resistance profiles were determined according to methods described in chapter III.

Results

Prevalence

Of the flocks on 32 farms that were surveyed, 16 farms were positive for *Campylobacter*. Of these 16 farms, 10 farms had flocks that were colonized by *C. jejuni* (63%) and 5 flocks were primarily (1 flock from company A had 6 *C. coli* isolates and 1 *C. jejuni* isolate) colonized by *C. coli* (31%). It was not possible to isolate the organism from 1 of the 16 farms. Flocks on 14 farms were sampled from Company A and 18 farms from Company B. Nine of 14 flocks sampled from Company A were positive for *Campylobacter*; 5 flocks were colonized by *C. jejuni* and 4 were positive for *C. coli*. Six of 18 flocks sampled from company B were positive for *Campylobacter*; 5 were colonized by *C. jejuni* and 1 *C. coli* (Results are combined for the two isolation sites as there were no differences between them, see Table 1).

Antibiotic Resistance

Resistance to fluoroquinolones was high among all isolates. There was no resistance to streptomycin in any of the isolates. Company A isolates had a higher level of resistance to all antibiotics than did company B isolates. Isolates from company A were 87% resistant to the quinolones whereas isolates from company B were 47% resistant to the quinolones. There were also several isolates (46%) from company A that were resistant to two or more different antibiotics, in addition to the quinolones. Resistance to the quinolones for *C. jejuni* and *C. coli* isolates was 67% and 78% respectively. *C. jejuni* isolates were more resistant to tetracycline while resistance to kanamycin, erythromycin, and fluoroquinolones was more prevalent in *C. coli* (table 2).

Discussion

The most common species of *Campylobacter* that colonizes chickens is *C. jejuni*, accounting for 80-90% of the isolates, while *C. coli* accounts for 10-20% of the isolates (Van Looveren et al., 2001; Wedderkopp et al., 2001). In this study, 63% of the flocks surveyed in eastern North Carolina were colonized by *C. jejuni* and the remaining 31% were colonized by *C. coli*. This represents a relatively high prevalence of *C. coli* in these flocks. Additional studies involving a greater number of flocks are needed to determine if *C. coli* is more prevalent in broilers raised in eastern North Carolina. A possible explanation for the prevalence of *C. coli* could be selection based on antibiotic use especially in company A. *C. coli* is inherently more resistant to antibiotics than *C. jejuni*.

A high level of antibiotic resistance was found in both the *C. jejuni* and *C. coli* isolates. Tetracycline resistance was more common in *C. jejuni* isolates and kanamycin and erythromycin resistance was more common in *C. coli* isolates. These antibiotic results are similar to a study conducted in Spain where *C. jejuni* isolates from broilers were more resistant to tetracycline than *C. coli* and less resistant to erythromycin and kanamycin (Saenz et al., 2000). The resistance in other countries such as Ireland appears to be much lower overall. In a study by Lucey et al. (2002) resistance of poultry isolates to fluoroquinolones was 30% and resistance to tetracycline was 24% overall in *C. jejuni* and *C. coli* isolates. Isolates from turkeys in previous studies by Smith et al. were more resistant to multiple antibiotics but were more sensitive to the quinolones than broiler isolates obtained in this study.

Variability in antibiotic resistance profiles between integrators is usually indicative of different frequencies and/or concentrations of antibiotics used within the company. Isolates from company A were more likely to be resistant than company B which may be indicative of a history of more frequent antibiotic use on these farms. Especially resistance to tetracycline, erythromycin, kanamycin, and quinolones.

In conclusion, we have shown the most common species of *Campylobacter* colonizing broilers raised in eastern North Carolina is *C. jejuni* which is in agreement with the literature (Van Looveren et al., 2001; Wedderkopp et al., 2001). Therefore the prevalence of *C. coli* in turkeys raised in North Carolina may not be indicative of larger numbers of *C. coli* in this region. However, we have also shown that *C. coli* was prevalent in broilers raised by company A which may be indicative of more frequent antibiotic use on these farms. There was a high level of antibiotic resistance in the broiler

isolates especially to quinolones. The resistance to quinolones was higher in these broiler isolates than in turkey isolates from the same region.

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Table 1. *Campylobacter* Colonization in Broilers

Company	Prevalence	*C. jejuni*	*C. coli*
A	9/14 (64%)	5	4
B	6/18 (33%)	5	1
Total	**16 farms**	**10**	**5**

Prevalence of *Campylobacter* by farm and species from broiler flocks involved in this study. No samples were purified from 1 farm. Cecal isolates were combined as there was generally no difference between the two isolation sites.

Table 2. Antibiotic Resistance in Broiler Isolates

Antibiotic[1]	Company A	Company B	*C. jejuni*	*C. coli*	Total
Str	0/61	0/43	0/72	0/32	**0**
Amp	33/61(54%)	25/43(58%)	40/72(56%)	19/32(59%)	**117/208(56%)**
Tet	40/61(66%)	18/43(42%)	52/72(72%)	6/32(19%)	**116/208(56%)**
Em	8/61(13%)	0/43	0/72	8/32(25%)	**16/208(8%)**
Kan	22/61(36%)	0/43	2/72(3%)	20/32(63%)	**44/208(21%)**
Na	53/61(87%)	20/43(47%)	48/72(67%)	25/32(78%)	**146/208(70%)**
Cipro	53/61(87%)	20/43(47%)	48/72(67%)	25/32(78%)	**146/208(70%)**

[1] str-streptomycin; amp-ampicillin; tet-tetracycline; em-erythromycin; kan-kanamycin; na-nalidixic acid; cipro-ciprofloxacin

Antibiotic resistance in broiler isolates by company and species. Total resistance is based on resistance by each company combined with resistance by each species.

Future Considerations

Future studies on *Campylobacter* in turkeys should be focused on the epidemiology of *Campylobacter* at the production level. There is a need to gain a better understanding of the transmission routes and risk factors that may be responsible for the introduction and spread of *Campylobacter* on the poultry farm. In addition, preliminary studies have shown that *Campylobacter*-free flocks have a higher performance standard providing an added incentive for producers (Reimers, Kathariou, Barnes, unpublished). It has also been shown by our first study that it is possible to consecutively raise turkey flocks that are *Campylobacter*-negative on a farm where other livestock are raised that may harbor *Campylobacter*. Future studies could focus on this system as a model and determine what measures were taken to prevent the colonization of the turkey flocks with *Campylobacter*. Several studies have indicated biosecurity measures play an important role. Different biosecurity measures need to be examined to determine those that are most effective. Studies of this nature have been done in broilers, but there is very little information available on the epidemiology of *Campylobacter* infection in turkey flocks.

Antibiotic resistance should also be addressed in future studies. In particular, researchers must work closely with the farmer to gain information on exactly what antibiotics or antimicrobials were administered and when. As shown in this research, the change in strain types throughout the production cycle coincided with different antibiotic resistance phenotypes. Better knowledge of what exactly is happening to the flock on the farm, in terms of treatments and everyday maintenance, may help clarify the reasons for the appearance and disappearance of different strain types.

Considerable efforts have been made in developing control strategies both on the farm and during processing of poultry. However, improvements still need to be made and knowledge has to be gained concerning the colonization of turkeys by *Campylobacter*. The best way to reduce the risk of campylobacteriosis for consumers is to produce poultry products that are free of *Campylobacter*. Thus, we must focus on strategies to prevent *Campylobacter* infection at the pre-harvest flock level.

Appendix A
Growth Curve of *C. jejuni* and *C. coli* from Cecal Isolates

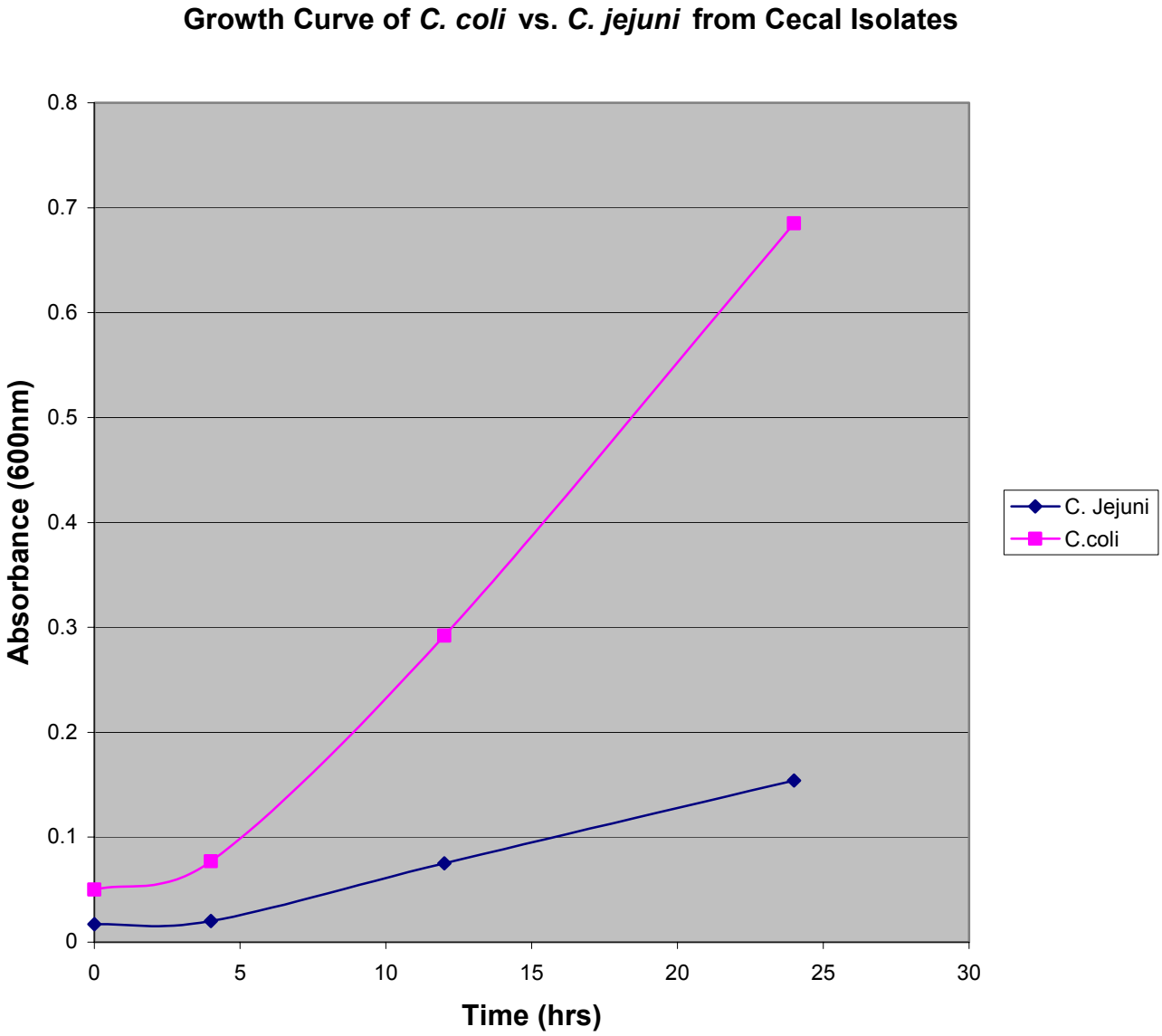

Fig 1. Growth curves were determined with two *C. coli* strains and two *C. jejuni* strains. All four strains were isolated from processing plant samples. The two *C. coli* strains were isolated from the cecum of one bird, whereas 1 *C. jejuni* strain was isolated from the Meckle's Diverticulum of the same bird and the other *C. jejuni* strain was isolated from Meckle's Diverticulum of a different bird.

Appendix B
Treatment Protocols

Table 1. Treatment record for commercial flock 1.

Week	Antibiotic Administered
1	-
2	-
3	lincomycin
4	tetracycline
5	-
6	copper sulfate
7-13	-

Table 2. Treatment record for commercial flock 2.

Week	Antibiotic Administered
1	Baetrol
2-4	-
5	HE vaccination, clinaform
6	lincomycin
6-9	-
10	copper sulfate
11	-
12	copper sulfate
13	-
14	copper sulfate
15	-