ABSTRACT

CRESPO RODRIGUEZ, MARIA DOLORES. *Salmonella* and *Campylobacter* Transmission Routes in Breeder Turkeys. (Under the direction of Dr. Douglas Smith and Dr. Sophia Kathariou).

Salmonella and Campylobacter species are leading causes of human diarrheal illness worldwide. Both pathogens are frequently found in the intestinal tracts of turkeys. However, the sources and routes of transmission of these organisms on poultry farms are not well understood. Most of the research has been performed with broilers while little information is available regarding turkey production. The objective of this study was to determine transmission routes through the different stages of turkey production, from rearing to breeding and hatching, and evaluate prevalence throughout the production chain. A flock of 140 turkey breeder poults was placed in a growout house after the housing environment was sanitized and tested for the presence of *Salmonella* and *Campylobacter*. Poults were separated by sex, then separated further into Treatment (82 hens and 22 toms) and Control (28 hens and 8 toms) groups and placed in different sides of the house. Treatment birds were inoculated via gavage at 10 days old and 12 weeks old with marker *Salmonella* and *Campylobacter* strains. Fecal droppings, intestinal samples, blood, organs, semen and the reproductive tract, as well as feed and drinkers, and vectors of transmission such as insects and mice, were tested for *Salmonella* and *Campylobacter* presence. During the breeding cycle, hens from the inoculated group were artificially inseminated with semen containing both pathogens. Eggs, offspring fecal samples and the reproductive tract were analyzed for evidence of vertical transmission of the bacteria. Processed carcasses from meat birds originating from the breeder flock were tested for prevalence of both pathogens. Assessing the carriage rate and reducing both the prevalence and levels of *Salmonella* and

Campylobacter in turkey and turkey products would reduce human exposure and economic costs associated with food-borne illness. *Campylobacter* was found to spread more rapidly than *Salmonella* and cross-contaminate turkeys throughout the growout house. In this study, naturally occurring strains of both pathogens seemed to successfully compete with the marker strains and persisted longer in the flock. Prevalence of *Salmonella* decreased over time, in contrast with a high prevalence of *Campylobacter* throughout the production cycle. Both pathogens were isolated from vectors present in the houses confirming that pests may contribute to spreading the bacteria. Vertical transmission of *Salmonella* and *Campylobacter* through contaminated semen could not be demonstrated in this study. A better understanding of the ecology and relation between these two pathogens is needed and may contribute to the implementation of effective intervention strategies to prevent the colonization of the flocks on farm, and consequently a reduction in foodborne disease risks related to poultry products.

Salmonella and *Campylobacter* Transmission Routes in Breeder Turkeys

by
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DEDICATION

To my beloved family.

BIOGRAPHY

Maria Dolores Crespo Rodriguez was born in Mos, Pontevedra, Spain. She earned her Bachelor of Science in Food Science and Technology at the Complutense University of Madrid in 2006. After graduation she obtained a research fellowship and joined the IIM-Spanish National Research Council in Vigo where she studied the biofilm formation of the foodborne pathogens *Staphylococcus aureus* and *Listeria monocytogenes* under different environmental conditions. She also worked in the food industry as a quality assurance and laboratory manager but she decided to increase her knowledge in molecular microbiology and she joined Dr. Kathariou's lab in 2010 as a research scholar. Maria earned her Master of Science in Methodologies and Applications in Molecular Biology at the University of Vigo in 2011 and then she joined the laboratory of Dr. Douglas Smith at North Carolina State University, Raleigh for Ph.D. Her doctorate work at NC State was focused on food safety and food microbiology studying *Salmonella* and *Campylobacter* routes of transmission in turkey production.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

REVIEW OF LITERATURE

1.1. INTRODUCTION

Salmonella and *Campylobacter* are leading causes of foodborne illness worldwide and both are associated with poultry meat and products [1–4]. Furthermore, the increasing importance of antibiotic resistant bacteria constitutes an emerging public health hazard [5]. Commercial poultry producers are facing the challenge of eliminating these pathogenic bacteria through the implementation of effective strategies.

The US poultry industry is the largest poultry producer in the world [6]. North Carolina is number two in the national ranking of total turkey production [7]**.** The acceptance and recognition by consumers of turkey meat as a nutritious and healthy product, with high protein and low fat content, good organoleptic properties, and lower price compared with other meats, has led to increased consumption. Turkey meat production was 1.3 billion pounds in the first quarter of 2014, and the predicted total production for the year is 5.7 billion pounds [8]. Data from the United States Department of Agriculture (USDA) from May 2014 estimated consumption of turkey in the US in 2013 was 16.0 pounds per person [9]. High consumption of turkey meat, with the likelihood of pathogen contamination, requires food safety interventions. Reduction or elimination of possible environmental reservoirs would be crucial for minimizing the introduction or reintroduction of both pathogens at the farm level. However, the implementation of successful interventions on the farm, requires better understanding of the environmental ecology aspects of *Salmonella* and *Campylobacter*.

Prevalence and persistence of colonization, identification of strains, and antibiotic resistance profiles of the pathogens in turkey flocks and the surrounding environment can provide valuable information regarding possible routes of transmission into the flock. That information could identify key on farm control strategies which could reduce foodborne disease associated with turkey. Most of the research and the scientific data currently available have been conducted on broilers, but little information is available regarding turkey production, especially through different life stages of the flock. The present study monitored a breeding turkey flock from day of hatch all through the rearing period, reproductive cycle (including artificial insemination), laying and hatching of fertile eggs, and monitoring of second generation offspring from hatch through brooding and rearing until processing. The longitudinal nature of the study allowed us to evaluate both potential horizontal and vertical transmission routes.

1.2. *CAMPYLOBACTER*

1.2.1. Campylobacter General Characteristics

Campylobacter spp. are a leading cause of bacterial gastrointestinal disease in humans. *Campylobacter* is a genus of small (0.2–0.9 μm wide, 0.2–5.0 μm long), curved, Gram negative, non-spore forming, microaerophilic and motile bacteria. Motility has been recognized as essential for colonization, and it has been reported that viscous conditions increase the motility capacity [10–12]. *Campylobacter* motility is given by a polar flagellum

which confers a very characteristic cork-screw-like motion [13]. The flagellum can be found on one, or both ends of the cell [13].

Campylobacters associated with human gastroenteritis are commonly known as thermophilic because of their optimum growth temperature at 42°C with a range between 37°C – 42°C. However, temperatures below 30°C do not support growth [14–16]. Its optimal pH for growth is between 6.5–7.5 and survival gets compromised below pH of 4.9 and above pH 9.0 [14]. *Campylobacter* spp. do not ferment or oxidize carbohydrates as an energy source, instead they use amino acids or intermediates from the tricarboxylic acid cycle [13]. Campylobacters are generally microaerophilic requiring reduced oxygen concentrations to be cultured and optimal growth conditions with 5% O2, 10% CO2 and 85% N [17, 18]. Biochemical tests for identification of species include catalase and oxidase activity, nitrate reduction and hippurate hydrolysis [13, 19]. The particular ability of *C. jejuni* for hydrolyzing hippurate due to the presence of the hippuricase gene, has been only found in *C. jejuni*, and is very useful for its identification and differentiation from other species such as *C. coli* [19].

1.2.2. Campylobacter Viable but Non-Culturable State

Campylobacter can adopt different morphologies depending on the age of the culture or the exposure to adverse conditions such as atmospheric or temperature stress. In fresh young cultures, *Campylobacter* is commonly found as a curved rod, spirally curved, comma shaped, S-shaped or gull-winged shaped. Older cultures or cells exposed to unfavorable conditions

can enter a non-culturable stage with a coccoidal shape [20–23]. Researchers have concluded that coccoidal forms are the result of the response to toxic oxygen species that lead to cell degeneration in culture [22, 24]. This stage represents a degenerative form of the organism rather than a dormant stage that cannot subsist for long periods of time under adverse conditions [23]. Several studies have shown a viable but non culturable (VBNC) state of *Campylobacter*; however the mechanisms of survival are not well understood [15, 25]. Nevertheless, it has been also suggested that cell morphology is not always related to culturability, and research suggest that VBNC campylobacters may have a role in transmission [15, 26].

1.2.3. Campylobacter Taxonomy

The family Campylobacteraceae includes two genera, *Campylobacter* and *Arcobacter*. The number of species and subspecies of *Campylobacter* have been increasing and varying with time. In 2011, 17 species and 6 subspecies were assigned to the genus *Campylobacter*, including *C. jejuni* subsp. *jejuni*, *C. jejuni* subsp. *doylei*, *C. coli*, *C. coli var.hyolilei*, *C. lari*, *C. upsaliensis*, *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis*, *C. curvus*, *C. concisus*, *C. gracillis*, *C. hominis*, *C. helveticus*, *C. hyointestinalis* subsp. *hyointestinalis*, *C. hyointestinalis* subsp. *lawsonii*, *C. canadensis*, *C. lanienae*, *C. mucosalis*, *C. showae*, *C. sputorum* bv. *sputorum*, *C. sputorum* bv. *paraureolyticus and C. rectus* [13, 27].

1.2.4. Campylobacter Implicated in Human Illness

Several risk factors for contracting campylobacteriosis include travelling to developing countries, having contact with pets, and consumption of contaminated water and foods including milk, vegetables, shellfish, and especially poultry [17, 28]. *C. jejuni* (subspecies *jejuni*) and *C. coli* are the two species most frequently reported in human illness. *C. jejuni* accounts for more than 85% of the reported cases of campylobacteriosis in the United States and *C. coli* causes most of the remaining cases [29, 30]. Other species also reported in humans diarrheal disease, but less frequently, are *C. lari* (more frequently associated with birds), *C. upsaliensis* (common in dogs), and *C. fetus* (an opportunistic human pathogen and a common cause of abortion in sheep and cattle) [31–34]. Although they infect humans less frequently, they can also cause severe extra-intestinal infections, usually related to increased human susceptibility factors such as pregnancy, young infants, elderly and immunocompromised people [35, 36].

1.2.5. Campylobacter Clinical Features

Symptoms of campylobacteriosis include abdominal cramps, malaise, fever and severe diarrhea that can be accompanied by mucus and blood, indicating invasion and ulceration of the colonic mucosa [37, 38]. Vomiting is not common [37]. The incubation period can vary, but frequently takes between 2-4 days, however can be as long as 10 days [39]. The infective dose for humans can be less than 500 cells [15, 40]. In most cases, campylobacteriosis is self-

limiting, lasting approximately one week or less, however immunocompromised individuals can suffer recurrent or chronic infections [38]. Serious complications such as bacteremia, septic abortion, pancreatitis, septic arthritis, and other extra-intestinal infections can occur [38, 40]. Death is rare. When pharmacological treatment is needed, the antibiotic of choice is erythromycin [41]. Fluoroquinolones and tetracyclines can be also used, but there was an increase in resistance to these drugs likely due to therapeutic use in the past years [41].

Campylobacteriosis can trigger the development of long term sequelae such as Guillain-Barré syndrome (GBS) or a variant known as Miller Fisher syndrome, reactive arthritis (Reiter's syndrome) and irritable bowel syndrome. The development of sequelae greatly increases the economic disease burden. GBS is a severe autoimmune disease that affects the peripheral nervous system leading to acute neuromuscular paralysis. About 1 in 1000 patients infected by *Campylobacter* develops the disorder [42]. With the reduction of poliomyelitis worldwide, GBS is currently the most common cause of acute flaccid paralysis [42]. It has been estimated that up to 40% Guillain-Barré syndrome cases are associated with campylobacteriosis, and the annual cost of these cases in the United States can be up to $1.8 billion [42, 43]. Acute reactive arthritis is characterized by joint inflammation. Based on prior literature, the annual incidence of reactive arthritis after *Campylobacter* infection can affect 4.3 per 100,000 [44]. Incidence has been estimated between 1 and 5% of people affected by campylobacteriosis with young adults being the population group most commonly affected [44]. Furthermore, 5% of the cases can relapse or become chronic [45].

1.3. *SALMONELLA*

1.3.1. Salmonella General Characteristics

Salmonella are Gram negative, 0.7-1.5 μm wide-2.0-5.0 μm long rod-shaped, facultative anaerobes, and non-spore forming intracellular pathogens belonging to the *Enterobacteriaceae* family. *Salmonella* is closely related to *Escherichia coli* and several studies about their clonal origins have estimated the divergence from a common ancestor about 120-160 million years ago [46, 47]. *Salmonella* is usually motile with peritrichous flagella. However, the poultry-adapted serovars *S.* Gallinarum and *S.* Pullorum are non-motile [48]. *Salmonella* has an optimum growth temperature of 37°C but can grow/survive in a wide range of temperatures including extreme conditions, from 2 to 54°C, however most serotypes are not able to grow below 7°C [49, 50]. It can also tolerate a wide range of pH, between 4-9.5 with an optimum growth at pH of 6.5-7.5, and it needs water activity above 0.93. In general, salmonellae are able to ferment D-glucose, producing acid and usually gas, but not lactose; they usually produce hydrogen sulfide; they reduce nitrates to nitrites; they decarboxylate lysine and ornithine; and are catalase positive, methyl red and Simmons citrate positive and oxidase, urea, indole and Voges Proskauer (VP) negative [51].

1.3.2. Salmonella Taxonomy

The nomenclature of the genus *Salmonella* has evolved through several variations to yield the current nomenclature system. In 1973, it was demonstrated that all serotypes of

Salmonella were related at species level, except for *S. bongory* [52]. Le Minor *et al.* and Reeves *et al*., in 1980s requested several changes in the taxonomy of the genus proposing the inclusion of two species of *Salmonella* [53]. The existence of two different taxonomic approaches in the scientific community with different interpretations of the Bacteriological Code led to discrepancies in *Salmonella* nomenclature. The Judicial Commission of the International Committee on the Systematics of Prokaryotes issued an Opinion in 2005 to resolve the nomenclature conflict. Nowadays, the occurrence of two species of *Salmonella*, *S. bongori* and *S. enterica*, has been accepted and derived in the taxonomy and nomenclature currently in use by the WHO and the CDC [53].

Phenotypic methods including biochemical reactions, phage typing, and serotyping have been traditionally used for identification and typing *Salmonella* isolates [54]. The current classification of *Salmonella enterica* in 6 subspecies is based on serotyping (also known as Kauffmann-White-Le Minor serotyping) [54]. *S. enterica* is further divided into six subspecies: *S. enterica* subsp. enterica (I), *S. enterica* subsp. salamae (II), *S. enterica* subsp. arizonae (IIIa), *S. enterica* subsp. diarizonae (IIIb), *S. enterica* subsp. houtenae (IV), and *S. enterica* subsp. indica (VI). Subespecies II, IIIa, IIIb, IV and VI and also the other species *S. bongori* (previously subspecies V) typically inhabit cold blooded animals and the environment [47, 55]. Serotyping is based on the immunologic reactivity, identified by agglutination reactions against specific antibodies, of two surface antigens, the O polysaccharide (O antigen or somatic antigen), that is the saccharidic structure of the long chain lipopolysaccharide (LPS) located in the outer membrane on the bacterial surface [56, 57], and the flagellin protein (H antigen). Most *Salmonella* have two different copies of the

gene encoding the flagellar protein (H antigens), known as phase 1 or H1 and phase 2 or H2 flagellar antigens, however only one flagellar antigen is expressed in every cell at a time [58]. This determines the diphasic subtypes of *Salmonella*. Monophasic *Salmonella*, expressing only one flagellar antigen, can be also found, and even triphasic and quadriphasic subtypes exist but they are rare [54]. Both, the O and H antigens are very variable, 46 O antigens and 114 H antigens have been recognized [54, 58]. More than 2,600 different serovars of *S. enterica* have been identified, and almost 60% of these serovars belong to *Salmonella enterica* subsp. *enterica* (I) [59, 60].

1.3.3. Salmonella Implicated in Human Illness

Most of the *Salmonella* infections (99%) in warm-blooded animals and humans have been caused by *S. enterica* subsp. *enterica* (I) and particularly the O-antigens serogroups A, B, C1, C2, D and E [55, 61, 62]. *S. enterica* serovars Typhimurium and Enteritidis have a wide host range and they have been responsible for most of the infections in humans [63, 64]. *Salmonella enterica* subsp. *enterica* serovars have been shown as both, animal host-adapted and also human host-adapted, causing infection in animals or humans respectively. *S.* Typhi and *S.* Paratyphi, the cause of typhoid and paratyphoid fever respectively, are exclusively human pathogens and do not have other hosts [65]. Examples of animal specific serovars are *S.* Gallinarum and *S.* Pullorum that cause fowl typhoid and pullorum disease in chickens and turkeys, and *S.* Choleraesuis that cause septicemia or enterocolitis, or both, in pigs. Nevertheless, some animal-specific serovars such as the bovine-adapted *S.* Dublin, the swine-

adapted *S.* Choleraesuis and the reptile-adapted *S.* Arizonae can sporadically cause severe illness in humans [62, 65]. *Salmonella* with a broad range of hosts (no host-specific) include the majority of serotypes (i.e. *S.* Typhimurium, *S.* Enteritidis) that are the causative agent of gastroenteritis in humans [66]. In 2011, the top four serotypes causing human illness in the United States were *S.* Enteritidis (17%), *S.* Typhimurium (13%), *S.* Newport (11%) and *S.* Javiana (6%) [67].

1.3.4. Non-Typhoidal Salmonellosis Clinical Features

Salmonella is the causative agent of human salmonellosis. Symptoms of infection begin 6-72 hours after consumption of *Salmonella*, including severe abdominal pain, headache, myalgia, fever, nausea, vomiting and non-bloody diarrhea. The infective dose necessary to cause infection can be variable depending on several factors such as the virulence of the particular serovar, the host susceptibility (affected by age and immune status) and matrix or vehicle of contamination (fatty foods or food with buffering capacities can facilitate the survival of *Salmonella* through the gastrointestinal tract). Infectious doses ranging from less than 100 cells to 10^9 cells have been reported [50, 68]. Gastroenteritis symptoms can be self-limiting in most cases after 4-7 days without antibiotic treatment and just requiring fluid therapy to prevent dehydration. However, severe infections can occur, especially in young infants, elderly and immunocompromised people, where infection can progress into septicemia, meningitis, and death. In these cases, antibiotic therapy is required. Drugs of choice include fluoroquinolones, third-generation cephalosporins, or ampicillin [69].

Sequelae after *Salmonella* infection include reactive arthritis, and inflammation in other tissues such as tendons, eyes, genitourinary tract, and skin have been reported [70].

1.4. EPIDEMIOLOGY ASPECTS OF *SALMONELLA* AND *CAMPYLOBACTER*

1.4.1. Public Health Significance of Foodborne Salmonella and Campylobacter

In 2011, the number of domestically acquired foodborne illnesses, hospitalizations and deaths caused by 31 major pathogens per year in the United States were estimated to be 9.4 million, 55,961 and 1,351, respectively. Non typhoidal *Salmonella* is responsible for 11% (1.0 million cases) of the foodborne illness, 35% (19,336 cases) of hospitalizations, and 28% (378 cases) of deaths, respectively. *Campylobacter* spp. are responsible of 9% (0.8 million cases) of foodborne disease, 15% (8,463 cases) of hospitalizations and 6% (76 cases) of deaths, respectively [71]. These estimates positioned both zoonotic bacteria in the top five causes of foodborne disease, hospitalization and deaths. In the ranking, *Salmonella* is the leading cause of hospitalization and death from all these known pathogens and surpassed only by norovirus in the case of illness. *Campylobacter* is the number four pathogen for causing illness, number three for hospitalizations, and number five for causing deaths [71].

1.4.2. Foodborne Disease Surveillance Systems

Surveillance of foodborne disease plays a key role in the food safety system, providing information about the occurrence of foodborne disease and outbreaks and thus contributing to

their prevention. Under-reported or under-diagnosed cases can undermine the surveillance system. In the US there are several surveillance systems coordinated by the Centers for Disease Control and Prevention (CDC), depending on data compiled by the state and local health agencies. Examples of these surveillance systems are: The Foodborne Diseases Active Surveillance Network (FoodNet), the National Antimicrobial Resistance Monitoring System (NARMS), the National Molecular Subtyping Network for Foodborne Disease Surveillance (PulseNet), the National Surveillance for Enteric Disease, the Foodborne Disease Outbreak Surveillance System (FDOSS) [72].

FoodNet

The Foodborne Diseases Active Surveillance Network (FoodNet) is a sentinel surveillance system, collecting information of laboratory confirmed infections caused by foodborne pathogens from 10 sites, covering 15% of the US total population (48 million people in 2011), that allows tracking the trends in foodborne disease. This network began in July 1995 with the collaboration of CDC, health departments of 10 states, the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS), and the Food and Drug Administration (FDA) [73].

1.4.3. Sources of Attribution and Health Burden Associated with Salmonella and Campylobacter Infections

Source of attribution refers to the estimation of the relative contribution of the multiple sources to the burden of human disease. The identification of specific food sources for attribution is a key step for the subsequent implementation of food safety control measures and interventions. Sources of attribution are estimated based on the analysis of outbreak data, identifying the food that has been the origin of infection. Complex or multi-ingredient foods have provided special challenges. Handling and consuming raw or undercooked poultry has been highly associated with *Campylobacter* and *Salmonella* infections [17, 39, 74]. Batz *et al.* (2012) estimated the disease burden for combinations of specific food and pathogen pairs in the United States [75]. The estimates for *Campylobacter* spp. and *S. enterica* attributed to poultry are shown in table 1.1. Estimations of the economic losses in the US due to burden of disease vary depending on the methodologies applied. Hoffman *et al.* in 2012 estimated a cost of illness per year of $14.0 billion ($4.4 to $33.0 billion), and a loss of 61,000 (19,397 to 144,974) quality-adjusted life year (QALYs) per year in the US. This burden of disease is caused by 14 of the 31 major pathogens related to foodborne disease reported by Scallan *et al.* in 2011 [71, 76]. *Salmonella* and *Campylobacter* can be found among the five pathogens accounting for approximately 90% of the QALY loss, together with norovirus, *Listeria* and *Toxoplasma* [76].

Table 1.1. Estimated annual disease burden, number of illness, hospitalization and deaths for *Campylobacter* spp. and *S. enterica* associated with poultry consumption by Batz *et al.* (2012) [75].

Pathogen-food combination	***Campylobacter*-poultry**	***S. enterica*–poultry**
Cost of illness ($ million)	1,257 (606–2,988)	693 (33–1,797)
No. of illnesses	608,231 (242,588–1,159,624)	215,109 (134,979–351,621)
No. of hospitalizations	6,091 (3,095–10,960)	4,048 (1,789–7,848)
No. of deaths	55 (0–239)	79 (0–212)

1.4.4. Salmonella and Campylobacter in Turkey Meat

Many studies have reported the prevalence of *Campylobacter* and *Salmonella* contamination in turkey meat with a wide variability of results in different geographical locations and studies. Possibly, variations can be also attributed to methods of evaluation or culture media used, location and time (at slaughter house or retail), and type of meat evaluated (i.e. whole carcass, legs, breast or ground meat). Logue *et al*. (2003) evaluated the prevalence of *Campylobacter* in prechill and postchill carcasses in the Midwestern US and found variation between processing plants. The lowest prevalence for *Campylobacter* detected was 19.8% (postchill) and the highest 41.8% (prechill) [77]. In a different study in the same area of US, Logue *et al*. (2003), found a prevalence of 16-17% of *Salmonella* in turkey carcasses [78]. A study by Cook *et al.* (2009) that analyzed *Salmonella* and *Campylobacter* in fresh turkey meat sold at retail in Canada reported *Campylobacter* presence in 46% of the samples tested, and *Salmonella* was detected in 24% of the samples

[79]. A different study, also in Canada, found a similar prevalence of both pathogens in the analyzed carcasses, 31.2% for *Salmonella* and 36.9% for *Campylobacter* [80]. A study in Southern Italy found 20.7% of *Campylobacter*-positive samples of turkey meat sold in retail outlets [81]. Alter *et al*. (2004) reported a 6.2 % *Campylobacter*-positive samples in retail turkey meat in Germany [82]. Another study, also in Germany, reported 34% of turkey meat at retail was contaminated with *Campylobacter*. In the same study, 29.2% of chilled turkey carcasses at the slaughterhouse were *Campylobacter*-positive [83].

In 2013, the Food and Drug Administration's Center for Veterinary Medicine (CVM) announced the publication of the 10th National Antimicrobial Resistance Monitoring System (NARMS) Retail Meat Annual Report. Surveillance data in 2011 for ground turkey meat found a percent of positive samples for *Campylobacter* and *Salmonella* of 2.3% and 12.3%, respectively [84].

1.4.5. Healthy People Initiative

The web site *HealthyPeople.gov* is managed by the US Department of Health and Human Services the web site with the objective of promoting health of the US population [85]. The Healthy People initiative includes multiple topics and objectives to increase food safety and reduce foodborne illness. Healthy people 2020 (launched in 2010) established objectives for reducing infections caused by key pathogens usually transmitted through food. The particular targets established were 11.4 cases per 100,000 for *Salmonella,* and 8.5 cases per 100,000 for *Campylobacter* [86]. Another objective was to reduce the number of outbreak-associated

infections due to bacteria associated with specific food commodity groups. Infections attributed to poultry mentioned were Shiga toxin-producing *E. coli* O157, *Campylobacter, Listeria*, or *Salmonella* species, with a target of 232 cases per year [86].

1.4.6. FoodNet Trends

Based on the progress report for 2013 from the Centers for Disease Control and Prevention (CDC), the estimated incidence of infection caused by *Campylobacter* increased 13% in 2013, compared to the 2006–2008 baseline period. However there was no change for *Salmonella* [87]. Compared to the period 2010-2012, campylobacteriosis increased 2% and salmonellosis decreased 9% in 2013. Both are still above the national HealthyPeople 2020 goals [87].

1.5. ROUTES OF TRANSMISSION OF *SALMONELLA* AND *CAMPYLOBACTER* INTO THE FLOCKS DURING THE TURKEY PRODUCTION CYCLE

1.5.1. Commercial Turkey Production Cycle

Turkey production comprises different stages, including breeder farms where fertile eggs are laid, hatcheries where eggs are incubated to generate poults, to the rearing and fattening farms for producing the meat turkeys. Foodborne pathogens can enter and contaminate a flock through many routes during the different growth stages of production and processing. However, sources of infection and modes of transmission of these pathogens on poultry

farms are not totally understood. Control strategies must consider all possible routes of transmission to successfully prevent the colonization of the flocks along all the stages of turkey production and processing. Furthermore, sanitation and biosecurity measures during all phases of production are also crucial to prevent colonization.

Hatchery

In commercial production, fertile eggs are provided by breeder farms. The incubation and hatching of eggs laid by the turkey breeder hens takes place in machines located in hatcheries. In general, turkey eggs hatch at 28 days. The first 25 days of the embryonic development take place in incubators or setters and then eggs are transferred to hatchers until day 28. Both setters and hatchers provide adequate conditions of temperature, humidity and ventilation to achieve optimum growth of the embryos. At day of hatch, poults are processed (i.e. sex sorted, vaccination, beak and toe trimming) prior to distribution to commercial growout facilities.

Meat-bird production

Generally, commercial turkey meat production covers two different phases: Brooding and growout. The brooding period starts at day of hatch and it can be variable in length depending on season or latitude, until 4 to 9 weeks. The first few weeks are critical and incorrect management can affect the flock health. Poults need to be in a clean and dry environment. Light conditions and adequate temperature, promote feed and water consumption, activity and growth, and minimize stress, which can negatively affect the

future growth and reproductive potential. The growout phase begins right after brooding. Turkeys are moved to a growout house for the finishing stage, where they grow until reaching the designated market weight.

Once turkeys achieve the market target weight, generally between 15 and 25 weeks of age, they are transported and processed at slaughter facilities. Turkeys are caught and loaded in trucks for transportation to the processing plant.

Parent Stock Breeder Flocks

Poults from hatcheries go through brooding and growing out stages as described for meat birds. Turkey hens reaching sexual maturity are moved to laying houses. Approximately at 15 weeks of age, breeding toms are selected based on physical characteristics and weight. Breeder hens are usually raised in open floor houses while toms are raised in floor pens in different facilities. Modern turkey breeding requires artificial insemination since turkeys weigh too much for natural mating. The typical ratio between breeder toms and hens to ensure fertility of hatching eggs is 1: 8-10. Lighting programs are used to optimize production. Turkeys are kept under several lighting schedules to develop sexual responses to a stimulatory day length. It is important to control length of daylight and also light intensity. Between 16-18 weeks to approximately 29 weeks, hens are moved to light-proof houses for a dark-out period (minimum of 12 weeks). Darkening periods are used to synchronize sexual development of the breeder hens. During this period the light hours received by the hens are reduced to a maximum of six hours per day, and after the dark-out period they return to 14 hours of continuous light. During the laying period is important not to decrease the day

length. Hens lay eggs through a production period of 28 to 30 weeks. A fertile egg is produced by a hen each 24 and 32 hours. Eggs are collected, cleaned and sanitized. Hatching eggs can be stored at 12.8-18.3°C and 70% humidity between three to ten days before incubation.

1.5.2. Modes of Transmission of Salmonella and Campylobacter in Poultry Flocks

Two different modalities of transmission are possible for the colonization of the flock by *Salmonella* and *Campylobacter*. Vertical transmission could occur from breeders to progeny, and horizontal transmission into birds from contact with environmental sources and other infected birds.

Vertical transmission of *Salmonella* and *Campylobacter*

Vertical transmission is one important source of contamination for *Salmonella* to the progeny due to infection of reproductive tissues and resulting contamination of fertile eggs. Breeders and hatcheries have been reported as vertical transmission sources for *Salmonella* in turkeys [88]. Successful methods for controlling *Salmonella* colonization in poultry breeders include strict biosecurity and hygiene measures, surveillance of *Salmonella* carriage, use of disinfectants for the eggs and sanitation of hatching cabinets, and the use of live *Salmonella* vaccines and competitive exclusion (CE) [89]. A potential factor for vertical transmission of *Salmonella* could be blood spots during the ovulatory process. It has been reported that blood

spots may facilitate the transmission of *Salmonella* into the eggs in hens if they are *Salmonella* positive in the ovary [90].

However, in the case of *Campylobacter* spp., the vertical transmission route still remains controversial. A study on the possible vertical transmission of *C. coli* to the eggs and chicken embryos in breeder hens showed that *Campylobacter* was only detected in fertile eggs and embryos using molecular techniques but not by plate culture, and *C. coli* could not grow in the embryos [91]. *C. jejuni* DNA have been detected by PCR in hatchery fluff and eggshells in turkey facilities [92, 93]. However, several studies in broilers and laying hens suggested that *Campylobacter* vertical transmission through eggs may be a rare event and its penetration ability into the egg is very limited [94–97]. On the contrary, some authors consider vertical transmission as a potential source of contamination to the offspring [98–100]. Since both pathogens have been recovered from semen and reproductive tract of hens, in both turkeys and broilers, evidence suggests vertical transmission [99, 101–104].

Horizontal transmission of *Salmonella* and *Campylobacter*

Horizontal transmission of *Salmonella* and *Campylobacter* can occur during all stages of turkey production. Horizontal spread of *Salmonella* has been reported in the hatchery where *Salmonella* can penetrate the fertile eggs through the eggshell, and poults can also be infected in the early posthatch period [105–108]. Environmental reservoirs may contribute to the spread of the pathogens. Birds colonized by *Salmonella* or *Campylobacter* can be the source of further flock infection and spread of bacteria [107]. The coprophagic nature of avian species facilitates the transmission and dissemination of the pathogens between birds in a

flock [103]. Evidence regarding the persistence of reservoirs has shown that cleaning and disinfecting poultry houses between flocks is not enough to prevent the colonization of the next flock [108]. On the complexity of possible environmental reservoirs, factors that allow survival and support transmission of *Salmonella* and *Campylobacter* in poultry farms are not completely understood. Multiple environmental sources have been associated with the transmission of these pathogens, including the facility environment, personnel, litter, drinkers and feeders, feed and water, air, presence of other animals (both domestic or wild) [80, 102, 105, 107, 109–112]. The presence of other animal species such as cows and pigs grown at the same farm has also been reported as a risk factor [113]. Pets including cats and dogs can be carriers of *Campylobacter* [113, 114]. Rodents can also act as reservoirs and vectors of transmission of both foodborne pathogens [114–116] and have an important role in spreading these pathogens. A study on *Salmonella* prevalence in layer hens found farms with higher density populations of rodents had significantly higher prevalence in flocks [117]. Insects are potential reservoirs and sources of transmission of C*ampylobacter* and *Salmonella*. Both pathogens have been previously recovered from flies, cockroaches and darkling beetles [118–126]. Studies have reported the capacity of flies contaminated with both pathogens to transmit them to poultry flocks [127–129].

1.5.3. Poultry Intestinal Colonization by Salmonella and Campylobacter

The process of pathogenic colonization is influenced by complex host-pathogen-environmental interactions. Gut health is closely related with commensal microflora that can

be impacted by environmental factors associated with production. In the gut ecosystem, pathogens may compete for nutrients with the previous existing microflora. It has been suggested that the presence of commensal microflora can alter the expression of receptors that pathogens use for invading the intestinal tract [113]. The important role of the beneficial microflora has been used to develop strategies for prevention of pathogen colonization, such as the use of competitive exclusion, prebiotics and probiotics, which have focused on supporting the growth and balance of beneficial microbial populations in the intestinal tract with a positive effect on immune system and health [113, 130].

Intestinal Colonization by *Salmonella*

Poultry colonization by *Salmonella* is influenced by several variables such as genetic susceptibility of the breed, age, immune status (as affected by stress level), and the *Salmonella* serovar and infectious dose [131]. During the first few days posthatch, birds are more susceptible to *Salmonella* colonization [131]. Studies in day old chickens have found that high doses of *S.* Enteritidis can cause high rate of mortality, while low infective doses can lead to asymptomatic carriers. However, in older birds symptoms are not observed after infection [132]. Furthermore, hens that become chronic carriers can produce eggs contaminated with *Salmonella* [133, 134]. The pathogenesis of *Salmonella* has been well described in mammalian models, however the information available for avian models is limited. Research in chickens to determine cellular responses due to *Salmonella* infection has shown similar responses as mammalian models, triggering the influx of phagocytic cells to the luminal surface of the intestine [63, 135]. *In vitro* studies have demonstrated that

heterophils (avian counterpart of mammalian neutrophils) and avian macrophages can kill *Salmonella* [63, 135]. *Salmonella* induces morphological changes for stimulating the uptake by the epithelial cells especially in ceca [132]. Lack of success clearing *Salmonella* by the immune system results in intestinal colonization and bacterial cells can disseminate from the intestine to the liver, spleen, and ovaries [63]. The ability of *Salmonella* to survive and replicate inside macrophages has been shown as important role in migration to other tissues in the host [135].

Intestinal Colonization by *Campylobacter*

C. jejuni typically colonizes the cecal and cloacal crypts of poultry and small intestines, although it can also be found less frequently in organs such as liver, gallbladder and spleen [103, 136]. *C. jejuni* locates principally in the mucous layer of the crypts instead of adhering directly to epithelial cells as happens in mammals before invading the cells, and it does not causes lesions or changes in the epithelial cells [136]. It has been reported that avian mucus (unlike human mucus) has the ability to inhibit *Campylobacter* from interacting with the surface of epithelial cells [137]. Also, *Campylobacter* may have several regulatory systems conferring protection against the hostile conditions inside and outside the host, but those mechanisms are not completely understood [138]. Chemotaxis, motility, flagella, adhesion, invasion and a multidrug efflux pump are multiple factors affecting colonization of *C. jejuni* [138]. As for *Salmonella*, poultry have been shown as asymptomatic carriers of *Campylobacter* after infection; further, cecal colonization is not always associated with detection in feces [136]. Van Deun *et al.* (2008) demonstrated that *C. jejuni* is able to persist

in the intestine after a fast replication in the mucus with invasion and evasion of the crypt epithelial cells [139]. Migration to spleen and liver was also observed [139]. Poultry colonization by *Campylobacter* is not detected in young birds until two to three weeks of age and that has been related with the protective effect of maternal antibodies [140].

1.5.4. Effect of Stress in Colonization

Colonization of animal intestines by enteric pathogens has been associated with an increased host susceptibility caused by environmental stress. Stress increases shedding of pathogens facilitating horizontal transmission between birds and increasing the possibility of carcass contamination during processing [113, 141–143]. There is also evidence that stress in hens can lead to contamination of egg contents [142]. Multiple stressors are possible, including climatic (extreme heat or cold, high humidity), environmental (poor ventilation, bright light, wet litter), nutritional (reduction of nutrients or feed intake), physiological (rapid growth, process of maturing sexually), social (overcrowding), and physical (handling, transport). Some of these stressors can be eliminated through good management practices but others cannot be avoided. Pre-slaughter events such as feed withdrawal, handling and transportation increase the vulnerability of birds to pathogenic infection and colonization. These stressors have been shown to promote negative changes on the beneficial microbial population in the gut, and also on the integrity of the epithelium [63, 113]. It has been proven that neuroendocrine stress hormones released by the host can trigger pathogenesis mechanisms in bacteria [144, 145]. The release of catecholamines during acute stress has

been related with changes in beneficial microflora and increased colonization by *Salmonella, Campylobacter,* and other pathogenic bacteria [142, 144].

1.5.5. Seasonality

The effect of season on pathogenic fecal shedding by poultry and other food animals is a key point for understanding epidemiological factors of *Salmonella* and *Campylobacter*. Increases in fecal shedding contribute to a higher environmental contamination by the pathogens, increasing the risk of exposure and infection of the flocks, and also elevating the risk of human infections. However, seasonal variation and the factors affecting the prevalence of these pathogens are not totally understood. Very different trends regarding prevalence of *Salmonella* and *Campylobacter* in association with climate and season have been described. A seasonal variation on prevalence of both pathogens in chicken flocks, with peaks in summer months, was observed in several studies at different geographical locations [110, 146–154]. However, other authors have found a higher incidence of *Salmonella* and *Campylobacter* on farms during fall or spring instead of the summer months [96, 155]. Logue *et al.* (2003) also observed a higher incidence of *Campylobacter* in turkey carcasses during the cooler months of the fall, winter and early spring [156]. Another study also found a peak in *Campylobacter* isolation during late fall and winter months [157]. Furthermore, Wallace *et al*. (1997) found a seasonal variation in the numbers of *Campylobacter* in intestine of chickens, however the prevalence of carriage was high all months of the study [158]. In contrast, in many other studies, a seasonal pattern was not

found for any of the pathogens [146, 149, 159, 160]. Possibly, a combination of climatic factors and other environmental and host physiological factors, influence the survival of the pathogens in the environment and the prevalence of colonization. Heat stress induced by high environmental temperatures causes depletion of the immune system that can lead to an increase in shedding and colonization rates of both pathogens in poultry [109, 145]. Furthermore, conditions in the surrounding environment such as litter pH and water activity can also contribute to bacterial survival and spread [109].

1.5.6. Prevalence of Colonization by Salmonella and Campylobacter

Salmonella and *Campylobacter* are commensal bacteria that can colonize the intestinal tract of different livestock animals such as swine, cattle, sheep and poultry causing little or no disease. Even though in most cases carriers were asymptomatic, the pathogens were excreted in feces in large numbers constituting a reservoir for disease [15, 161, 162]. Both pathogens can be found in wild and domestic animals. Poultry have been cited as the most frequent hosts for *Campylobacter* possibly due to their higher body temperature, approximately 42°C, ideal for *Campylobacter* growth [15]. *Salmonella* have been found at higher prevalence levels at early ages in the flock and decrease in prevalence at slaughter age [163–164]. *Campylobacter* spp. commonly persist during the entire lifetime once it colonizes the host [124, 164–165]. The prevalence of colonization by *Salmonella* and *Campylobacter* may vary between flocks and farm geographical location. Sampling and culture methods used can also lead to variability. A study conducted in eastern North Carolina found a high

prevalence of *Campylobacter* colonization in conventionally reared turkeys of 87% [166]. Arsenault *et al.* (2007) in a study performed in Canada found a prevalence of *Salmonella*-positive turkey flocks of 54% [80]. A broiler study found a higher prevalence of *Salmonella* in fecal samples in conventional (38.8%) in comparison with organic (5.6%) farms [167]. However, Luangtongkum *et al*. (2006) reported a high prevalence of *Campylobacter* colonization in both organically and conventionally reared turkeys [168]. Nayak *et al.* (2003) found a wide variability (0 to 21%) on the prevalence of *Salmonella* between flocks located at the same turkey farms [112].

1.5.7. Salmonella and Campylobacter Survival in the Environment

Although *Campylobacter* spp. have been reported sensitive to environmental conditions such as temperature, oxygen exposure, and dryness, some studies have shown survival in adverse conditions allowing their persistence in environmental reservoirs. Campylobacters require specific parameters for laboratory growth including temperatures above 30°C (optimum 37°C-42°C), microaerobic conditions and high moisture. Ultraviolet B (UVB) radiation exposure also reduce *C. jejuni* survival [169]. Thus, the survival outside a host can be compromised. However, Rollins and Colwell (1986) observed the survival for months of *C. jejuni* at 4°C in aquatic environments, in a viable but non culturable state (VBNC) [170]. The VBNC state is thought to play a key role in environmental survival [171]. Chan *et al.* (2001) also described how *Campylobacter* can still be physiologically active at temperatures lower than 30°C, especially at 4°C which allows the pathogen to survive in processing

conditions [172]. Lee *et al.* (1998) demonstrated the survival of *C. jejuni* in chicken skin at -20°C and -70°C, and possible growth at 4°C [173]. In a study with broilers in North Carolina, *Campylobacter* was isolated from environmental samples including grass [174]. Also, *C. jejuni* survived adverse conditions, forming biofilms where cells were confined in a matrix of extracellular polymeric molecules, which conferred protection against environmental stress; this could explain the persistence of the pathogen in the environment [171, 175–178].

Salmonella seems to be able to easily survive in different environments and has developed strategies of resistance to several adverse conditions of stress, such as nutrient limitation, changes in water activity, pH and temperature, presence of antimicrobials, oxidation [179]. Most *Salmonella* have been found to survive for long periods of time in dry matrices, dust and water rapidly multiply when favorable conditions develop [180]. The ability of *Salmonella* to form biofilms, which allows the organism to survive in the environment (host and non-host related) and in food processing facilities has also been reported [181].

1.6. *SALMONELLA* AND *CAMPYLOBACTER* CONTAMINATION DURING PROCESSING

1.6.1. Turkey Processing Basic Steps

Because *Salmonella* and *Campylobacter* are commensal bacteria of the intestinal tract of poultry, further contamination and cross-contamination of carcasses occur during processing. The basic steps of turkey processing include:

1- Live receiving
2- Hanging
3- Stunning
4- Bleeding
5- Scalding
6- Picking (defeathering)
7- Transfer
8- Evisceration
9- Chilling

Logue *et al.* (2007) reported that samples taken at post-defeathering, evisceration (pre-wash) and post-chill had higher prevalence of *Salmonella* [77]. The same steps or sample areas have been also associated with cross-contamination of poultry carcasses with *Campylobacter* [15]. Cross-contamination of carcasses with both pathogens during defeathering has been associated with contamination of the rubber fingers in contact with the

carcasses, presence of contaminated scald water that is reused, and also the release of feces from the cloaca by pathogen-positive birds [15, 182–185]. The evisceration process has been shown to contribute to carcass contamination due to escape of intestinal contents onto the carcass or with equipment and surfaces [15, 77, 156, 185–187]. At the end of the slaughter process, chilling have also been found to increase cross-contamination with *Salmonella* and *Campylobacter* [15, 39, 185, 188, 189].

1.6.2. Pre-slaughter Events

Because birds are asymptomatic carriers is an additional difficulty since is not possible to detect infection by inspecting their physical appearance. *Salmonella* and *Campylobacter* can be present in feathers and skin of positive flocks and steps of transport, holding prior to slaughter and other successive steps during processing may contribute to the spread of the pathogens to other birds and/or cross-contamination of carcasses [190, 191]. Results from several studies have shown the relevance of interventions on farm to prevent flock colonization by *Salmonella* and *Campylobacter*. These measures decreased the probability of carcass contamination and cross-contamination during processing [192–194].

Feed withdrawal

Prior to slaughter, an approximately 8-12 hour feed withdrawal has been implemented with the objective of emptying the intestinal tract. It has been reported that preharvest feed withdrawal increased the incidence of *Salmonella* and *Campylobacter* in broiler crops, which

increased the risk of contamination of carcasses during processing [15, 39, 136, 193, 195–197]. Extensive feed withdrawal periods in broilers have shown to increase carcass contamination due to intestinal rupture during processing [194, 198, 199]. Proper methods and times along with acids added into water during feed withdrawal have been shown to reduce *Salmonella* and *Campylobacter* at slaughter [194, 200–202].

Transportation

Transportation from the farm to the processing plant constitutes a risk for cross-contamination of *Salmonella* and *Campylobacter* within and between flocks. Consequently, hygiene control measures, including cleaning and disinfection of trucks or transport vehicles and crates has been reported as necessary to limit cross-contamination. Several broiler studies have reported transportation as a source of contamination [186, 203–206]. However, turkey studies have not found correlation between pre-slaughter events such as feed withdrawal, catching and loading, transportation, and holding time in the prevalence of *Salmonella* in turkeys [207, 208].

1.6.3. Regulations Applying During Slaughter

The USDA Food Safety and Inspection Service (FSIS) has been given the role of ensuring the wholesomeness, safety and proper labeling of meat, poultry and processed eggs at national level [209]. USDA-FSIS personnel rely on prevention-based policies and practices to limit foodborne diseases [209]. The National food safety initiative “From Farm

to Table" in 1997 was created with the goal of reducing the incidence of foodborne illness, and improving the safety of the food supply in the US. In 1997 USDA-FSIS began the implementation of HACCP and pathogen reduction requirements for the meat and poultry industries [210]. The same year, a zero-tolerance policy for visible fecal contamination was implemented for poultry carcasses [211].

More recently, USDA-FSIS implemented new performance standards for *Salmonella* and *Campylobacter* in chilled carcasses at young chicken and turkey slaughter establishments. The new standards set a tolerance for *Salmonella* of no more than five positive samples in 51 tested for young chickens, and no more than four positives in 56 samples tested for turkeys. The prevalence allowed to pass for *Campylobacter* must be no more than eight positive samples in the 51 analyzed for young chickens, and no more than four positive samples in 56 samples tested for turkeys [212].

1.7. IMPLICATIONS

Many aspects of *Salmonella* and *Campylobacter* colonization of poultry are not completely understood, including pathogen survival in the environment, host-pathogen interactions, and the complex ecosystem of the host intestine. Further research will provide knowledge of colonization dynamics and the development of more effective strategies towards their eradication from poultry flocks. This together with the progress in molecular biology and next-generation high-throughput sequencing technologies should allow improved food safety and public health.

1.8. REFERENCES AND NOTES

1. Lindmark, H., S. Boqvist, M. Ljungström, P. Agren, B. Björkholm, and L. Engstrand. 2009. Risk factors for campylobacteriosis: An epidemiological surveillance study of patients and retail poultry. J. Clin. Microbiol. 47: 2616–2619.

2. Greig, J.D., and A. Ravel. 2009. Analysis of foodborne outbreak data reported internationally for source attribution. Int. J. Food Microbiol. 130: 77–87.

3. Friedman, C.R., R.M. Hoekstra, M. Samuel, R. Marcus, J. Bender, B. Shiferaw, S. Reddy, S.D. Ahuja, D.L. Helfrick, F. Hardnett, M. Carter, B. Anderson, and R. V Tauxe. 2004. Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. Clin. Infect. Dis. 38 Suppl 3: S285–S296.

4. Pires, S.M., H. Vigre, P. Makela, and T. Hald. 2010. Using outbreak data for source attribution of human salmonellosis and campylobacteriosis in Europe. Foodborne Pathog. Dis. 7: 1351–1361.

5. US Department of Health and Human Services. Centers for Disease Control and Prevention (CDC). Antibiotic Resistance Threats in the United States, 2013. Accessed May, 2014. http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf.

6. United States Department of Agriculture-Economic Research Service. Accessed June, 2014. http://www.ers.usda.gov/topics/animal-products/poultry-eggs.

7. North Carolina Poultry Federation. US Poutry and Eggs association. Accessed June, 2014. http://www.ncpoultry.org/facts/facts.cfm.

8. USDA. Livestock, Dairy, and Poultry Outlook No. (LDPM-239) 18 pp, May 2014. http://www.ers.usda.gov/publications/ldpm-livestock,-dairy,-and-poultry-outlook.

9. USDA. World Agricultural Supply and Demand Estimates. Accessed June, 2014. http://www.usda.gov/oce/commodity/wasde/latest.pdf.

10. Guerry, P. 2007. *Campylobacter* flagella: Not just for motility. Trends Microbiol. 15: 456–461.

11. Ferrero, R.L., and A. Lee. 1988. Motility of *Campylobacter jejuni* in a viscous environment: Comparison with conventional rod-shaped bacteria. J. Gen. Microbiol. 134: 53–59.

12. Shigematsu, M., A. Umeda, S. Fujimoto, and K. Amako. 1998. Spirochaete-like swimming mode of *Campylobacter jejuni* in a viscous environment. J. Med. Microbiol. 47: 521–526.

13. Debruyne, L., D. Gevers, and P. Vandamme. 2008. Taxonomy of the family Campylobacteracea. In: Nachamkin I, MJ Blaser, editors. *Campylobacter*. Washington, DC: ASM Press. pp. 3–25.

14. Silva, J., D. Leite, M. Fernandes, C. Mena, P.A. Gibbs, and P. Teixeira. 2011. *Campylobacter* spp. as a foodborne pathogen: A Review. Front. Microbiol. 2:200.

15. Keener, K.M., M.P. Bashor, P.A. Curtis, B.W. Sheldon, and S. Kathariou. 2004. *Campylobacter* and poultry processing. Compr. Rev. Food Sci. Food Saf. 3: 105–116.

16. Park, S.F. 2002. The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. Int. J. Food Microbiol. 74: 177–188.

17. Altekruse, S.F., N.J. Stern, P.I. Fields, and D.L. Swerdlow. 1999. *Campylobacter jejuni*-An emerging foodborne pathogen. Emerg. Infect. Dis. 5: 28–35.

18. Garénaux, A., F. Jugiau, F. Rama, R. de Jonge, M. Denis, M. Federighi, and M. Ritz. 2008. Survival of *Campylobacter jejuni* strains from different origins under oxidative stress conditions: Effect of temperature. Curr. Microbiol. 56: 293–297.

19. Snelling, W.J., M. Matsuda, J.E. Moore, and J.S.G. Dooley. 2005. Under the microscope: *Campylobacter jejuni*. Lett. Appl. Microbiol. 41: 297–302.

20. Buck, G.E., K.A. Parshall, and C.P. Davis. 1983. Electron microscopy of the coccoid form of *Campylobacter jejuni*. J. Clin. Microbiol. 18: 420–421.

21. Beumer, R.R., J. De Vries, and F.M. Rombouts. 1992. *Campylobacter jejuni* non-culturable coccoid cells. Int. J. Food Microbiol. 15: 153–163.

22. Moran, A.P., and M.E. Upton. 1987. Factors affecting production of coccoid forms by *Campylobacter jejuni* on solid media during incubation. J. Appl. Bacteriol. 62: 527–537.

23. Boucher, S.N., E.R. Slater, A.H. Chamberlain, and M.R. Adams. 1994. Production and viability of coccoid forms of *Campylobacter jejuni*. J. Appl. Bacteriol. 77: 303–307.

24. Moran, A.P., and M.E. Upton. 1986. A comparative study of the rod and coccoid forms of *Campylobacter jejuni* ATCC 29428. J. Appl. Bacteriol. 60: 103–110.

25. Cappelier, J.M., and M. Federighi. 1998. Demonstration of viable but non culturable state for *Campylobacter jejuni*. Rev. Med. Vet. (Toulouse). 149: 319–326.

26. Jones, K. 2001. Campylobacters in water, sewage and the environment. Symp. Ser. Soc. Appl. Microbiol. : 68S–79S.

27. Vandamme P. and De Ley, J. 1991. Proposal for a New Family, Campylobacteraceae. Int. J. Syst. Bacteriol. 41: 451–455.

28. Damborg, P., K.E.P. Olsen, E. Møller Nielsen, and L. Guardabassi. 2004. Occurrence of *Campylobacter jejuni* in pets living with human patients infected with *C. jejuni*. J. Clin. Microbiol. 42: 1363–1364.

29. CDC. Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet Surveillance Report for 2011 (Final Report). Atlanta, Georgia: U.S. Department of Health and Human Services, CDC. 2012.

30. Friedman, C.R., J. Neimann, H.C. Wegener, and R. V. Tauxe. 2000. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. Washington, USA: ASM International. pp. 121–138.

31. Patrick, M.E., M.J. Gilbert, M.J. Blaser, R. V Tauxe, J.A. Wagenaar, and C. Fitzgerald. 2013. Human infections with new subspecies of *Campylobacter fetus*. Emerg. Infect. Dis. 19: 1678–1680.

32. Labarca, J.A., J. Sturgeon, L. Borenstein, N. Salem, S.M. Harvey, E. Lehnkering, R. Reporter, and L. Mascola. 2002. *Campylobacter upsaliensis*: Another pathogen for consideration in the United States. Clin. Infect. Dis. 34: E59–E60.

33. Blaser, M.J. 1998. Editorial Response : *Campylobacter fetus* — Emerging infection and model system for bacterial pathogenesis at mucosal surfaces. Clin Infect Dis. 27: 256–258.

34. Oporto, B., and A. Hurtado. 2011. Emerging thermotolerant *Campylobacter* species in healthy ruminants and swine. Foodborne Pathog. Dis. 8: 807–813.

35. Werno, A.M., J.D. Klena, G.M. Shaw, and D.R. Murdoch. 2002. Fatal case of *Campylobacter lari* prosthetic joint infection and bacteremia in an immunocompetent patient. J. Clin. Microbiol. 40: 1053–1055.

36. Söderström, C., C. Schalén, and M. Walder. 1991. Septicaemia caused by unusual *Campylobacter* species (*C. laridis* and *C. mucosalis*). Scand. J. Infect. Dis. 23: 369–371.

37. Butzler, J.P. 2004. *Campylobacter*, from obscurity to celebrity. Clin. Microbiol. Infect. 10: 868–876.

38. Papaconstantinou, H.T., and J.S. Thomas. 2007. Bacterial colitis. Clin. Colon Rectal Surg. 20: 18–27.

39. White, P.L., A.R. Baker, and W.O. James. 1997. Strategies to control *Salmonella* and *Campylobacter* in raw poultry products. Rev. Sci. Tech. 16: 525–541.

40. Peterson, M.C. 1994. Clinical aspects of *Campylobacter jejuni* infections in adults. West. J. Med. 161: 148–152.

41. Allos, B.M. 2001. *Campylobacter jejuni* infections: Update on emerging issues and trends. Clin. Infect. Dis. 32: 1201–1206.

42. Nyati, K.K., and R. Nyati. 2013. Role of *Campylobacter jejuni* infection in the pathogenesis of Guillain-Barre syndrome: An update. Biomed Res. Int. 852195: 13 pages, doi:10.1155/2013/852195.

43. Yan, S.S., M.L. Pendrak, S.L. Foley, and J.H. Powers. 2005. *Campylobacter* infection and Guillain–Barré syndrome: Public health concerns from a microbial food safety perspective. Clin. Appl. Immunol. Rev. 5: 285–305.

44. Pope, J.E., A. Krizova, A.X. Garg, H. Thiessen-Philbrook, and J.M. Ouimet. 2007. *Campylobacter* Reactive Arthritis: A Systematic Review. Semin. Arthritis Rheum. 37: 48–55.

45. Bremell, T., A. Bjelle, and A. Svedhem. 1991. Rheumatic symptoms following an outbreak of *Campylobacter* enteritis: A five year follow up. Ann. Rheum. Dis. 50: 934–938.

46. Ochman, H., S. Elwyn, and N.A. Moran. 1999. Calibrating bacterial evolution. Proc. Natl. Acad. Sci. U. S. A. 96: 12638–12643.

47. Cotter, P.A., and V.J. DiRita. 2000. Bacterial virulence gene regulation: An evolutionary perspective. Annu. Rev. Microbiol. 54: 519–565.

48. Thomson, N.R., D.J. Clayton, D. Windhorst, G. Vernikos, S. Davidson, C. Churcher, M.A. Quail, M. Stevens, M.A. Jones, M. Watson, A. Barron, A. Layton, D. Pickard, R.A. Kingsley, A. Bignell, L. Clark, B. Harris, D. Ormond, Z. Abdellah, K. Brooks, I. Cherevach, T. Chillingworth, J. Woodward, H. Norberczak, A. Lord, C. Arrowsmith, K. Jagels, S. Moule, K. Mungall, M. Sanders, S. Whitehead, J.A. Chabalgoity, D. Maskell, T. Humphrey, M. Roberts, P.A. Barrow, G. Dougan, and J. Parkhill. 2008. Comparative genome analysis of

Salmonella Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. Genome Res. 18: 1624–1637.

49. Ricke, S.C., O.K. Koo, S. Foley, and R. Nayak. 2013. *Salmonella*. In: Guide to Foodborne Pathogens. John Wiley & Sons. pp. 112–137.

50. Bell, C., and A. Kyriakides. 2002. *Salmonella*: A practical approach to the organism and its control in foods. Practical Food Microbiology Series. Blackwell Science Ltd., Oxford, United Kingdom.

51. Global Salm-Surv. A global *Salmonella* surveillance and laboratory support project of the World Health Organization. Laboratory Protocols Level 1 Training Course Identification of Salmonella 4th Ed. April 2003. http://www.antimicrobialresistance.dk/.

52. Crosa, J.H., D.J. Brenner, W.H. Ewing, and S. Falkow. 1973. Molecular relationships among the *Salmonelleae*. J. Bacteriol. 115: 307–315.

53. Tindall, B.J., P.A.D. Grimont, G.M. Garrity, and J.P. Euzéby. 2005. Nomenclature and taxonomy of the genus *Salmonella*. Int. J. Syst. Evol. Microbiol. 55: 521–524.

54. Wattiau, P., C. Boland, and S. Bertrand. 2011. Methodologies for *Salmonella enterica* subsp. enterica subtyping: Gold standards and alternatives. Appl. Environ. Microbiol. 77: 7877–7885.

55. Brenner, F.W., R.G. Villar, F.J. Angulo, R. Tauxe, and B. Swaminathan. 2000. *Salmonella* nomenclature. J. Clin. Microbiol. 38: 2465–2467.

56. Reeves, P.R., M. Hobbs, M.A. Valvano, M. Skurnik, C. Whitfield, D. Coplin, N. Kido, J. Klena, D. Maskell, C.R.H. Raetz, and P.D. Rick. 1996. Bacterial polysaccharide synthesis and gene nomenclature. Trends Microbiol. 4: 495–503.

57. Schnaitman, C.A., and J.D. Klena. 1993. Genetics of lipopolysaccharide biosynthesis in enteric bacteria. Microbiol. Rev. 57: 655–682.

58. McQuiston, J.R., R. Parrenas, M. Ortiz-Rivera, L. Gheesling, F. Brenner, and P.I. Fields. 2004. Sequencing and comparative analysis of flagellin genes fliC, fljB, and flpA from *Salmonella*. J. Clin. Microbiol. 42: 1923–1932.

59. Grimont, P.A.D., and F.-X. Weill. 2007. Antigenic formulae of the *Salmonella* serovars. 9th ed. WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur, Paris, France.

60. Guibourdenche, M. Roggentin, P., M. Mikoleit, P. Fields, J. Bockemühl, P. Grimont, and F. Weill. 2010. Supplement 2003 – 2007 (No. 47) to the White-Kauffmann-Le Minor scheme. Res. Microbiol. 161: 26 – 29.

61. Porwollik, S., E.F. Boyd, C. Choy, P. Cheng, L. Florea, E. Proctor, and M. McClelland. 2004. Characterization of *Salmonella enterica* subspecies I genovars by use of microarrays. J. Bacteriol. 186: 5883–5898.

62. Jacobsen, A., R.S. Hendriksen, and Aaresturp. 2011. The *Salmonella* enterica Pan-genome. Microb. Ecol. : 487–504.

63. Dunkley, K.D., T.R. Callaway, V.I. Chalova, J.L. McReynolds, M.E. Hume, C.S. Dunkley, L.F. Kubena, D.J. Nisbet, and S.C. Ricke. 2009. Foodborne *Salmonella* ecology in the avian gastrointestinal tract. Anaerobe. 15: 26–35.

64. WHO. Media Center. Fact sheet N°139. Updated August 2013. Accessed June, 2014. http://www.who.int/mediacentre/factsheets/fs139/en/.

65. Chiu, C.-H., L.-H. Su, and C. Chu. 2004. *Salmonella enterica* serotype Choleraesuis: Epidemiology, pathogenesis, clinical disease, and treatment. Clin. Microbiol. Rev. 17: 311–322.

66. Velge, P., A. Wiedemann, M. Rosselin, N. Abed, Z. Boumart, A.M. Chausse, O. Grepinet, F. Namdari, S.M. Roche, A. Rossignol, and I. Virlogeux-Payant. 2012. Multiplicity of *Salmonella* entry mechanisms, a new paradigm for *Salmonella* pathogenesis. Microbiologyopen. 1: 243–258.

67. Centers for Disease Control and Prevention. National Enteric Disease Surveillance: *Salmonella* Annual Report, 2011. http://www.cdc.gov/ncezid/dfwed/pdfs/salmonella-annual-report-2011-508c.pdf.

68. Blaser, M.J., and L.S. Newman. 1982. A review of human salmonellosis: I. Infective Dose. Rev. Infect. Dis. 4: 1096–1106.

69. Acheson, D., and E.L. Hohmann. 2001. Nontyphoidal salmonellosis. Clin. Infect. Dis. 32: 263–269.

70. Townes, J.M. 2010. Reactive arthritis after enteric infections in the United States: The problem of definition. Clin. Infect. Dis. 50: 247–254.

71. Scallan, E., R.M. Hoekstra, F.J. Angulo, R. V. Tauxe, M.A. Widdowson, S.L. Roy, J.L. Jones, and P.M. Griffin. 2011. Foodborne illness acquired in the United States — Major pathogens. Emerg. Infect. Dis. 17: 1–21.

72. CDC. Foodborne Illness Surveillance, Response, and Data Systems. Accessed June, 2014. http://www.cdc.gov/foodborneburden/surveillance-systems.html.

73. CDC. Foodborne Diseases Active Surveillance Network (FoodNet). Accessed June 2013. http://www.cdc.gov/foodnet/.

74. Domingues, A.R., S.M. Pires, T. Halasa, and T. Hald. 2012. Source attribution of human campylobacteriosis using a meta-analysis of case-control studies of sporadic infections. Epidemiol. Infect. 140: 970–981.

75. Batz, M.B., S. Hoffmann, and J.G. Morris, Jr. 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. J. Food Prot. 75: 1278–1291.

76. Hoffmann, S., M.B. Batz, and J.G. Morris, Jr. 2012. Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. J. Food Prot. 75: 1292–1302.

77. Logue, C.M., and C.W. Nde. 2007. *Salmonella* contamination of turkey from processing to final product- A process to product perspective. Foodborne Pathog. Dis. 4: 491–504.

78. Logue, C.M., J.S. Sherwood, P.A. Olah, L.M. Elijah, and M.R. Dockter. 2003. The incidence of antimicrobial-resistant *Salmonella* spp on freshly processed poultry from US Midwestern processing plants. J. Appl. Microbiol. 94: 16–24.

79. Cook, A., R. Reid-Smith, R. Irwin, S.A. McEwen, A. Valdivieso-Garcia, and C. Ribble. 2009. Antimicrobial resistance in *Campylobacter*, *Salmonella*, and *Escherichia coli* isolated from retail turkey meat from southern Ontario, Canada. J. Food Prot. 72: 473–481.

80. Arsenault, J., A. Letellier, S. Quessy, V. Normand, and M. Boulianne. 2007. Prevalence and risk factors for *Salmonella* spp. and *Campylobacter* spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. Prev. Vet. Med. 81: 250–264.

81. Nobile, C.G.A., R. Costantino, A. Bianco, C. Pileggi, and M. Pavia. 2013. Prevalence and pattern of antibiotic resistance of *Campylobacter* spp. in poultry meat in Southern Italy. Food Control. 32: 715–718.

82. Alter, T., M. Gurtler, F. Gaull, A. Johne, and K. Fehlhaber. 2004. Comparative analysis of the prevalence of *Campylobacter* spp. in retail turkey and chicken meat. Arch. Lebensmittelhyg. 55: 60–63.

83. Atanassova, V., F. Reich, L. Beckmann, and G. Klein. 2007. Prevalence of *Campylobacter* spp. in turkey meat from a slaughterhouse and in turkey meat retail products. FEMS Immunol. Med. Microbiol. 49: 141–145.

84. US Food and Drug Administration (FDA). 2011 NARMS Retail Meat Annual Report.

85. Healthy People 2020. Accessed June, 2014. http://www.healthypeople.gov/2020/default.aspx.

86. Healthy People 2020. Food Safety, Objectives. Accessed June, 2014. http://www.healthypeople.gov/2020/topics-objectives/topic/food-safety/objectives.

87. Centers for Disease Control and Prevention (CDC). FoodNet. Data, statistics and publications. Trends and tables. http://www.cdc.gov/foodnet/data/trends/trends-2013.html.

88. Kumar, M.C., M.D. York, J.R. McDowell, and B.S. Pomeroy. 1971. Dynamics of *Salmonella* infection in fryer roaster turkeys. Avian Dis. 15: 221–232.

89. Sanchez, S., C.L. Hofacre, M.D. Lee, J.J. Maurer, and M.P. Doyle. 2002. Animal sources of salmonellosis in humans. J. Am. Vet. Med. Assoc. 221: 492–497.

90. Smith, D.P., and M.T. Musgrove. 2008. Effect of blood spots in table egg albumen on *Salmonella* growth. Poult. Sci. 87: 1659–1661.

91. Rossi, D.A., B.B. Fonseca, R.T. de Melo, G. da S. Felipe, P.L. da Silva, E.P. Mendonça, A.L.L. Filgueiras, and M.E. Beletti. 2012. Transmission of *Campylobacter coli* in chicken embryos. Brazilian J. Microbiol. 43: 535–543.

92. Hiett, K.L., N.A. Cox, and N.J. Stern. 2002. Direct polymerase chain reaction detection of *Campylobacter* spp. in poultry hatchery samples. Avian Dis. 46: 219–223.

93. Perko-Mäkelä, P., P. Isohanni, M. Katzav, M. Lund, M.-L. Hänninen, and U. Lyhs. 2009. A longitudinal study of *Campylobacter* distribution in a turkey production chain. Acta Vet. Scand. 51: 18.

94. Callicott, K.A., V. Friethriksdóttir, J. Reiersen, R. Lowman, J. Bisaillon, E. Gunnarsson, E. Berndtson, K.L. Hiett, D.S. Needleman, and N.J. Stern. 2006. Lack of evidence for vertical transmission of *Campylobacter* spp. in chickens. Appl. Environ. Microbiol. 72: 5794–5798.

95. Sahin, O., P. Kobalka, and Q. Zhang. 2003. Detection and survival of *Campylobacter* in chicken eggs. J. Appl. Microbiol. 95: 1070–1079.

96. Doyle, M.P. 1984. Association of *Campylobacter jejuni* with laying hens and eggs. Appl. Environ. Microbiol. 47: 533–536.

97. Shanker, S., A. Lee, and T.C. Sorrell. 1986. *Campylobacter jejuni* in broilers: The role of vertical transmission. J. Hyg. (Lond). 96: 153–159.

98. Hiett, K.L., N.A. Cox, R.J. Buhr, and N.J. Stern. 2002. Genotype analyses of *Campylobacter* isolated from distinct segments of the reproductive tracts of broiler breeder hens. Curr. Microbiol. 45: 400–404.

99. Cole, K., A.M. Donoghue, P.J. Blore, and D.J. Donoghue. 2004. Isolation and prevalence of *Campylobacter* in the reproductive tracts and semen of commercial turkeys. Avian Dis. 48: 625–630.

100. Cox, N.A., L.J. Richardson, J.J. Maurer, M.E. Berrang, P.J. Fedorka-Cray, R.J. Buhr, J.A. Byrd, M.D. Lee, C.L. Hofacre, P.M. O'Kane, A.M. Lammerding, A.G. Clark, S.G. Thayer, and M.P. Doyle. 2012. Evidence for horizontal and vertical transmission in *Campylobacter* passage from hen to her progeny. J. Food Prot. 75: 1896–1902.

101. Buhr, R.J., N.A. Cox, N.J. Stern, M.T. Musgrove, J.L. Wilson, and K.L. Hiett. 2002. Recovery of *Campylobacter* from segments of the reproductive tract of broiler breeder hens. Avian Dis. 46: 919–924.

102. Cox, N.A., N.J. Stern, K.L. Hiett, and M.E. Berang. 2002. Identification of a new source of *Campylobacter* contamination in poultry: Transmission from breeder hens to broiler chickens. Avian Dis. 46: 535–541.

103. Newell, D.G., and C. Fearnley. 2003. Sources of *Campylobacter* colonization in broiler chickens. Appl. Environ. Microbiol. 69: 4343–4351.

104. Cox, N.A., L.J. Richardson, R.J. Buhr, and P.J. Fedorka-Cray. 2009. *Campylobacter* species occurrence within internal organs and tissues of commercial caged Leghorn laying hens. Poult. Sci. 88: 2449–2456.

105. Cason, J.A., N.A. Cox, and J.S. Bailey. 1994. Transmission of *Salmonella* typhimurium during hatching of broiler chicks. Avian Dis. 38: 583–588.

106. Padron, M. 1990. *Salmonella typhimurium* penetration through the eggshell of hatching eggs. Avian Dis. 34: 463–465.

107. Heyndrickx, M., D. Vandekerchove, L. Herman, I. Rollier, K. Grijspeerdt, and L. De Zutter. 2002. Routes for *Salmonella* contamination of poultry meat: Epidemiological study from hatchery to slaughterhouse. Epidemiol. Infect. 129: 253–265.

108. Van Immerseel, F., J. De Buck, F. Pasmans, L. Bohez, F. Boyen, F. Haesebrouck, and R. Ducatelle. 2004. Intermittent long-term shedding and induction of carrier birds after infection of chickens early posthatch with a low or high dose of *Salmonella enteritidis*. Poult. Sci. 83: 1911–1916.

109. Hoover, N.J., P.B. Kenney, J.D. Amick, and W.A. Hypes. 1997. Preharvest sources of *Salmonella* colonization in turkey production. Poult. Sci. 76: 1232–1238.

110. Annan-Prah, A., and M. Janc. 1988. The mode of spread of *Campylobacter jejuni/coli* to broiler flocks. J. Vet. Med. 35: 11–18.

111. Bull, S.A., V.M. Allen, G. Domingue, F. Jørgensen, J.A. Frost, R. Ure, R. Whyte, D. Tinker, J.E.L. Corry, J. Gillard-King, and T.J. Humphrey. 2006. Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. Appl. Environ. Microbiol. 72: 645–652.

112. Nayak, R., P.B. Kenney, J. Keswani, and C. Ritz. 2003. Isolation and characterisation of *Salmonella* in a turkey production facility. Br. Poult. Sci. 44: 192–202.

113. Humphrey, T., S. O'Brien, and M. Madsen. 2007. Campylobacters as zoonotic pathogens: A food production perspective. Int. J. Food Microbiol. 117: 237–257.

114. Ellis-Iversen, J., F. Jorgensen, S. Bull, L. Powell, A.J. Cook, and T.J. Humphrey. 2009. Risk factors for *Campylobacter* colonisation during rearing of broiler flocks in Great Britain. Prev. Vet. Med. 89: 178–184.

115. Fernie, D.S., and R.W. Park. 1977. The isolation and nature of campylobacters (microaerophilic vibrios) from laboratory and wild rodents. J. Med. Microbiol. 10: 325–329.

116. Lapuz, R., H. Tani, K. Sasai, K. Shirota, H. Katoh, and E. Baba. 2008. The role of roof rats (*Rattus rattus*) in the spread of *Salmonella* Enteritidis and *S*. Infantis contamination in layer farms in eastern Japan. Epidemiol. Infect. 136: 1235–1243.

117. Lapuz, R.R., D. V. Umali, T. Suzuki, K. Shirota, and H. Katoh. 2012. Comparison of the prevalence of *Salmonella* infection in layer hens from commercial layer farms with high and low rodent densities. Avian Dis. 56: 29–34.

118. Hiett, K.L., N.J. Stern, N.A. Cox, M.T. Musgrove, and S. Ladely. 2002. Molecular subtype analyses of *Campylobacter* spp. from Arkansas and California poultry operations. Appl. Environ. Microbiol. 68: 6220–6236.

119. Rosef, O., and G. Kapperud. 1983. House flies (Musca domestica) as possible vectors of *Campylobacter fetus* subsp. jejuni. Appl. Environ. Microbiol. 45: 381–383.

. Hald, B., H. Skovgård, K. Pedersen, and H. Bunkenborg. 2008.. Poult. Sci. 87: 1428–1434.

121. Skovgård, H., K. Kristensen, and B. Hald. 2011. Retention of *Campylobacter* (Campylobacterales: Campylobacteraceae) in the house fly (Diptera: Muscidae). J. Med. Entomol. 48: 1202–1209.

122. Szalanski, A.L., C.B. Owens, T. McKay, and C.D. Steelman. 2004. Detection of *Campylobacter* and *Escherichia coli* O157 : H7 from filth flies by polymerase chain reaction. Med. Vet. Entomol. 18: 241–246.

123. Umunnabuike, A.C., and E.A. Irokanulo. 1986. Isolation of *Campylobacter* subsp. *jejuni* from oriental and american cockroaches caught in kitchens and poultry houses in Vom, Nigeria. Int. J. Zoonoses. 13: 180–186.

124. Jacobs-Reitsma, W.F., A.W. van de Giessen, N.M. Bolder, and R.W. Mulder. 1995. Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. Epidemiol. Infect. 114: 413–421.

125. Choo, L.C., A.A. Saleha, S.S. Wai, and N. Fauziah. 2011. Isolation of *Campylobacter* and *Salmonella* from houseflies (*Musca domestica*) in a university campus and a poultry farm in Selangor, Malaysia. Trop. Biomed. 28: 16–20.

126. Olsen, A.R., and T.S. Hammack. 2000. Isolation of *Salmonella* spp. from the housefly, *Musca domestica* L., and the dump fly, *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae), at caged-layer houses. J. Food Prot. 63: 958–960.

127. Holt, P.S., C.J. Geden, R.W. Moore, and R.K. Gast. 2007. Isolation of *Salmonella enterica* serovar Enteritidis from houseflies (*Musca domestica*) found in rooms containing *Salmonella* serovar Enteritidis-challenged hens. Appl. Environ. Microbiol. 73: 6030–6035.

128. Shane, S.M., M.S. Montrose, and K.S. Harrington. 1985. Transmission of *Campylobacter jejuni* by the housefly (*Musca domestica*). Avian Dis. 29: 384–391.

129. Hald, B., H. Skovgård, D.D. Bang, K. Pedersen, J. Dybdahl, J.B. Jespersen, and M. Madsen. 2004. Flies and *Campylobacter* infection of broiler flocks. Emerg. Infect. Dis. 10: 1490–1492.

130. Yang, Y., P.A. Iji, and M. Choct. 2009. Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. Worlds. Poult. Sci. J. 65: 97–114.

131. Foley, S.L., R. Nayak, I.B. Hanning, T.J. Johnson, J. Han, and S.C. Ricke. 2011. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. Appl. Environ. Microbiol. 77: 4273–4279.

132. Bohez, L., R. Ducatelle, F. Pasmans, N. Botteldoorn, F. Haesebrouck, and F. Van Immerseel. 2006. *Salmonella enterica* serovar Enteritidis colonization of the chicken caecum requires the HilA regulatory protein. Vet. Microbiol. 116: 202–210.

133. Guard-Petter, J. 2001. The chicken, the egg and *Salmonella enteritidis*. Environ. Microbiol. 3: 421–430.

134. Gantois, I., R. Ducatelle, F. Pasmans, F. Haesebrouck, R. Gast, T.J. Humphrey, and F. Van Immerseel. 2009. Mechanisms of egg contamination by *Salmonella* Enteritidis. FEMS Microbiol. Rev. 33: 718–738.

135. Henderson, S.C., D.I. Bounous, and M.D. Lee. 1999. Early events in the pathogenesis of avian salmonellosis. Infect. Immun. 67: 3580–3586.

136. Sahin, O., T.Y. Morishita, and Q. Zhang. 2002. *Campylobacter* colonization in poultry: Sources of infection and modes of transmission. Anim. Health Res. Rev. 3: 95–105.

137. Byrne, C.M., M. Clyne, and B. Bourke. 2007. *Campylobacter jejuni* adhere to and invade chicken intestinal epithelial cells in vitro. Microbiology. 153: 561–569.

138. Hermans, D., K. Van Deun, A. Martel, F. Van Immerseel, W. Messens, M. Heyndrickx, F. Haesebrouck, and F. Pasmans. 2011. Colonization factors of *Campylobacter jejuni* in the chicken gut. Vet. Res. 42: 82.

139. Van Deun, K., F. Pasmans, R. Ducatelle, B. Flahou, K. Vissenberg, A. Martel, W. Van den Broeck, F. Van Immerseel, and F. Haesebrouck. 2008. Colonization strategy of *Campylobacter jejuni* results in persistent infection of the chicken gut. Vet. Microbiol. 130: 285–297.

140. Sahin, O., N. Luo, S. Huang, and Q. Zhang. 2003. Effect of *Campylobacter*-specific maternal antibodies on *Campylobacter jejuni* colonization in young chickens. Appl. Environ. Microbiol. 69: 5372–5379.

141. Soleimani, A.F., I. Zulkifli, M. Hair-Bejo, M. Ebrahimi, S.D. Jazayeri, S.R. Hashemi, A. Meimandipour, and Y.M. Goh. 2012. Epigenetic modification: Possible approach to reduce *Salmonella enterica* serovar enteritidis susceptibility under stress conditions. Avian Pathol. 41: 351–354.

142. Humphrey, T. 2006. Are happy chickens safer chickens? Poultry welfare and disease susceptibility. Br. Poult. Sci. 47: 379–391.

143. Bull, S.A., A. Thomas, T. Humphrey, J. Ellis-Iversen, A.J. Cook, R. Lovell, and F. Jorgensen. 2008. Flock health indicators and *Campylobacter* spp. in commercial housed broilers reared in Great Britain. Appl. Environ. Microbiol. 74: 5408–5413.

144. Freestone, P.P.E., S.M. Sandrini, R.D. Haigh, and M. Lyte. 2008. Microbial endocrinology: How stress influences susceptibility to infection. Trends Microbiol. 16: 55–64.

145. Verbrugghe, E., F. Boyen, W. Gaastra, L. Bekhuis, B. Leyman, A. Van Parys, F. Haesebrouck, and F. Pasmans. 2012. The complex interplay between stress and bacterial infections in animals. Vet. Microbiol. 155: 115–127.

146. Jacobs-Reitsma, W.F., N.M. Bolder, and R.W.A.W. Mulder. 1994. Cecal carriage of *Campylobacter* and *Salmonella* in Dutch broiler flocks at slaughter: A one-year study. Poult. Sci. 73: 1260–1266.

147. Stern, N.J., M.R. Clavero, J.S. Bailey, N.A. Cox, and M.C. Robach. 1995. *Campylobacter* spp. in broilers on the farm and after transport. Poult. Sci. 74: 937–941.

148. Boysen, L., H. Vigre, and H. Rosenquist. 2011. Seasonal influence on the prevalence of thermotolerant *Campylobacter* in retail broiler meat in Denmark. Food Microbiol. 28: 1028–1032.

149. Wedderkopp, A., K.O. Gradel, J.C. Jorgensen, and M. Madsen. 2001. Pre-harvest surveillance of *Campylobacter* and *Salmonella* in Danish broiler flocks: A 2-year study. Int. J. Food Microbiol. 68: 53–59.

150. Rasschaert, G., K. Houf, J. Van Hende, and L. De Zutter. 2006. *Campylobacter* contamination during poultry slaughter in Belgium. J. Food Prot. 69: 27–33.

151. Ellerbroek, L.I., J.A. Lienau, and G. Klein. 2010. *Campylobacter* spp. in broiler flocks at farm level and the potential for cross-contamination during slaughter. Zoonoses Public Health. 57: e81–e88.

152. Jonsson, M.E., M. Norström, M. Sandberg, A.K. Ersbøll, and M. Hofshagen. 2010. Space-time patterns of *Campylobacter* spp. colonization in broiler flocks, 2002-2006. Epidemiol. Infect. 138: 1336–1345.

153. Jorgensen, F., J. Ellis-Iversen, S. Rushton, S.A. Bull, S.A. Harris, S.J. Bryan, A. Gonzalez, and T.J. Humphrey. 2011. Influence of season and geography on *Campylobacter*

jejuni and *C. coli* subtypes in housed broiler flocks reared in Great Britain. Appl. Environ. Microbiol. 77: 3741–3748.

154. McBride, G.B., B. Brown, and B.J. Skura. 1978. Effect of bird type, growers and season on the incidence of salmonellae in turkeys. J. Food Sci. 43: 323–326.

155. Rodriguez, A., P. Pangloli, H.A. Richards, J.R. Mount, and F.A. Draughon. 2006. Prevalence of *Salmonella* in diverse environmental farm samples. J. Food Prot. 69: 2576–2580.

156. Logue, C.M., J.S. Sherwood, L.M. Elijah, P.A. Olah, and M.R. Dockter. 2003. The incidence of *Campylobacter* spp. on processed turkey from processing plants in the midwestern United States. J. Appl. Microbiol. 95: 234–241.

157. Bolton, F.J., D. Coates, D.N. Hutchinson, and A.F. Godfree. 1987. A study of thermophilic campylobacters in a river system. J. Appl. Bacteriol. 62: 167–176.

158. Wallace, J.S., K.N. Stanley, J.E. Currie, P.J. Diggle, and K. Jones. 1997. Seasonality of thermophilic *Campylobacter* populations in chickens. J. Appl. Microbiol. 82: 219–224.

159. Gregory, E., H. Barnhart, D.W. Dreesen, N.J. Stern, and J.L. Corn. 1997. Epidemiological study of *Campylobacter* spp. in broilers: Source, time of colonization, and prevalence. Avian Dis. 41: 890–898.

160. Humphrey, T.J., A. Henley, and D.G. Lanning. 1993. The colonization of broiler chickens with *Campylobacter jejuni*: Some epidemiological investigations. Epidemiol. Infect. 110: 601–607.

161. Gopinath, S., S. Carden, and D. Monack. 2012. Shedding light on *Salmonella* carriers. Trends Microbiol. 20: 320–327.

162. Baird-Parker, A.C. 1990. Foodborne salmonellosis. Lancet. 336: 1231–1235.

163. Danguy des Deserts, J., R.H. Davies, K. Vaughan, I. Mclaren, P. Canning, A. Wintrip, D. Mueller-Doblies, and J.J. Carrique-Mas. 2011. A longitudinal study of *Salmonella* infection in different types of turkey flocks in Great Britain. Zoonoses Public Health. 58: 200–208.

164. Wesley, I. V., and W.T. Muraoka. 2009. Time of entry of *Salmonella* and *Campylobacter* into the turkey brooder house. Food Bioprocess Technol. 4: 616–623.

165. Berrang, M.E., D.P. Smith, W.R. Windham, and P.W. Feldner. 2004. Effect of intestinal content contamination on broiler carcass *Campylobacter* counts. J. Food Prot. 67: 235–238.

166. Wright, S.L., D.K. Carver, R.M. Siletzky, S. Romine, W.E.M. Morrow, and S. Kathariou. 2008. Longitudinal study of prevalence of *Campylobacter jejuni* and *Campylobacter coli* from turkeys and swine grown in close proximity. J. Food Prot. 71: 1791–1796.

167. Alali, W.Q., S. Thakur, R.D. Berghaus, M.P. Martin, and W.A. Gebreyes. 2010. Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. Foodborne Pathog. Dis. 7: 1363–1371.

168. Luangtongkum, T., T.Y. Morishita, A.J. Ison, S. Huang, P.F. McDermott, and Q. Zhang. 2006. Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. Appl. Environ. Microbiol. 72: 3600–3607.

169. Obiri-Danso, K., N. Paul, and K. Jones. 2001. The effects of UVB and temperature on the survival of natural populations and pure cultures of *Campylobacter jejuni*, *Camp. coli*, *Camp. lari* and urease-positive thermophilic campylobacters (UPTC) in surface waters. J. Appl. Microbiol. 90: 256–267.

170. Rollins, D.M., and R.R. Colwell. 1986. Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. Appl. Environ. Microbiol. 52: 531–538.

171. Murphy, C., C. Carroll, and K.N. Jordan. 2006. Environmental survival mechanisms of the foodborne pathogen *Campylobacter jejuni*. J. Appl. Microbiol. 100: 623–632.

172. Chan, K.F., H. Le Tran, R.Y. Kanenaka, and S. Kathariou. 2001. Survival of clinical and poultry-derived isolates of *Campylobacter jejuni* at a low temperature (4 degrees C). Appl. Environ. Microbiol. 67: 4186–4191.

173. Lee, A., S.C. Smith, and P.J. Coloe. 1998. Survival and growth of *Campylobacter jejuni* after artificial inoculation onto chicken skin as a function of temperature and packaging conditions. J. Food Prot. 61: 1609–1614.

174. Thakur, S., J. Brake, S. Keelara, M. Zou, and E. Susick. 2013. Farm and environmental distribution of *Campylobacter* and *Salmonella* in broiler flocks. Res. Vet. Sci. 94: 33–42.

175. Joshua, G.W.P., C. Guthrie-Irons, A. V. Karlyshev, and B.W. Wren. 2006. Biofilm formation in *Campylobacter jejuni*. Microbiology. 152: 387–96.

176. Reuter, M., A. Mallett, B.M. Pearson, and A.H.M. van Vliet. 2010. Biofilm formation by *Campylobacter jejuni* is increased under aerobic conditions. Appl. Environ. Microbiol. 76: 2122–2128.

177. Hanning, I., R. Jarquin, and M. Slavik. 2008. *Campylobacter jejuni* as a secondary colonizer of poultry biofilms. J. Appl. Microbiol. 105: 1199–1208.

178. Trachoo, N., J.F. Frank, and N.J. Stern. 2002. Survival of *Campylobacter jejuni* in biofilms isolated from chicken houses. J. Food Prot. 65: 1110–1116.

179. Spector, M.P., and W.J. Kenyon. 2012. Resistance and survival strategies of *Salmonella enterica* to environmental stresses. Food Res. Int. 45: 455–481.

180. Liebana, E., L. Garcia-Migura, C. Clouting, F.A. Clifton-Hadley, M. Breslin, and R.H. Davies. 2003. Molecular fingerprinting evidence of the contribution of wildlife vectors in the maintenance of *Salmonella* Enteritidis infection in layer farms. J. Appl. Microbiol. 94: 1024–1029.

181. Steenackers, H., K. Hermans, J. Vanderleyden, and S.C.J. De Keersmaecker. 2012. *Salmonella* biofilms: An overview on occurrence, structure, regulation and eradication. Food Res. Int. 45: 502–531.

182. Nde, C.W., J.M. McEvoy, J.S. Sherwood, and C.M. Logue. 2007. Cross contamination of turkey carcasses by *Salmonella* species during defeathering. Poult. Sci. 86: 162–167.

183. Berrang, M.E., R.J. Buhr, J.A. Cason, and J.A. Dickens. 2001. Broiler carcass contamination with *Campylobacter* from feces during defeathering. J. Food Prot. 64: 2063–2066.

184. Reich, F., V. Atanassova, E. Haunhorst, and G. Klein. 2008. The effects of *Campylobacter* numbers in caeca on the contamination of broiler carcasses with *Campylobacter*. Int. J. Food Microbiol. 127: 116–120.

185. Mead, G.C. 2005. Food safety control in the poultry industry. Boca Raton, FL : CRC Press ; Cambridge : Woodhead Pub., 2005.

186. Mead, G.C., W.R. Hudson, and M.H. Hinton. 1994. Use of a marker organism in poultry processing to identify sites of cross-contamination and evaluate possible control measures. Br. Poult. Sci. 35: 345–354.

187. Smith, D.P., J.K. Northcutt, J.A. Cason, A. Hinton, R.J. Buhr, and K.D. Ingram. 2007. Effect of external or internal fecal contamination on numbers of bacteria on prechilled broiler carcasses. Poult. Sci. 86: 1241–1244.

188. Smith, D.P., J.A. Cason, and M.E. Berrang. 2005. Effect of fecal contamination and cross-contamination on numbers of coliform, *Escherichia coli*, *Campylobacter*, and *Salmonella* on immersion-chilled broiler carcasses. J. Food Prot. 68: 1340–1345.

189. Stopforth, J.D., R. O'Connor, M. Lopes, B. Kottapalli, W.E. Hill, and M. Samadpour. 2007. Validation of individual and multiple-sequential interventions for reduction of microbial populations during processing of poultry carcasses and parts. J. Food Prot. 70: 1393–1401.

190. Kotula, K.., and Y. Pandya. 1995. Bacterial contamination of broiler chickens before scalding. J. Food Prot. 58: 1326–1329.

191. Berrang, M.E., R.J. Buhr, and J.A. Cason. 2000. *Campylobacter* recovery from external and internal organs of commercial broiler carcass prior to scalding. Poult. Sci. 79: 286–290.

192. Campbell, D.F., S.S. Green, C.S. Custer, and R.W. Johnston. 1982. Incidence of *Salmonella* in fresh dressed turkeys raised under *Salmonella*-controlled and uncontrolled environments. Poult. Sci. 61: 1962–1967.

193. Corrier, D.E., J.A. Byrd, B.M. Hargis, M.E. Hume, R.H. Bailey, and L.H. Stanker. 1999. Presence of *Salmonella* in the crop and ceca of broiler chickens before and after preslaughter feed withdrawal. Poult. Sci. 78: 45–49.

194. Compliance guideline for controlling *Salmonella* and *Campylobacter* in poultry. Third Edition. May 2010. Food Safety and Inspection Service, US Department of Agriculture, Washington, DC.

195. Byrd, J.A., D.E. Corrier, M.E. Hume, R.H. Bailey, L.H. Stanker, and B.M. Hargis. 1998. Effect of feed withdrawal on *Campylobacter* in the crops of market-age broiler chickens. Avian Dis. 42: 802–806.

196. Ramirez, G.A., L.L. Sarlin, D.J. Caldwell, C.R. Yezak, Jr., M.E. Hume, D.E. Corrier, J.R. Deloach, and B.M. Hargis. 1997. Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. Poult. Sci. 76: 654–656.

197. Wesley, I. V, W.T. Muraoka, D.W. Trampel, and H.S. Hurd. 2005. Effect of preslaughter events on prevalence of *Campylobacter jejuni* and *Campylobacter coli* in market-weight turkeys. Appl. Environ. Microbiol. 71: 2824–2831.

198. Northcutt, J.K., S.I. Savage, and L.R. Vest. 1997. Relationship between feed withdrawal and viscera condition of broilers. Poult. Sci. . 76 : 410–414.

199. Bilgili, S.F., and J.B. Hess. 1997. Tensile strength of broiler intestines as influenced by age and feed withdrawal. J. Appl. Poult. Res. 6: 279–283.

200. Byrd, J.A., B.M. Hargis, D.J. Caldwell, R.H. Bailey, K.L. Herron, J.L. McReynolds, R.L. Brewer, R.C. Anderson, K.M. Bischoff, T.R. Callaway, and L.F. Kubena. 2001. Effect

of lactic acid administration in the drinking water during preslaughter feed withdrawal on *Salmonella* and *Campylobacter* contamination of broilers. Poult. Sci. 80: 278–283.

201. Mohyla, P., S.F. Bilgili, O.A. Oyarzabal, C.C. Warf, and G.K. Kemp. 2007. Application of acidified sodium chlorite in the drinking water to control *Salmonella* serotype Typhimurium and *Campylobacter jejuni* in commercial broilers. J. Appl. Poult. Res. 16: 45–51.

202. Avila, L.A.F. de, V.P. do Nascimento, C.W. Canal, C.T.P. Salle, and H.L. de S. Moraes. 2003. Effect of acidified drinking water on the recovery of *Salmonella enteritidis* from broiler crops. Rev. Bras. Ciência Avícola. 5: 183–188.

203. Berrang, M.E., J.K. Northcutt, D.L. Fletcher, and N.A. Cox. 2003. Role of dump cage fecal contamination in the transfer of *Campylobacter* to carcasses of previously negative broilers. J. Appl. Poult. Res. 12: 190–195.

204. Slader, J., G. Domingue, F. Jørgensen, K. McAlpine, R.J. Owen, F.J. Bolton, and T.J. Humphrey. 2002. Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. Appl. Environ. Microbiol. 68: 713–719.

205. Corry, J.E.L., V.M. Allen, W.R. Hudson, M.F. Breslin, and R.H. Davies. 2002. Sources of *Salmonella* on broiler carcasses during transportation and processing: Modes of contamination and methods of control. J. Appl. Microbiol. 92: 424–432.

206. Rigby, C.E., J.R. Pettit, A.H. Bentley, J.L. Spencer, M.O. Salomons, and H. Lior. 1982. The relationships of salmonellae from infected broiler flocks, transport crates or processing plants to contamination of eviscerated carcases. Can. J. Comp. Med. 46: 272–278.

207. Rostagno, M.H., I. V Wesley, D.W. Trampel, and H.S. Hurd. 2006. *Salmonella* prevalence in market-age turkeys on-farm and at slaughter. Poult. Sci. 85: 1838–1842.

208. Wesley, I. V, E. Harbaugh, D.W. Trampel, F. Rivera, M.H. Rostagno, and H.S. Hurd. 2006. Effect of preslaughter events on the prevalence of *Salmonella* in market-weight turkeys. J. Food Prot. 69: 1785–1793.

209. USDA - US Department of Agriculture. Food Safety. Accessed June, 2014. http://www.usda.gov/wps/portal/usda/usdahome?navid=food-safety.

210. Food Safety From Farm to Table: A National Food Safety Initiative. Report to the president. May 1997. http://www.cdc.gov/ncidod/foodsafe/report.htm.

211. USDA-FSIS, 1996. Pathogen reduction; hazard analysis and critical control point (HACCP) systems. Federal Register 61, 38806–38989.

212. Federal Register Notice, Vol. 75, No. 93, 27288, May 14, 2010 (Docket No. FSIS-2009-0034), "New Performance Standards for *Salmonella* and *Campylobacter* in Young Chicken and Turkey Slaughter Establishments".

CHAPTER II

ROUTES OF TRANSMISSION OF *SALMONELLA* AND *CAMPYLOBACTER* IN BREEDER TURKEYS

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2.1. SUMMARY

Salmonella and *Campylobacter* are frequent colonizers of the intestinal tracts of poultry and have been associated with significant foodborne disease. This project included monitoring of turkey breeder hens and toms from day of hatch to 65 weeks of age with the objective of determining routes of transmission for *Salmonella* and *Campylobacter* throughout the turkey production cycle. Breeder poults were separated by sex and then in two groups (control and inoculated) for each sex. The inoculated group was orally gavaged with marker strains of both pathogens. The inoculated groups (toms and hens) were placed on the opposite side of a growout house from the uninoculated groups. Fecal samples, intestinal samples and organs, feed, drinkers and other vectors such as insects and mice were analyzed at different time points until 65 weeks. Monitoring results showed that *Campylobacter* spread rapidly and cross-contaminated turkeys throughout the growout house. For both *Salmonella* and *Campylobacter*, naturally occurring strains that were isolated seemed to outcompete marker strains after several weeks post inoculation and persisted in the flock. The most common naturally occurring strains isolated were *C. jejuni* (tetracycline resistant), *C. coli* (kanamycin resistant), and *S.* Agona. Pathogens were also isolated from pest vectors in the houses, confirming the importance of proper pest control and biosecurity to control the spread of the bacteria.

2.2. DESCRIPTION OF PROBLEM

Salmonella and *Campylobacter* are foodborne zoonotic pathogens of public health relevance worldwide, both ranking among the top five pathogens contributing to foodborne disease in the US [1–3]. Frequent colonization of intestinal tracts of poultry with these bacteria makes poultry meat an important source of infection [1, 2, 4]. Furthermore, colonization of the flock by these pathogens at the pre-harvest level has been found to be associated with contamination of poultry carcasses during processing [5–13]. Since 2011, USDA Food Safety and Inspection Service (FSIS) regulations require turkey processing companies to maintain low levels of *Salmonella* and *Campylobacter* in processed products as monitored by testing programs [14]. A number of methods including chemical interventions have been used to control these pathogens at the plant. However, reducing or eliminating both pathogens from birds prior to processing is potentially more beneficial than excessive plant interventions. Further understanding about entry, transmission, and overall prevalence of both pathogens in the production chain may help to determine risk factors and controls for pre-harvest food safety, and lead to methods for prevention and/or reduction of pathogenic bacteria colonizing poultry. Many different routes of transmission for these pathogens into the flock such as vertical transmission, pests, wild animals, feed and water, farm workers, environment, and lack of hygiene, have been previously considered and investigated, especially in chickens [15–20]. However, there is little information regarding turkey flocks. The objective of this project was to determine routes of transmission for *Salmonella* and *Campylobacter* throughout turkey production and processing.

2.3. MATERIALS AND METHODS

2.3.1. Inoculation and Monitoring

This study began with the placement of a flock of 140 Nicholas turkey breeder poults [21] in a growout house of a Turkey Research Unit at North Carolina State University, Raleigh [22]. Prior to beginning the project, the house was spray disinfected [23], and environmental samples including drinkers and feed were analyzed for the presence of *Salmonella* and *Campylobacter*. At arrival, artificial straw from the transportation boxes containing feces, and fecal samples during the first week of life were analyzed for both pathogens. Poults were placed into pens separated by sex and then separated further into two groups: Inoculated (82 hens and 22 toms) and Control (28 hens and 8 toms) (Fig. 2.1.). Inoculated and control groups were separated by two plastic curtains, a tray containing PI quat 20® for feet immersion was located between both sides for passing through when leaving the inoculated side. The use of boot covers and other protective equipment (gloves, coveralls) was required to enter the pens of inoculated turkeys, and protective garments were removed between leaving the inoculated side of the house to prevent cross-contamination. Inoculated toms (IT) and hens (IH) were orally gavaged at 10 days old (3/30/12) with 0.1 mL of an inoculum containing marker *Salmonella* (approx. 10^7 cfu/mL) and *Campylobacter* strains (approx. 10^7 cfu/mL) (Table 2.1). Poults were not inoculated sooner to prevent mortality. Also, *Campylobacter* colonization is frequently found around weeks 3 to 4 in broiler chickens, likely associated with the disappearance of maternal antibodies against *Campylobacter* [24], so inoculation was performed before that time range. *S. enterica* ser.

Enteritidis nalidixic acid resistant (from now *S.* Enteritidis NAL^R) and *C. coli* resistant to gentamicin and kanamycin (GK) [25] were administered to IH, and *S. enterica* ser. Typhimurium (from now *S.* Typhimurium NAL^R) and a strain of *C. jejuni* resistant to tetracycline, streptomycin, kanamycin and quinolones (nalidixic acid and ciprofloxacin) (TSKQ) to IT. Control toms (CT) and hens (CH) were orally inoculated with the same volume of phosphate-buffered saline (PBS) [26] to simulate the same level of stress due to handling. Turkeys were fed a diet formulated without growth promoters or other antibiotics which could have affected colonization by the bacteria inoculated. At 12 weeks (6/11/12) birds in the same pen were distributed in two pens to reduce the number of turkeys per pen. The distribution of pens in the house and number of turkeys per pen is shown in figure 2.2. Due to the reduction in number of positive fecal samples for marker strains, a second inoculation was carried out at the same time of handling the birds. IH and IT were gavaged this second time with 2 mL of an inoculum containing the same strains of bacteria used for the first inoculation but in higher concentration (approx. 10^8 cfu/mL) (Table 2.1). For this second inoculation a higher level of inoculum was used due to the greater weight and age of the poults.

When the hens reached sexual maturity, at 21 weeks, both groups, IH and CH, were moved to a dark-out house, where a step down lighting program was applied. The house had solid side walls and light treps in fans and inlet systems for a total light control. Hours of light were controlled by a time switch [27]. Hens received 8 hours of light per 24 hours day for the first five weeks, and then the hours of light were reduced half an hour per week until week 12, where they received 4.5 hours of light per day. IH and CH were in two different

rooms, but hens of each group were together in one room, not in different pens. Thus, for this period of time fecal samples were reduced to two pooled samples for CH, and 3-4 pooled samples for IH.

2.3.2. Inoculum Preparation

S. enterica ser. Enteritidis and Typhimurium, both resistant to 200 µg/mL of nalidixic acid, were grown overnight in brain heart infusion (BHI) broth [28] at 37°C in a water bath with agitation. *Campylobacter* strains were initially grown in Mueller-Hinton agar (MHA) plates [29] at 42°C for 48 hours in microaerobic conditions using Ziploc bags filled with a microaerobic gas mixture (5% O2, 10% CO2, 85% N) [30] and then transferred into Mueller-Hinton broth (MHB) [28] and incubated at 42°C for 24 hours in microaerobic conditions. The inoculum of each bacteria was plated (100 µL) onto brilliant green sulfa agar plates (BGS) [31] for enumeration of *Salmonella*, and MHA in the case of *Campylobacter*, to calculate the concentration of bacteria per milliliter. *Campylobacter* inoculum was also observed under the microscope prior to inoculation to check morphology, motility and density of cells. For the first inoculation (day 10), both marker *Salmonella* inoculums were diluted 1:10 in BHI after overnight growth (10^8-10^9 cfu/mL). Then, 1 mL of the *S.* Enteritidis and *C. coli* inoculums was added into 8 mL of PBS. The same dilution was made for S. Typhimurium and *C. jejuni*. For inoculation at 12 weeks, both *Salmonella* inoculums were diluted 1:2 in BHI after growing overnight. Then, the same volumes of *Salmonella* and *Campylobacter* inoculums were mixed.

2.3.3. Fecal Analysis

Pooled fecal droppings from each pen were analyzed starting the first week and then weekly until week 15 (from April 2012 to July 2012), biweekly in July (weeks 17 and 19) and August (weeks 21 and 23), and one time in September (week 27), October (week 32) and December (week 39) 2012, and May 2013 (week 61). In week 19 independent fecal droppings per pen were analyzed for *Campylobacter* which increased the number of samples analyzed for *Campylobacter* (n=200) in comparison with *Salmonella* (n=184). For the last sample analyzed at week 61, only the hens were available, toms were sacrificed around week 50 (March 2013). Fecal droppings from the same pen were collected in a 50 mL centrifuge plastic tube, mixed with a sterile cotton swab and directly streaked onto Campy Cefex agar (CCA) [31] containing gentamicin 200 µg/mL, or nalidixic acid 20 µg/mL, respectively, for the selective identification of marker strains of *Campylobacter* inoculated into the poults. CCA was no longer used after week 21 for detection of marker strains of *Campylobacter* due to an excessive growth of background microflora, modified cefoperazone charcoal deoxycholate agar (mCCDA) was used instead. For the detection of other campylobacters the samples were streaked onto mCCDA [29]. Enumeration of *Campylobacter* was performed for occasional samples in independent fecal droppings (Table 2.2.). For enumeration, 1 gram of feces was combined with 9 mL of buffered peptone water (BPW) 1% and homogenized, serial dilutions were plated (0.1 mL) onto mCCDA. The detection limit was 1.0×10^2 cfu/g. All plates were incubated at 42°C for 48 hours under microaerobic conditions. One

Campylobacter colony per plate was sub-cultured on MHA for purification and further characterization including antibiotic susceptibility test and species determination.

For *Salmonella* identification, fecal samples were diluted in BPW 1% in a ratio 1:10 for a first preenrichment step [29], the sample was stomached for 60 seconds and incubated at 37^{o}C for 24 hours. This initial step was followed by a selective enrichment, 0.5 mL of the previously enriched solution was added into tetrathionate (TT) broth base tubes [32] supplemented with 0.2 mL of iodine solution [32], and 0.1 mL was added into Rappaport-Vassiliadis (RV) broth [32]. Tubes were vortexed and incubated at 42°C for 24 hours. After incubation a loopful from each of the tubes was streaked onto BGS agar without antibiotics. Samples were then streaked onto BGS containing NAL 200 µg/mL for detection of the marker strains used to inoculate the turkeys, and incubated at 37^{o}C for 24 hours. Presumptive *Salmonella* colonies were stabbed and streaked into triple sugar iron (TSI) agar and lysine iron agar (LIA) slants. Slants were incubated for 24 hours at 37°C. Isolates giving typical reactions for *Salmonella* were streaked onto nutrient agar (NA) for purification, and confirmed by serology with *Salmonella* Poly O [28] and Poly H [29] antiserum. *Salmonella* and *Campylobacter* isolates were preserved in cryovials containing BHI broth with 20% glycerol at -80°C.

2.3.4. Blood, Organs and Intestinal Samples Collection and Analysis

Blood samples were taken at week 11 after inoculation with marker strains from all the groups, and week 21 from both groups of hens (inoculated and control). Blood was drawn (8-

10 mL) from the wing vein. Skin was sprayed with 70% alcohol and allowed a 30 seconds contact time, followed by the application of povidone-iodine solution 10% that was allowed at least one minute contact time before vein puncture. Blood was directly plated (0.1 mL) onto CCA for *Campylobacter* detection, and 4 mL were used for enrichment to investigate each of the pathogens. A 1:10 dilution using Bolton broth [29] was performed for *Campylobacter* enrichment and incubated in microaerobic conditions at 42°C for 24-48 hours followed by plating of 0.1 mL onto CCA and incubation at 42°C for 48 hours. *Salmonella* detection was performed as described for fecal samples.

Turkeys were periodically euthanized by electrocution between weeks 11 and 65 (Table 2.3.) for collection and analysis of intestinal samples and organs. Guidelines of the approved Institutional Use and Animal Care Committee's protocol at North Carolina State University were followed. The number of samples analyzed was limited due to the need of maintaining the flock for monitoring through the reproductive stages, eggs analysis and hatchability study (not discussed in this chapter). Absence of corneal reflex and vital signs were examined before proceeding to spray the carcass exteriors with 70% ethanol and opening the abdominal cavity. Spleen, liver and gallbladder, ceca and jejunum, were aseptically collected and placed into sterile Whirl-pak bags, labeled accordingly, and packed on ice for transportation to the laboratory. Ceca and jejuna fecal contents were directly streaked onto mCCDA agar plates. Previous studies have reported that direct plating of fecal or cecal samples yielded better recovery of *Campylobacter* than enrichment [33–36]. Spleen was immersed in alcohol for 30 seconds, rinsed in saline solution and then aseptically divided in two longitudinal pieces for the investigation of each pathogen. Liver was also aseptically divided in two pieces and the

one containing the gallbladder was used for *Campylobacter* detection. Samples were individually weighed, macerated with a rubber mallet and BPW 1% was added in a ratio of three times the weight of the sample for the preenrichment of *Salmonella*. Next steps of selective enrichment and plating were performed the same as for the fecal samples. For *Campylobacter* enrichment, three times the weight of the sample of Bolton broth with selective supplements [29] and laked horse blood 5% [37] was added to the samples, stomached and incubated in microaerobic conditions at 42 ^{o}C for 48 hours. Samples were plated onto CCA or mCCDA agar plates.

2.3.5. Vectors and Other Samples Tested

Feed (25g), swabs from drinkers (two swabs per drinker/pen), wood shavings (collected in a whirl-pak bag) and other possible vectors of transmission such as insects and mice were periodically tested for the presence of *Salmonella* and *Campylobacter*. The different samples analyzed are shown in table 2.4. Glue traps [38] were placed in several locations of both sides of the growout house, where inoculated and control turkeys were located. Insects were separated by species using forceps. The number of insects analyzed was variable (Table 2.4.). Two mice were analyzed, one was found in a glue trap, and the other one was killed by the farm crew. Whole insects and mice were initially macerated with a rubber mallet, and processed as previously described for organs and intestinal samples, using an enrichment step for both pathogens.

2.3.6. Campylobacter and Salmonella Subtyping

Species of *Campylobacter* isolates were determined by multiplex polymerase chain reaction (PCR). For these purpose primers were used to amplify the *C. jejuni* specific *hip* gene (hipF 5'-ATG ATG GCT TCT TCG GAT AG-3' and hipR 5'-GCT CCT ATG CTT ACA ACT GC-3'), and the *C. coli ceu* gene (ceuF 5'-ATG AAA AAA TCT TTA GTT TTT GCA-3' and ceuR 5'-GAT TTT ATT ATT TGT AGC AGC G-3') [39–41]. Genomic DNA from *C. coli* strain D124 and *C. jejuni strain* NCTC 11168 were included as positive controls; negative controls (no DNA) was performed for each reaction. *Salmonella* isolates were serotyped by SMART, a multiplex PCR and capillary electrophoresis analysis [42].

2.3.7. Campylobacter Antibiotic Resistance Test

Campylobacter isolates were tested for resistance to a panel of antibiotics (Table 2.5.) by agar dilution method. All isolates were also grown on MHA plates to ensure viability. *C. jejuni* ATCC 33560 [43], sensitive to all the antibiotics tested, was included in the test as a quality control strain.

2.3.8. Pulsed-Field Gel Electrophoresis

Genomic DNA fingerprinting of *Campylobacter* and *Salmonella* isolates was determined by Pulsed-field gel electrophoresis (PFGE) in particular cases to check identity or similarity

of strains. DNA was restricted using *Sma*I [44] for *Campylobacter* and XbaI for *Salmonella* [45].

2.3.9. Statistical Analysis

Frequencies of detection observed for both pathogens were reported. Ceca and jejuna frequencies of detection were compared using two-sided Fisher's exact test. Fisher's test was performed using JMP 11 software [46]. Significance was defined at $P \leq 0.05$. Clonal relationships of *Campylobacter* isolates based on PFGE banding patterns were calculated using BioNumerics [47].

2.4. RESULTS AND DISCUSSION

2.4.1. Monitoring of Salmonella in Fecal Samples

A total of 184 fecal samples were analyzed for *Salmonella* from week 3 to week 61. Of those, 102 (55.4%) were positive. Marker strains were isolated in 45 (44.1%) of the positive samples, and the remaining 57 (55.9%) were naturally occurring strains (susceptible to nalidixic acid) (Table 2.6.). Table 2.6. shows the number of positive fecal samples for *Salmonella* per group. From the 57 isolates classified as naturally occurring (susceptible to nalidixic acid), a subset of 29 representative isolates was selected, including isolates from each group and from different dates of fecal analysis. The other 28 isolates (49.1% of the naturally occurring) were not further characterized. Subtyping was performed at the Russell

Research Center (USDA-ARS), Athens, GA. The serotypes found in each group of birds are shown in figure 2.3. Subtyping of naturally occurring isolates revealed that 26 (89.7%) from the total subtyped isolates (n=29) were *S.* Agona, two (6.9%) were *S.* Typhimurium, and one (3.4%) was *S.* Liverpool (Table 2.7.). Further characterization by PFGE revealed that the two *S.* Typhimurium isolates from IT (susceptible to nalidixic acid) had the same DNA fingerprint as the marker strain inoculated into the toms (nalidixic acid resistant) (Fig. 2.4.), indicating that they were the marker strain that probably lost the ability to be resistant to nalidixic acid. Although nalidixic acid resistance of *Salmonella* is frequently associated with point mutations in the quinolone resistance determining regions (QRDR) [48, 49], resistance to quinolones can also be mediated by plasmids [50, 51]. However, the origin of the resistance in the marker strains used in this study is unknown. After the first inoculation, the marker strains persisted in fecal samples for 5 and 10 weeks in hens and toms respectively, and the marker strains were not isolated from control birds at any time (Table A.1.). However, naturally occurring *Salmonella* strains were isolated in control hens at week 3 (week 1 after the first inoculation) and week 9 (week 7 after inoculation) in control toms (Table A.1.). Marker strains persisted in fecal samples of the inoculated turkeys up to week 19 (seven weeks after the second inoculation) (Table A.1.). Marker *Salmonella* strains were not isolated from control birds, and naturally occurring strains of *Salmonella* persisted in both control toms and hens. Further characterization revealed these isolates were *S.* Agona. *S.* Agona was isolated from all the groups of turkeys (Fig. 2.3.). This serotype has been previously reported to be found commonly colonizing swine, broilers, and turkeys [52, 53].

A detailed list of fecal samples analyzed for *Salmonella* and the positive isolates per group of birds is presented in table A.2.

2.4.2. Monitoring of Campylobacter in Fecal Samples

A total of 200 fecal samples were analyzed for *Campylobacter* from week 3 to week 61. From them, 144 (72%) were identified as positive, with 57 of the 144 (39.6%) characterized as *C. coli*, 73 (50.7%) as *C. jejuni*, and 14 (9.7%) that were not further characterized. Moreover, 59 of the 144 positive isolates (41%) were marker strains. A detailed analysis of the isolates taking into account each of the four groups of turkeys, Control Toms (CT), Control Hens (CH), Inoculated Hens (IH), and Inoculated Toms (IT) is shown in Table 2.8. *C. jejuni* was detected in higher rates than *C. coli* for all groups, except for CT where *C. coli* was more frequently found (Table 2.8.). Enumeration results for occasional fecal samples are shown in table 2.2. In weeks 4 and 19, *Campylobacter* was found in levels of 10^3 to 10^7 cfu/g of feces, in contrast with levels between 10^2 to10^5 cfu/g in weeks 47 and 61 (Table 2.2.). Marker strains of *Campylobacter* inoculated into turkey poults were isolated from fecal samples until week 6 and 7 in IT and IH, respectively (five and six weeks after the first inoculation in IT and IH, respectively) (Table A.1.). However, the strain inoculated into the toms (*C. jejuni*) was also isolated from hens' droppings, and vice versa, at week 4 (week 3 after the first inoculation) (Table A.1.). Both marker strains were isolated from control birds, hens and toms at week 4 (week 3 after gavage on the inoculated birds). The recovery of marker *Campylobacter* strains from control groups, and from groups in which the marker

strains were not inoculated originally was also supported by PFGE analysis (data not shown). Naturally occurring strains of *Campylobacter* were isolated from control birds, both toms and hens from week 4 (Table A.1.). From the 19 *Campylobacter*-positive fecal samples from CT, 16 isolates were further characterized (species and antibiotic susceptibility profile). Of the 16 characterized, seven (43.8%) of them were marker strains, three (60% of the total *C. jejuni* recovered from CT) were *C. jejuni* TSKQ (the marker strain inoculated into the IT), and four isolates (36.4 % of the total *C. coli* recovered in CT) were *C. coli* GK (the marker strain inoculated into the IH). In CH, from the 27 positive samples, 23 (85.2%) were further characterized. From the characterized isolates, four (17.4%) were marker strains, three of them were *C. jejuni* TSKQ (21.4% of the *C. jejuni* isolated in CH), and one was *C. coli* GK (11.1% of the *C. coli* isolates in CH) (Table 2.8.). After the second inoculation, *C. coli* GK (marker strain inoculated into the IH) was no longer recovered from fecal samples; however, *C. jejuni* TSKQ (the marker strain inoculated into the toms) was isolated from fecal samples up to week 21 (nine weeks after second inoculation) (Table A.1.). In IT, *C. jejuni* TSKQ was recovered in 12 of the 23 speciated isolates (75% of the total *C. jejuni*), and two isolates were *C. coli* GK (28.6% of the *C. coli* recovered in IT) (Table 2.8.). In IH, 14 from the 68 speciated isolates (46.7% of the *C. coli* recovered in IH) were *C. coli* GK (marker strain inoculated in IH). In contrast with the 20 *C. jejuni* TSKQ isolates (marker strain inoculated into IT) (52.6% of the *C. jejuni* isolates recovered in IH) (Table 2.8.). These findings may suggest a higher colonization ability of the marker strain used for inoculating the toms. This strain of *C. jejuni* was originally recovered from turkey hosts, in contrast with the *C. coli* GK strain that was originally recovered from chickens. Host association could be one explanation

for the higher recovery rate of the *C. jejuni* TSKQ strain. A strong host-association of *Campylobacter* has been previously reported by Sheppard *et al*. in 2010 [54]. Relative frequencies of *Campylobacter* recovered from fecal samples, calculated based on the total number of isolates characterized per group, are represented in figure 2.5. Marker strains inoculated into the toms (*C. jejuni* TSKQ) and hens (*C. coli* GK) were isolated in all the groups (Fig. 2.5.). The relative frequencies recovered per group (calculated based on the total number of isolates characterized) for *C. jejuni* TSKQ were 52.2% in IT samples, 29.4% in IH isolates, 18.8% in CT isolates, and 13.0% in CH isolates (Fig. 2.5.). *C. coli* GK was recovered in 25.0% of CT, 20.6% of IH, 8.7% of IT, and 4.4% of CH samples (Fig. 2.5.). *C. jejuni* TSKQ was recovered in higher frequencies than *C. coli* GK in all groups, except for CT, with 18.8% and 25.0%, respectively (Fig. 2.5.). Moreover, naturally occurring strains *C. jejuni* T (tetracycline resistant) and *C. coli* K (kanamycin resistant) were recovered in fecal samples from all the groups (Fig. 2.5.). *C. coli* K was recovered in higher frequencies than *C. jejuni* T for all groups except for CH (34.8%) where the recovery of *C. jejuni* T was higher (39.1%) (Fig. 2.5.). The difference was bigger in CT with 43.8% of *C. coli* K isolates, in contrast with 12.5% of *C. jejuni* T. A detailed list of *Campylobacter* isolates recovered from fecal samples are reported in table A.3. Further characterization results (by PFGE) of some naturally occurring isolates were compared with other isolates with the same antibiotic profile isolated from fecal samples of turkeys grown at the same farm either in the same house previously, or in other houses during the study; isolates were the same strain. *C. coli* K recovered from flies were found to be also the same strain that was isolated in previous studies (data not shown). The detection of the same strains of *Campylobacter* colonizing

turkeys at different locations on the farm may indicate the persistence in the environment of certain strains [55, 56]. These findings are in accordance with previous studies in broilers showing that environmental reservoirs may play a key role in transmission of *Campylobacter* into the flocks [18, 55]. A high prevalence of *Campylobacter* resistant to tetracycline and kanamycin in both conventional and organic poultry production have been previously reported [57].

Our findings indicate that *Campylobacter* spread rapidly throughout the growout house and the flock was colonized by several naturally occurring strains of *Campylobacter*, which seem to successfully compete with the marker strains after a few weeks and persist in the flock. It is not known how these naturally occurring strains reached the farm, but it seems evident that both antimicrobial resistance profiles, tetracycline and kanamycin, can persist in the farm environment without current antibiotic selection pressure as previously suggested by some authors [57].

2.4.3. Frequencies of Detection of Campylobacter and Salmonella over Time

The observed relative frequencies of detection of both pathogens in the different groups were interesting and different patterns were exhibited. To estimate this prevalence, the number of positive samples per month and group were compared to the total number of fecal samples analyzed. A complicating factor was that the number of pens per group was different leading to a larger number of samples of IH (6 pens), in contrast to only one pen of CT. There was a tendency for *Salmonella* and *Campylobacter* to peak at different times (Fig. 2.6.

and 2.7., respectively). *Campylobacter* had a maximum number of positive isolates in April 2012 (week 3 to 6), right after inoculation with marker strains, but this was also true for the control groups which were colonized with naturally occurring strains. In June (weeks 11 to 14) the number detected was lower or *Campylobacter* was absent in some groups (Fig. 2.7.). There was an increase in background microflora on the plates that could have had a negative effect on *Campylobacter* detection in both inoculated and control groups; nevertheless, in the control groups two different media was used (mCCDA and Campy Cefex) without detection using either medium. In contrast, *Salmonella* detection seemed to increase more discretely in April and May (except for the CH group) and then reached a maximum detection in June for all groups, coinciding with low or absence of detection of *Campylobacter* (Fig. 2.7.). In July (weeks 15-17-19), frequency of detection of *Campylobacter* was higher and reached a second peak in August (weeks 21-23), and prevalence of *Salmonella* in turn decreased in July, reaching low levels or decreasing below detection levels from August 2012 to February 2013 (week 47) (Fig. 2.6. and 2.7.). The only exception was the CT group where *Salmonella* detection remained high for a longer time, until December 2012 (week 39), and then became undetectable in February 2013 (Fig. 2.6.). The lack of detection of *Salmonella* in hens, especially in IH, was also intriguing since artificial insemination with semen containing marker strains of *Salmonella* (*S.* Enteritidis NAL^R) and *Campylobacter* (*C. coli* GK) was performed between December 2012 and January 2013, which should have increased the possibilities of infection (Artificial insemination of hens is covered in Chapter 3). Also, *C. coli* GK was not detected in feces during that period. Prevalence of *Campylobacter* in both groups of toms, CT and IT, was similarly high between August and October, but not detected

in December, and again high in February (Fig. 2.7.). In hens, CH and IH, frequencies of detection were high from August to February 2013 and then there was a decrease in May 2013 (week 61) (Fig. 2.7.). In IH, an opposite tendency was observed again at this time point (May 2013), with an increase in *Salmonella* detection (Fig. 2.6.). This pattern has been observed for *Salmonella* in previous studies where *Salmonella* colonizing ceca reduced as the juvenile animals developed into adults [16, 58]. In general, these observations point to the high complexity of the population dynamics in the intestinal tract colonization and subsequent detection.

2.4.4. Campylobacter and Salmonella in Blood, Organs and Intestinal Samples

Campylobacter and *Salmonella* were not detected in blood or any of the spleens analyzed (Table A.4.). *Salmonella* was not isolated from liver. *C. jejuni* was recovered from a liver-gallbladder of CH only once (Table A.4.). Although organs were aseptically collected before ceca to prevent contamination, samples were further manipulated in the laboratory which could have increased the risk of external cross-contamination. Because *Campylobacter* was never recovered from any other liver-gallbladder samples during the project, likely this was a result of cross-contamination. *Salmonella* and *Campylobacter* were successfully recovered from organs of broilers in previous studies [59–63]. However, differences in colonization kinetics can be attributed to different strains of bacteria, host breed, age, and the time passed after inoculation [64–66]. In this study samples were analyzed 10 weeks after inoculation which may affect the systemic survival of the bacteria.

In the case of intestinal samples, 51 of the 67 ceca analyzed (76.1%) were positive for *Campylobacter*, in contrast with seven (10.4%) positive samples for *Salmonella* (Table 2.9.). Similar rates of cecal colonization by both pathogens were previously described in broilers, however no association was observed between concurrent colonization [67]. The carriage of *Campylobacter* and *Salmonella* in jejuna was lower than in ceca. From the 27 jejuna samples analyzed, seven were positive for *Campylobacter* (25.9%), and one was positive for *Salmonella* (3.7%) (Table 2.9.). Moreover, *S.* Agona was isolated from ceca of IH, ceca of CT and IT and jejunum of CT (Table A.4.).

A significant difference ($p<0.05$) between the carriage rates in ceca and jejuna was evident for *Campylobacter*, but not for *Salmonella*. However, differences in colonization between sex were not found to be significant. Different carriage rates observed between groups for *Salmonella* and *Campylobacter* in ceca and jejuna are represented in table 2.9. These finding suggested that ceca analysis was a good indicator of *Campylobacter* colonization as indicated in previous studies [5, 68]. Regarding the antibiotic resistance profile of intestinal isolates, marker strains of *Campylobacter* were not detected. *C. jejuni* TSKQ was recovered in one cecum from IH, however PFGE analysis showed that it was not the marker strain inoculated into the toms. This isolate will be further characterized by multi-locus sequence typing (MLST) to confirm its identity and discard a possible temperate bacteriophage interference that may had altered the band pattern during PFGE [69, 70]. Naturally occurring *C. coli* K was recovered in seven of seven (100%) ceca samples characterized from IT, three of four (75%) from CT, eight of 16 (50%) from CH, and eight of 21 (38.1%) in IH (Fig. 2.8.). In jejuna samples, *C. coli* K was recovered from one of three

(25%) of jejuna analyzed from IT, but it was not detected in the other groups (Fig. 2.9.). *C. jejuni* T was recovered in 100% of the jejuna isolates from CT and CH, 66.7% from IT and 50% from IH, in contrast with a low frequency of detection in ceca samples with only 9.5% from IH and no detection in ceca from the other groups (Fig. 2.9.). Moreover, 50% and 42.9% of ceca isolates from CH and IH respectively were pan-sensitive *C. jejuni* (Fig. 2.8.). This antibiotic resistance profile was also observed in one isolate from jejunum. However, it was not recovered from any of the ceca or jejuna samples from both tom groups (Fig. 2.8. and 2.9.). The majority of these *C. jejuni* pan-sensitive isolates were detected at the end of the study after the toms were terminated. It was intriguing the low frequency of detection of pan-sensitive strains of *C. jejuni* (detected only in both groups of hens) in fecal samples (Fig. 2.5.), and the higher frequency of detection in ceca later in the study (at 65 weeks) (Fig. 2.8.). This observation could be explain by a low level of colonization by these pan-sensitive strains at the beginning of the study, that could increase over time and reaching higher concentrations in cecal contents, or also due to new contamination events by pan-sensitive strains in the flock.

Poults were inoculated with both organisms at a time to achieve intestinal colonization with both pathogens. The final objective of the second inoculation was to maintain a positive flock all the way through reproductive stages which would allow us to assess vertical transmission of both pathogens from the breeders to their offspring, which will be investigated in a separate analysis of data from this study (Chapter 3 and 4). It was also intended to check the occurrence of extra-intestinal infection, or dissemination and colonization of other organs and tissues such as spleen, liver, gallbladder, and the

reproductive tract. The unbalance design of the project, with different number of birds and pens per group (IH=82, IT=22, CH=28, CT=8), was a limitation for statistical analysis purposes. This was also an impediment for a more extended analysis of organs at the beginning of the project since it was a requirement to keep the flock until reproduction, for assessment of vertical transmission, and it limited the number of samples of spleen, liver and gallbladder, reproductive tract, and intestinal samples analyzed, to evaluate intestinal colonization and dissemination to other organs. These aspects should be considered in future studies, where a balance design will allow a better analysis and comparison between groups and a group size big enough to allow periodic testing by necropsy. Other issues of inoculating both pathogens at the same time are the impossibility of establishing correlations between the infections with every one of the pathogens, or evaluating the effects that colonization by one of them may have in the subsequent colonization by the other. The occurrence of colonization with field strains of *Campylobacter* and *Salmonella*, and the possibility of outcompetition, must be taken into account for next studies. In our project, some of the field *Campylobacter* strains were also isolated from turkeys in previous studies carried out at the same farm, which may indicate a certain endemic nature. The repetitive isolation of the same strains from successive flocks has been previously reported [71]. Field strains can be better adapted to a particular environment, or be more efficient colonizing turkeys than the marker strains used due to host association [54, 72]. *S.* Enteritidis and *S.* Typhimurium, both nalidixic acid resistant, and the *C. coli* GK were originally isolated from chicken sources . Furthermore, genomic attributes associated with host specificity could be

also implicated [73]. Further investigation is needed to understand the ecology and mechanisms of survival of these pathogens in the environment.

2.4.5. Vectors and Other Samples Tested

Both species of *Campylobacter* were isolated from flies in one sample analyzed in October 2012, but not in any other insects analyzed (Table 2.4.). Antibiotic resistance testing showed that the *C. coli* isolate was resistant to kanamycin. *C. jejuni* detected was not further characterized. *Campylobacter* and *Salmonella* were not detected in any feed or drinkers tested. *Salmonella* was isolated from flies *(S.* Agona), and from a mouse caught in the proximity of the control hens pens *(S.* Enteritidis NAL^R) (Table 2.4.).

One difficulty found during the detection of *Campylobacter* from vector samples was the use of enrichment media, which led to the overgrowth of background microflora in the plates. This difficulty can be addressed in the future performing a direct plating of the sample, complementarily to the enrichment step.

2.5. CONCLUSIONS AND APPLICATIONS

1. Monitoring indicated that *Campylobacter* spread more rapidly than *Salmonella* and cross-contaminated turkeys throughout the growout house.
2. Naturally occurring strains of both pathogens seemed to outcompete marker strains after a few weeks and persisted longer in the flock.

3. *Salmonella* showed a reduction in prevalence over time while a high prevalence of *Campylobacter* was observed throughout.
4. Naturally occurring strains of both pathogens were isolated from ceca and jejuna of older breeder hens and toms indicating a persistent reservoir. Moreover, cecal carriage of *Campylobacter* was higher than observed for *Salmonella*.
5. Both pathogens were isolated from several vectors present in the houses confirming that pests and poor biosecurity may contribute to spreading the bacteria.

2.6. ACKNOWLEDGMENTS

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Table 2.1. *Campylobacter* and *Salmonella* strains used for turkey inoculation.

Sex of inoculated turkeys	***Campylobacter* strain and antibiotic profile**	***mL of inoculum gavaged per bird (cfu/mL)***		***Salmonella* serotype (Nalidixic acid resistant)**	***mL of inoculum gavaged (cfu/mL)***	
		First inoculation (10 days)	***Second inoculation (12 weeks)***		***First inoculation (10 days)***	***Second inoculation (12 weeks)***
Toms	*C. jejuni* 10882 TSKQ*	0.1 ($9.2x10^6$)	2 ($1.3x10^8$)	*S.* Typhimurium NAL*	0.1 ($9.4x10^6$)	2 ($5.6 x10^8$)
Hens	*C. coli* 12456 GK*	0.1 ($1.4x10^7$)	2 ($4.8x10^8$)	*S.* Enteritidis NAL*	0.1 ($1.2x10^7$)	2 ($3.6x10^8$)

* T, Tetracycline; S, Streptomycin; K, Kanamycin; Q, Quinolones (Nalidixic acid and Ciprofloxacin); G, Gentamicin; NAL, Nalidixic acid.

Table 2.2. Enumeration of *Campylobacter* from independent fecal samples performed occasionally.

Week	Group	Source	cfu/g levels (approx.)
4	IT	Fecal	1.5×10^7
4	IT	Fecal	7.5×10^6
4	IH	Fecal	3.0×10^7
19	IH	Fecal	5.7×10^5
19	IH	Fecal	9.4×10^7
19	IH	Fecal	1.3×10^5
19	IH	Fecal	8.8×10^5
19	IH	Fecal	1.5×10^6
19	IH	Fecal	3.0×10^6
19	IH	Fecal	1.3×10^6
19	IH	Fecal	4.2×10^3
19	IH	Fecal	2.8×10^5
19	IH	Fecal	1.3×10^5
19	IH	Fecal	9.6×10^5
19	IH	Fecal	4.0×10^6
19	IT	Fecal	1.1×10^5
19	IT	Fecal	9.0×10^4
19	IT	Fecal	3.0×10^5
47	CH	Fecal	1.9×10^5
47	CH	Fecal	6.0×10^2
47	CH	Fecal	$<1.0 \times 10^2$
47	IH	Fecal	2.6×10^5
47	IH	Fecal	1.3×10^5
47	IT	Fecal	1.5×10^2
61	CH	Fecal	1.0×10^4

IT, Inoculated Toms; IH, Inoculated Hens, CH, Control Hens.

Table 2.3. Number of blood, organs and intestinal samples analyzed at different time points during the project from the different groups of turkeys.

	June (2012)		August (2012)		September (2012)	November (2012)		December (2012)		March (2013)			June (2013)		
	week 11		**week 20**		**week 25**	**week 33**		**week 38**		**week 50**			**week 65**		
	IH	IT	CH	IH	IT	CT	IT	CT	IT	IH	CT	IT	IH	CH	Total
Blood	9	3	2	2	-	-	-	-	-	-	-	-	-	-	16
Ceca	3	1	2	2	2	1	2	3	2	5	2	2	20	20	67
Jejuna	3	1	2	2	2	1	2	3	2	5	2	2	-	-	27
Spleen	3	1	2	2	2	1	2	-	-	-	-	-	-	-	13
Liver-Gallbladder	3	1	2	2	2	1	2	3	2	5	2	-	-	-	25

IH, Inoculated Hens; IT, Inoculated Toms; CH, Control Hens; CT, Control Toms.

Table 2.4. Vectors and other environmental samples analyzed during the project.

Sampling Date	Vectors-Environmental Sample	*Campylobacter* and/or *Salmonella* Detected
Mar12	Feed (25g), drinkers (2 swabs/drinker/pen), wood shavings	Negative
May12	Flies (10), feed (25g)	*Salmonella* (flies)
Sep12	Flies (5), cricket (1), roaches (3)	Negative
Oct12	Roaches (4), cricket (3), camel crickets (3)	Negative
Oct12	Flies (1), roaches (7), mouse (1), camel crickets (3)	Negative
Oct12	Flies (5), roaches (11), spiders (2), feed (25g), drinkers (2 swabs/drinker/pen)	*Campylobacter* (flies)
Feb13	Feed (25g), drinkers (2 swabs/drinker/pen), wood shavings	Negative
Mar13	Mouse (1)	*Salmonella*
Jun13	Flies (5), roaches (5)	Negative
Jun13	Flies (6), roaches (4)	Negative

Table 2.5. Minimum inhibitory concentration of antibiotics tested for *Campylobacter* isolates.

Antibiotic	Concentration (µg/mL)
Kanamycin[1]	25
Tetracycline[2]	16
Erythromycin[2]	10
Streptomycin[2]	15
Nalidixic Acid[1]	20
Ciprofloxacin[1]	4
Gentamicin[1]	200

[1] [26]; [2] [74].

Table 2.6. Marker and naturally occurring *Salmonella* recovered from fecal samples analyzed from the different groups of turkeys (hens and toms, control and inoculated) from week 3 (April 2012) to week 61 (May 2013).

Group	Fecal samples	POS (%)	Marker (% of isolates from positive samples)	Naturally Occurring (susceptible to NAL*) (% of isolates from positive samples)
CT	20	14 (70.0)	0	14 (100)
CH	36	21 (58.3)	0 (0)	21 (100)
IH	96	50 (52.1)	31 (62)	19 (38)
IT	32	17 (53.1)	14 (82.4)	3 (17.6)
Total	184	102 (55.4)	45 (44.1)	57 (55.9)

*NAL, nalidixic acid 200ppm.
CT, Control Toms; CH, Control Hens; IH, Inoculated Hens; IT, Inoculated Toms.

Table 2.7. Naturally occurring serotypes of *Salmonella* isolated from fecal samples.

	***S.* Agona**	***S.* Typhimurium**	***S.* Liverpool**
Number of isolates	26	2	1
% of isolates from serotyped samples (n=29)	89.7	6.9	3.4

Table 2.8. Number of fecal isolates analyzed for *Campylobacter*, positive isolates, species (*C. coli*, *C. jejuni*), isolates where species and antibiotic resistance profile were not characterized (NC), and frequencies from fecal samples distributed by sex and group.

	CT	**CH**	**IH**	**IT**	**Total**
Number of samples analyzed per group (% of isolates from the total 200 samples)	27 (13.5)	38 (19.0)	99 (49.5)	36 (18.0)	200
Campylobacter-positive samples (% of isolates from the total samples analyzed/group)	19 (70.4)	27 (71.1)	75 (75.8)	23 (63.9)	144 (72)
C. coli (% of isolates from the total speciated isolates)	11 (68.8)	9 (39.1)	30 (40.0)	7 (30.4)	57 (43.8)
C. jejuni (% of isolates from the total speciated isolates)	5 (31.3)	14 (60.9)	38 (50.7)	16 (69.6)	73 (56.2)
Isolates not further characterized (% of isolates from the positive samples)	3 (15.8)	4 (14.8)	7 (9.3)	0 (0)	14 (9.7)
C. coli GK (marker inoculated into IH) (% of isolates from the total *C. coli*)	4 (36.4)	1 (11.1)	14 (46.7)	2 (28.6)	21 (36.8)
C. jejuni TSKQ (marker inoculated into IT) (% of isolates from the total *C. jejuni*)	3 (60.0)	3 (21.4)	20 (52.6)	12 (75.0)	38 (52.1)
Total marker strains (% of isolates from the total speciated samples)	7 (43.8)	4 (17.4)	34 (50.0)	14 (60.9)	59 (45.4)

CT, Control Toms; CH, Control Hens; IH, Inoculated Hens; IT, Inoculated Toms.

Table 2.9. *Campylobacter* positive samples from the total number of jejuna and ceca analyzed per group of turkeys and % of isolates from total samples analyzed (in parenthesis).

	CT	**CH**	**IH**	**IT**	**Positive/Total analyzed (%)**
Campylobacter Cecum	6/6 (100)	16/22 (72.7)	21/30 (70.0)	8/9 (88.9)	51/67 (76.1)
Campylobacter Jejunum	1/6 (16.7)	1/2 (50.0)	2/10 (20.0)	3/9 (33.3)	7/27 (25.9)
Salmonella Cecum	2/6 (33.3)	0/22 (0)	4/30 (13.3)	1/9 (11.1)	7/67 (10.4)
Salmonella Jejunum	1/6 (16.7)	0/2 (0)	0/10 (0)	0/9 (0)	1/27 (3.7)

CT, Control Toms; CH, Control Hens; IH, Inoculated Hens; IT, Inoculated Toms.

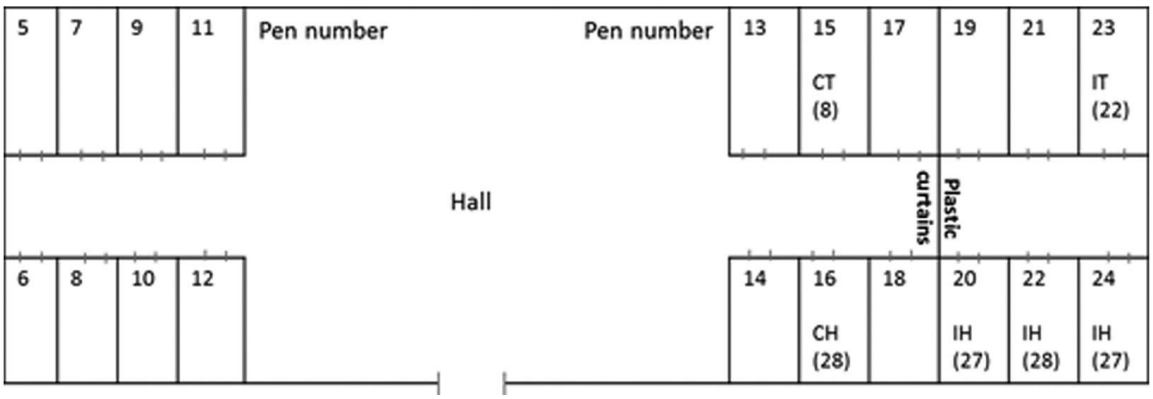

Fig. 2.1. Distribution of pens of Control and Inoculated turkeys in the growout house from week 1 to week 11. In parenthesis is indicated the number of turkeys per pen.
CT, Control Toms; CH, Control Hens; IH, Inoculated Hens; IT, Inoculated Toms.

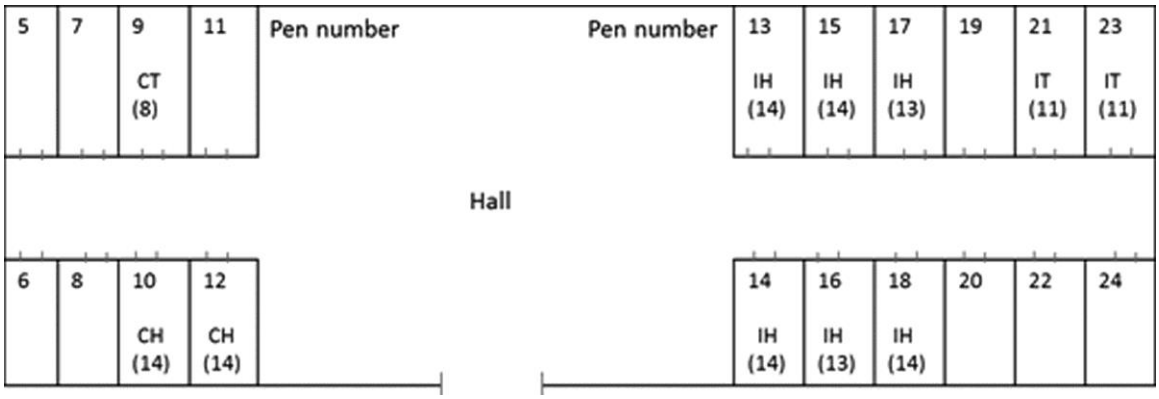

Fig. 2.2. Distribution of pens of Control and Inoculated turkeys in the growout house at week 12. In parenthesis is indicated the number of turkeys per pen. CT, Control Toms; CH, Control Hens; IH, Inoculated Hens; IT, Inoculated Toms.

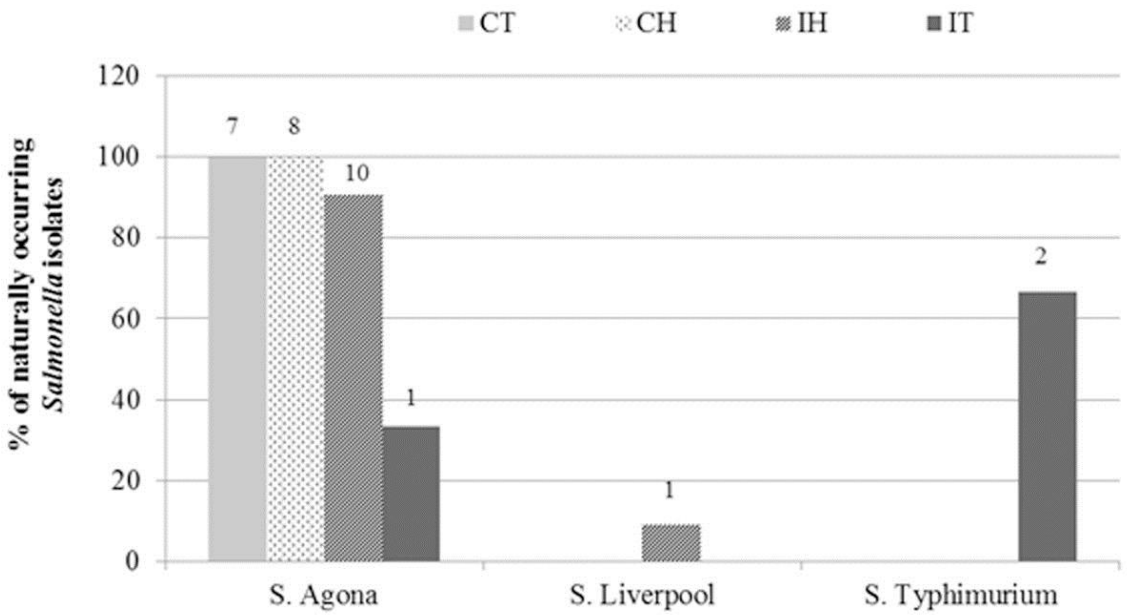

Fig. 2.3. Naturally occurring *Salmonella* serotypes isolated from fecal samples of each group of breeder turkeys. Relative frequencies were calculated from the total number of naturally occurring isolates per group (CT=7, CH=8, IH=11, IT=3). CT, Control Toms; CH, Control Hens; IH, Inoculated Hens; IT, Inoculated Toms.

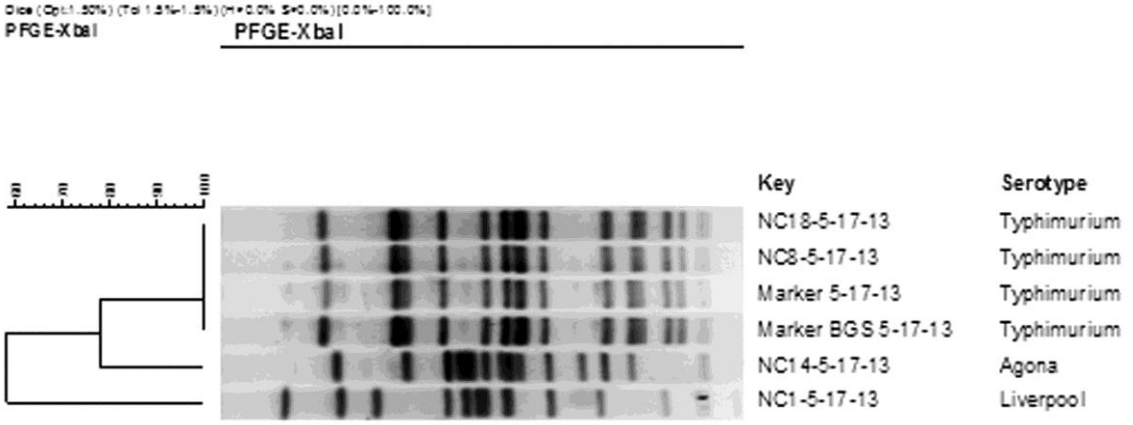

Fig. 2.4. PFGE results showing the band pattern of *S*. Typhimurium marker strains (Marker), the two nalidixic acid sensitive *S*. Typhimurium isolates (NC18-5-17-13 and NC8-5-17-13), and other two isolates (*S*. Agona and *S*. Liverpool). PFGE performed at the Russell Research Center (USDA-ARS), Athens, GA.

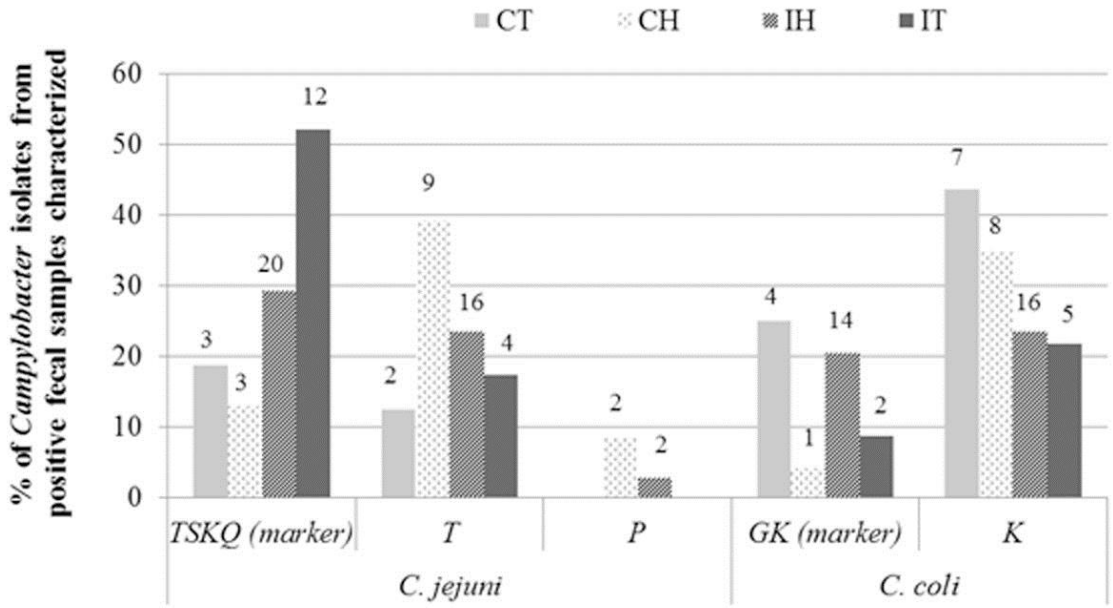

Fig. 2.5. *Campylobacter* species and antibiotic susceptibility profile of isolates from fecal samples. Frequencies were calculated based on the total positive isolates characterized (species and antibiotic susceptibility profile) per group (CT=16, CH=23, IH=68, IT=23). CT, Control Toms; CH, Control Hens; IH, Inoculated Hens; IT, Inoculated Toms. T, Tetracycline; S, Streptomycin; K, Kanamycin; Q, Quinolones (Nalidixic acid and Ciprofloxacin); G, Gentamicin; P, Pan-sensitive; NC, Not characterized.

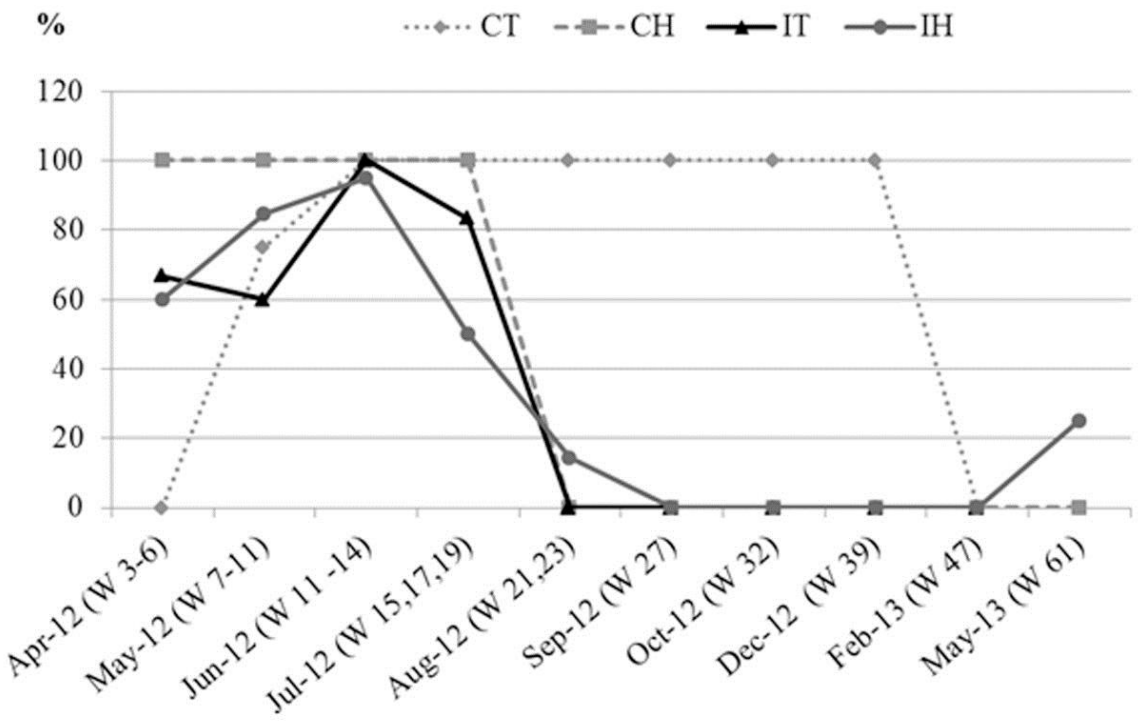

Fig. 2.6. Relative frequencies of detection of *Salmonella* in fecal samples on the different groups of turkeys. CT, Control Toms; CH, Control Hens; IT, Inoculated Toms; IH, Inoculated Hens.

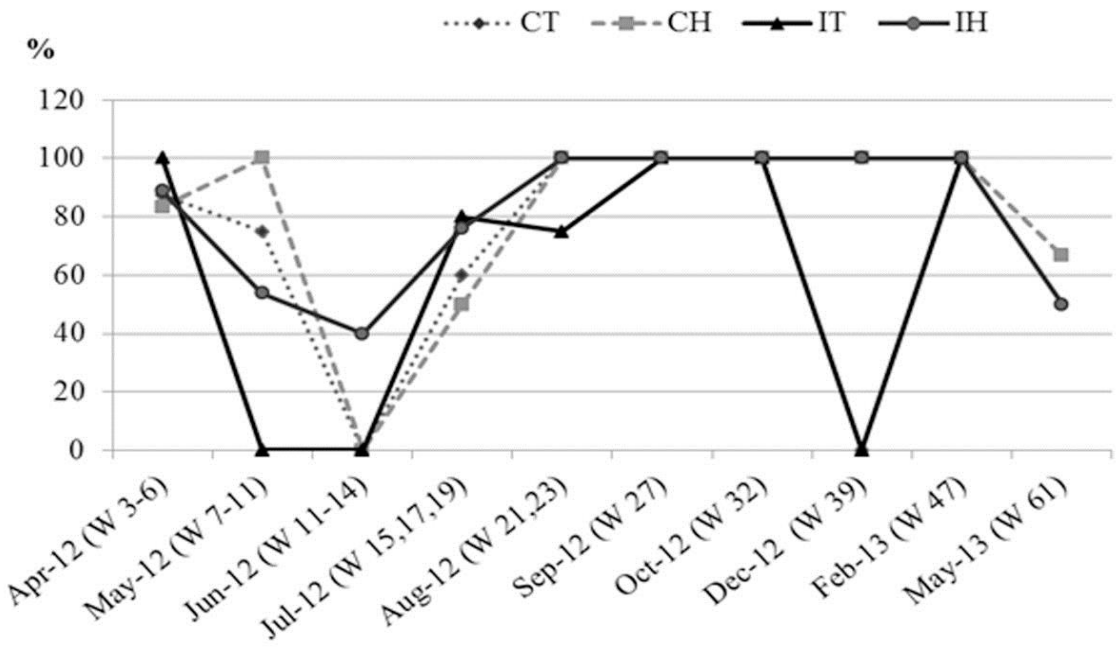

Fig. 2.7. Relative frequencies of detection of *Campylobacter* in fecal samples on the different groups of turkeys. Fecal samples were analyzed from April 2012 to May 2013. CT, Control Toms; CH, Control Hens; IT, Inoculated Toms; IH, Inoculated Hens.

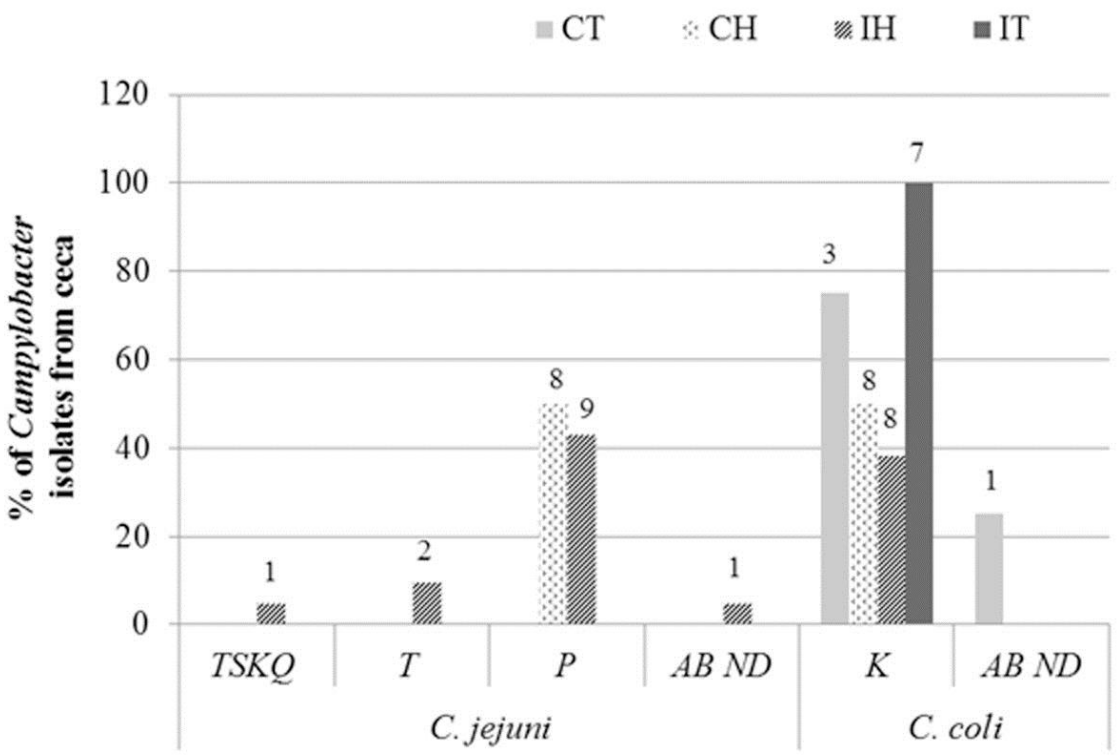

Fig. 2.8. Species and antibiotic resistance profile of *Campylobacter* isolates from ceca. Relative frequencies were calculated from the total positive characterized cecal samples per group (CT=4, CH=16, IH=21, IT=7). CT, Control Toms; CH, Control Hens; IH, Inoculated Hens; IT, Inoculated Toms. AB ND, Antibiotic susceptibility not determined. T, Tetracycline; S, Streptomycin; K, Kanamycin; Q, Quinolones (Nalidixic acid and Ciprofloxacin); G, Gentamicin; P, Pan-sensitive.

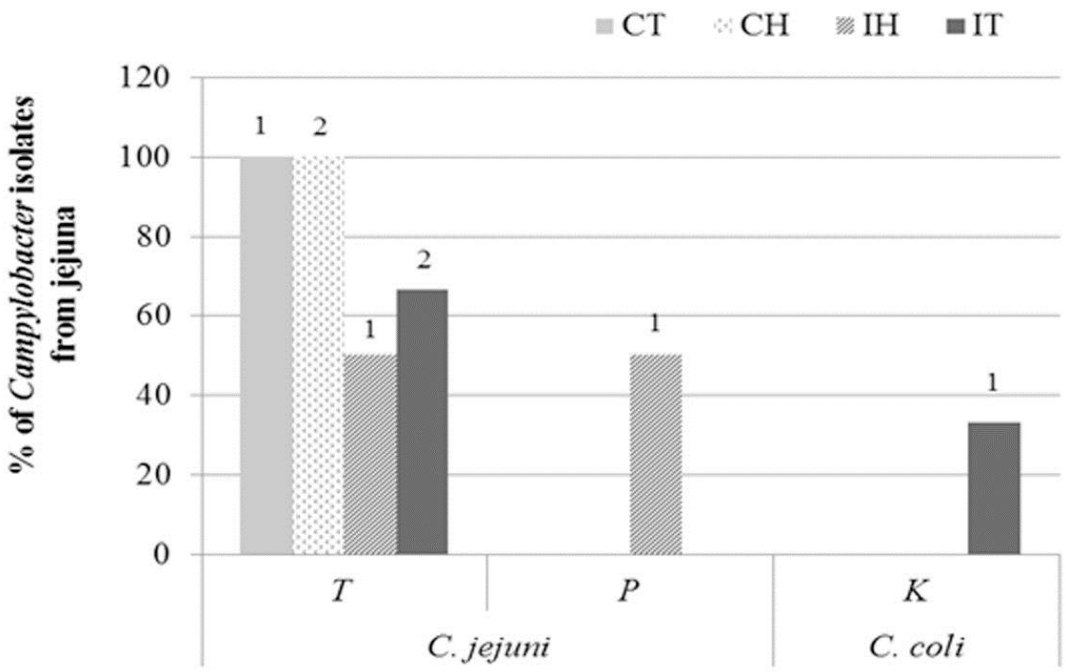

Fig. 2.9. Species and antibiotic resistance profile of *Campylobacter* isolated from jejunum. Relative frequencies were calculated from the total positive samples characterized per group (CT=1, CH=2, IH=2, IT=3). CT, Control Toms; CH, Control Hens; IH, Inoculated Hens; IT, Inoculated Toms. T, Tetracycline; K, Kanamycin; P, Pan-sensitive.

2.7. REFERENCES AND NOTES

1. Schlundt, J., H. Toyofuku, J. Jansen, and S.A. Herbst. 2004. Emerging food-borne zoonoses. Rev. Sci. Tech. 23: 513–533.

2. Skovgaard, N. 2007. New trends in emerging pathogens. Int. J. Food Microbiol. 120: 217–224.

3. Scallan, E., R.M. Hoekstra, F.J. Angulo, R. V. Tauxe, M.A. Widdowson, S.L. Roy, J.L. Jones, and P.M. Griffin. 2011. Foodborne illness acquired in the United States — Major pathogens. Emerg. Infect. Dis. 17: 1–21.

4. Wingstrand, A., J. Neimann, J. Engberg, E.M. Nielsen, P. Gerner-Smidt, H.C. Wegener, and K. Mølbak. 2006. Fresh chicken as main risk factor. Emerg Infect Dis. 12: 280–284.

5. Wallace, J.S., K.N. Stanley, and K. Jones. 1998. The colonization of turkeys by thermophilic campylobacters. J. Appl. Microbiol. 85: 224–230.

6. Corry, J.E., and H.I. Atabay. 2001. Poultry as a source of *Campylobacter* and related organisms. Symp. Ser. Soc. Appl. Microbiol. : 96S–114S.

7. Johannessen, G.S., G. Johnsen, M. Okland, K.S. Cudjoe, and M. Hofshagen. 2007. Enumeration of thermotolerant *Campylobacter* spp. from poultry carcasses at the end of the slaughter-line. Lett. Appl. Microbiol. 44: 92–97.

8. Smith, D.P., J.K. Northcutt, J.A. Cason, A. Hinton, R.J. Buhr, and K.D. Ingram. 2007. Effect of external or internal fecal contamination on numbers of bacteria on prechilled broiler carcasses. Poult. Sci. 86: 1241–1244.

9. Hue, O., V. Allain, M.J. Laisney, S. Le Bouquin, F. Lalande, I. Petetin, S. Rouxel, S. Quesne, P.-Y. Gloaguen, M. Picherot, J. Santolini, S. Bougeard, G. Salvat, and M. Chemaly. 2011. *Campylobacter* contamination of broiler caeca and carcasses at the slaughterhouse and correlation with *Salmonella* contamination. Food Microbiol. 28: 862–868.

10. Corry, J.E.L., V.M. Allen, W.R. Hudson, M.F. Breslin, and R.H. Davies. 2002. Sources of *Salmonella* on broiler carcasses during transportation and processing: Modes of contamination and methods of control. J. Appl. Microbiol. 92: 424–432.

11. Berrang, M.E., D.P. Smith, W.R. Windham, and P.W. Feldner. 2004. Effect of intestinal content contamination on broiler carcass *Campylobacter* counts. J. Food Prot. 67: 235–238.

12. Berrang, M.E., R.J. Buhr, J.A. Cason, and J.A. Dickens. 2001. Broiler carcass contamination with *Campylobacter* from feces during defeathering. J. Food Prot. 64: 2063–2066.

13. Heyndrickx, M., D. Vandekerchove, L. Herman, I. Rollier, K. Grijspeerdt, and L. De Zutter. 2002. Routes for *Salmonella* contamination of poultry meat: Epidemiological study from hatchery to slaughterhouse. Epidemiol. Infect. 129: 253–265.

14. Federal Register Notice, Vol. 75, No. 93, 27288, May 14, 2010 (Docket No. FSIS-2009-0034), "New Performance Standards for *Salmonella* and *Campylobacter* in Young Chicken and Turkey Slaughter Establishments".

15. Pearson, A.D., M.H. Greenwood, R.K. Feltham, T.D. Healing, J. Donaldson, D.M. Jones, and R.R. Colwell. 1996. Microbial ecology of *Campylobacter jejuni* in a United Kingdom chicken supply chain: intermittent common source, vertical transmission, and amplification by flock propagation. Appl. Environ. Microbiol. 62: 4614–4620.

16. Jacobs-Reitsma, W.F., N.M. Bolder, and R.W.A.W. Mulder. 1994. Cecal carriage of *Campylobacter* and *Salmonella* in Dutch broiler flocks at slaughter: A one-year study. Poult. Sci. 73: 1260–1266.

17. Pearson, A.D., M. Greenwood, T.D. Healing, D. Rollins, M. Shahamat, J. Donaldson, and R.R. Colwell. 1993. Colonization of broiler chickens by waterborne *Campylobacter jejuni*. Appl. Environ. Microbiol. 59: 987–996.

18. Hiett, K.L., N.J. Stern, N.A. Cox, M.T. Musgrove, and S. Ladely. 2002. Molecular subtype analyses of *Campylobacter* spp. from Arkansas and California poultry operations. Appl. Environ. Microbiol. 68: 6220–6236.

19. Cox, N.A., L.J. Richardson, J.J. Maurer, M.E. Berrang, P.J. Fedorka-Cray, R.J. Buhr, J.A. Byrd, M.D. Lee, C.L. Hofacre, P.M. O'Kane, A.M. Lammerding, A.G. Clark, S.G. Thayer, and M.P. Doyle. 2012. Evidence for horizontal and vertical transmission in *Campylobacter* passage from hen to her progeny. J. Food Prot. 75: 1896–1902.

20. Jacobs-Reitsma, W.F., A.W. van de Giessen, N.M. Bolder, and R.W. Mulder. 1995. Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. Epidemiol. Infect. 114: 413–421.

21. Aviagen Turkeys, Inc. Lewisburg, West Virginia, USA.

22. Turkey Research Unit (TEU). 4601 Mid Pines Road, Raleigh, NC (NCSU).

23. Virkon®S. Dupont. Distributed by Neogen, Lexington, KY.

24. Sahin, O., Q. Zhang, J.C. Meitzler, B.S. Harr, T.Y. Morishita, and R. Mohan. 2001. Prevalence, antigenic specificity, and bactericidal activity of poultry anti-*Campylobacter* maternal antibodies. Appl. Environ. Microbiol. 67: 3951–3957.

25. Cox, N.A., L.J. Richardson, M.E. Berrang, R.J. Fedorka-Cray, and R.J. Buhr. 2009. *Campylobacter coli* naturally resistant to elevated levels of gentamicin as a marker strain in poultry research. J. Food Prot. 72: 1288–1292.

26. Fisher Biotech, Fair Lawn, NJ.

27. Tork Model 1101. Tork, Mt. Vernon, NY.

28. BD and company Sparks, MD.

29. Oxoid Ltd, Hampshire, England.

30. Airgas, Durham, NC.

31. Acumedia, Neogen, Lansing, MI.

32. Hardy Diagnostics, Santa Maria, CA.

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

34. Musgrove, M.T., M.E. Berrang, J.A. Byrd, N.J. Stern, and N.A. Cox. 2001. Detection of *Campylobacter* spp. in ceca and crops with and without enrichment. Poult. Sci. 80: 825–828.

35. Rodgers, J.D., F.A. Clifton-Hadley, C. Marin, and A.B. Vidal. 2010. An evaluation of survival and detection of *Campylobacter jejuni* and *C. coli* in broiler caecal contents using culture-based methods. J. Appl. Microbiol. 109: 1244–1252.

36. Daczkowska-Kozon, E.G., and K.S. Wojciech Sawicki. 2010. The caeca – niche supporting survival of *Campylobacter* spp. in commercially reared broiler chickens. Polish J. food Nutr. Sci. 60: 265–271.

37. Thermo Fisher Scientific, Remel Products, Lenexa, Kansas.

38. Mouse and insect glue trap. J.T. Eaton & Co., Inc. Twinsburg, OH.

39. Houng, H.S., O. Sethabutr, W. Nirdnoy, D.E. Katz, and L.W. Pang. 2001. Development of a ceuE-based multiplex polymerase chain reaction (PCR) assay for direct detection and

differentiation of *Campylobacter jejuni* and *Campylobacter coli* in Thailand. Diagn. Microbiol. Infect. Dis. 40: 11–19.

40. Marshall, S.M., P.L. Melito, D.L. Woodward, W.M. Johnson, F.G. Rodgers, and M.R. Mulvey. 1999. Rapid idenntification of *Campylobacter, Arcobacter,* and *Helicobacter* isolates by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene. J. Clin. Microbiol. 37: 4158–4160.

41. Gonzalez, I., K.A. Grant, P.T. Richardson, S.F. Park, and M.D. Collins. 1997. Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the ceuE gene encoding a putative virulence determinant. J. Clin. Microbiol. 35: 759–763.

42. Leader, B.T., J.G. Frye, J. Hu, P.J. Fedorka-Cray, and D.S. Boyle. 2009. High-throughput molecular determination of *Salmonella enterica* serovars by use of multiplex PCR and capillary electrophoresis analysis. J. Clin. Microbiol. 47: 1290–1299.

43. American Type Culture Collection.

44. New England BioLabs Inc.

45. Roche Diagnostics GmbH, Roche Applied Science, 68298 Mannheim, Germany.

46. JMP®, Version 11. SAS Institute Inc., Cary, NC.

47. BioNumerics, version 4.6, Applied Maths, Saint-Marten-Latem, Belgium.

48. Eaves, D.J., L. Randall, D.T. Gray, A. Buckley, M.J. Woodward, A.P. White, and L.J. V Piddock. 2004. Prevalence of mutations within the quinolone resistance-determining region of gyrA , gyrB , parC , and parE and association with antibiotic resistance in quinolone-resistant *Salmonella enterica*. Antimicrob. Agents Chemother. 48: 4012–4015.

49. Piddock, L.J. V, V. Ricci, I. McLaren, and D.J. Griggs. 1998. Role of mutation in the gyrA and parC genes of nalidixic-acid-resistant *Salmonella* serotypes isolated from animals in the United Kingdom. J. Antimicrob. Chemother. 41: 635–641.

50. Jacoby, G.A. 2005. Mechanisms of resistance to quinolones. Clin. Infect. Dis. 41 (Suppl : S120–S126.

51. Gunell, M., M.A. Webber, P. Kotilainen, A.J. Lilly, J.M. Caddick, J. Jalava, P. Huovinen, A. Siitonen, A.J. Hakanen, and L.J. V Piddock. 2009. Mechanisms of resistance in nontyphoidal *Salmonella enterica* strains exhibiting a nonclassical quinolone resistance phenotype. Antimicrob. Agents Chemother. 53: 3832–3836.

52. Foley, S.L., A.M. Lynne, and R. Nayak. 2008. *Salmonella* challenges : Prevalence in swine and poultry and potential pathogenicity of such isolates. J. Anim. Sci. 86: E149–E162.

53. Irwin, R.J., C. Poppe, S. Messier, G.G. Finley, and J. Oggel. 1994. A national survey to estimate the prevalence of *Salmonella* species among Canadian registered commercial turkey flocks. Can. J. Vet. Res. 58: 263–267.

54. Sheppard, S.K., F. Colles, J. Richardson, A.J. Cody, R. Elson, A. Lawson, G. Brick, R. Meldrum, C.L. Little, R.J. Owen, M.C.J. Maiden, and N.D. McCarthy. 2010. Host association of *Campylobacter* genotypes transcends geographic variations. Appl. Environ. Microbiol. 76: 5269–5277.

55. Petersen, L. and A.W. 2001. Evidence that certain clones of *Campylobacter jejuni* persist during successive broiler flock rotations. 67: 2739–2745.

56. Kudirkiene, E., M. Malakauskas, A. Malakauskas, a M. Bojesen, and J.E. Olsen. 2010. Demonstration of persistent strains of *Campylobacter jejuni* within broiler farms over a 1-year period in Lithuania. J. Appl. Microbiol. 108: 868–877.

57. Luangtongkum, T., T.Y. Morishita, A.J. Ison, S. Huang, P.F. McDermott, and Q. Zhang. 2006. Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. Appl. Environ. Microbiol. 72: 3600–3607.

58. Wesley, I. V., and W.T. Muraoka. 2009. Time of entry of *Salmonella* and *Campylobacter* into the turkey brooder house. Food Bioprocess Technol. 4: 616–623.

59. Cox, N.A., L.J. Richardson, R.J. Buhr, and P.J. Fedorka-Cray. 2009. *Campylobacter* species occurrence within internal organs and tissues of commercial caged Leghorn laying hens. Poult. Sci. 88: 2449–2456.

60. Richardson, L.J., N.A. Cox, R.J. Buhr, and M.A. Harrison. 2011. Isolation of *Campylobacter* from circulating blood of commercial broilers. Avian Dis. 55: 375–378.

61. Cox, N.A., L.J. Richardson, R.J. Buhr, J.K. Northcutt, J.S. Bailey, P.F. Cray, and K.L. Hiett. 2007. Recovery of *Campylobacter* and *Salmonella* serovars from the spleen, liver and gallbladder, and ceca of six-and eight-week-old commercial broilers. J. Appl. Poult. Res. 16: 477–480.

62. Cox, N.A., C.L. Hofacre, J.S. Bailey, R.J. Buhr, J.L. Wilson, K.L. Hiett, L.J. Richardson, M.T. Musgrove, D.E. Cosby, J.D. Tankson, Y.L. Vizzier, P.F. Cray, L.E. Vaughn, P.S. Holt, and D. V Bourassaa. 2005. Presence of *Campylobacter jejuni* in various organs one hour, one

day, and one week following oral or intracloacal inoculations of broiler chicks. Avian Dis. 49: 155–158.

63. Cox, N.A., L.J. Richardson, R.J. Buhr, J.S. Bailey, J.L. Wilson, and K.L. Hiett. 2002. Detection of *Campylobacter jejuni* in various lymphoid organs of broiler breeder hens after oral or intravaginal inoculation. Poult. Sci. 85: 1378–1382.

64. Newell, D.G., and C. Fearnley. 2003. Sources of *Campylobacter* colonization in broiler chickens. Appl. Environ. Microbiol. 69: 4343–4351.

65. Berchieri, A., P. Wigley, K. Page, C.K. Murphy, and P.A. Barrow. 2001. Further studies on vertical transmission and persistence of *Salmonella enterica* serovar Enteritidis phage type 4 in chickens. Avian Pathol. 30: 297–310.

66. Gast, R.K., and P.S. Holt. 1998. Persistence of *Salmonella enteritidis* from one day of age until maturity in experimentally infected layer chickens. Poult. Sci. 77: 1759–1762.

67. Rasschaert, G., K. Houf, J. Van Hende, and L. De Zutter. 2007. Investigation of the concurrent colonization with *Campylobacter* and *Salmonella* in poultry flocks and assessment of the sampling site for status determination at slaughter. Vet. Microbiol. 123: 104–109.

68. Wallace, J.S., K.N. Stanley, J.E. Currie, P.J. Diggle, and K. Jones. 1997. Seasonality of thermophilic *Campylobacter* populations in chickens. J. Appl. Microbiol. 82: 219–224.

69. Barton, C., L.-K. Ng, S.D. Tyler, and C.G. Clark. 2007. Temperate bacteriophages affect pulsed-field gel electrophoresis patterns of *Campylobacter jejuni*. J. Clin. Microbiol. 45: 386–391.

70. Gibson, J., E. Lorenz, and R.J. Owen. 1997. Lineages within *Campylobacter jejuni* defined by numerical analysis of pulsed-field gel electrophoretic DNA profiles. J. Med. Microbiol. 46: 157–163.

71. Lee, B.C., N. Reimers, H.J. Barnes, C. D'Lima, D. Carver, and S. Kathariou. 2005. Strain persistence and fluctuation of multiple-antibiotic resistant *Campylobacter coli* colonizing turkeys over successive production cycles. Foodborne Pathog. Dis. 2: 103–110.

72. Rabsch, W., H.L. Andrews, R.A. Kingsley, R. Prager, H. Tschäpe, L.G. Adams, and A.J. Bäumler. 2002. *Salmonella enterica* serotype Typhimurium and its host-adapted variants. Infect. Immun. 70: 2249–2255.

73. Crespo-Rodriguez, M.D., E. Altermann, J. Olson, R.M. Siletzky, and S. Kathariou. 2012. Genomic attributes associated with host adaptation in *Campylobacter jejuni* and

Campylobacter coli from poultry and swine (P1-83). Poster presented at the Annual meeting of the International Association of Food Protection, Jul 23, 2012, Providence, RI .

74. Sigma Chemical Co., St. Louis, MO.

CHAPTER III

EVALUATION OF VERTICAL TRANSMISSION OF *SALMONELLA* AND *CAMPYLOBACTER* IN BREEDER TURKEYS

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Key words: *Salmonella*, *Campylobacter*, vertical transmission, breeder turkeys, hatching eggs.

Primary audience: researchers, flock supervisors, veterinarians.

Publication status: To be submitted to Journal of Applied Poultry Research.

3.1. SUMMARY

Salmonella and *Campylobacter* are pathogens of concern frequently associated with poultry products. Pre-harvest control in breeder flocks and their offspring is crucial to reduce the transmission of these bacteria. However, the direct vertical transmission of these organisms through fertile turkey eggs to the offspring has not been demonstrated. The purpose of this study was to determine if *Campylobacter* and *Salmonella* was transmitted through eggs and offspring of turkeys after artificial inoculation of breeder hens with semen containing marker strains of both bacteria (inoculated hens), as compared to uninoculated control hens. Eggs were collected and eggshells and yolks were checked for the presence of marker strains of *Salmonella* and *Campylobacter*. Eggs from both groups of hens were also incubated. At day of hatch, the number of hatched and unhatched eggs was recorded. Swabs from the hatchery trays with eggshells and fecal residues were taken, and paper pads with fecal droppings from both groups of poults, from inoculated and control hens, were analyzed. Reproductive tract (RT) segments, including ovary and ovarian follicles, and upper level of the tract (from infundibulum to isthmus), of hens from both breeder flocks were analyzed at 65 weeks. Semen and testes from toms were also analyzed. No evidence of vertical transmission of these pathogens through artificial insemination was demonstrated. However, *Salmonella* and *Campylobacter* were found in RT of hens and semen from toms, indicating a potential route of transmission of these pathogens to the offspring.

3.2. DESCRIPTION OF PROBLEM

Salmonella and *Campylobacter* are leading causes of gastroenteritis worldwide and both are frequently found colonizing the intestinal tract of poultry. They have also been isolated from the avian reproductive tract indicating a possible source of contamination of hatching eggs and subsequent transmission to the offspring [1–5]. Furthermore, the isolation of *Salmonella* and *Campylobacter spp.* from semen may suggest the possibility of transmission to the reproductive tract of hens via insemination [4, 6, 7]. Even though the outer and inner shell membranes of eggs can offer protection against bacterial penetration, several studies have demonstrated a rapid penetration of *Salmonella* into the egg through pores or cracks in the shell [8–11]. Sanitation of hatching eggs will not offer any protection to the developing embryo if bacteria have penetrated to the internal contents [12]. Furthermore, warm incubation temperatures may enhance the multiplication of *Salmonella*. Although *Salmonella* is not found in high frequencies in eggs yolks in naturally contaminated eggs, the nutrient environment in the yolk is favorable for bacterial growth [9]. The vertical transmission of *Campylobacter*, through ovaries or oviduct to the egg, remains controversial. Although vertical transmission has been considered as a potential source of contamination to the offspring by some authors [4, 13, 14], it has been reported by others that transmission of *Campylobacter* through the eggs is a rare and unlikely event [15–17]. Nevertheless, there is little research concerning the mechanism of transmission of these pathogens in turkey hatching eggs. The present study was part of a project where a turkey breeder flock was monitored from day one to week 65 of life and evaluated for transmission routes of

Salmonella and *Campylobacter*. The current study was performed during the reproductive cycle of breeder turkeys, between weeks 32 to 65. The objectives of this research were to evaluate vertical transmission of both pathogens to eggs and offspring, and to evaluate any negative effects due to the presence of these pathogens with reference to egg fertility and hatchability.

3.3. MATERIALS AND METHODS

3.3.1. Artificial Insemination and Egg Analysis

A nalidixic acid-resistant strain of *Salmonella enterica* serovar Enteritidis and a gentamicin resistant strain of *Campylobacter coli* [18] were used to inoculate 80 breeder hens via semen through artificial insemination. These inoculated hens (IH) were orally gavaged with the same marker strains at the beginning of the study, at 10 days and 12 weeks (Chapter 2). A 0.5 mL aliquot of each bacterial culture, *S.* Enteritidis (approx. 10^8 cfu/mL) and *C. coli* (approx. 10^7 cfu/mL) were added to 2 mL of semen pooled from several inoculated toms (IT). IT were initially orally inoculated with marker strains of *S*. Typhimurium and *C. jejuni* (Chapter 2). A control flock of 20 hens, located in a different area of the same turkey house, was also inseminated using non inoculated semen, from the control toms (CT), that was diluted with Minnesota Turkey Growers Association (MTGA) semen extender containing gentamicin [19], added in the same ratio 1:2 (v/v). Prior to the insemination of IH, adding marker strains of *Salmonella* and *Campylobacter* into the semen, both groups of hens were inseminated twice (week 35). IH were artificially inseminated a total of four times using the

semen containing both marker strains at weeks 39, 40, 42, and 43. Insemination was performed with insemination styrene tubes of 10.3 cm in length and 0.208 cm of inside diameter [20], filled with approximately 0.035 mL of semen. Prior to the beginning of inseminations using the pathogens, a set of six eggs from CH and 20 eggs from IH was analyzed (eggs from both groups were unwashed). Eggs were collected every day by the farm crew who identified the pen number and date on every egg with a marker. Eggs were kept in a cool room (12.8°C) until the day of analysis. Eggs were analyzed in sets of six eggs from CH, and 20 unwashed eggs from IH. Only one set of 20 washed eggs from IH was analyzed due to lack of eggs, and two further sets of 16 eggs were used (Table 3.1.). A total of 24 eggs from CH, and 142 eggs from IH were analyzed (90 unwashed and 52 sanitized with a quaternary ammonia product following manufacturer's dosage instructions [21]) (Table 3.1.).

A shell crush method as described by Musgrove et al., 2005 was used for the bacterial recovery from the eggshells and yolks [22]. Eggs were cracked on the edge of a sterile beaker, egg whites were discarded and egg yolks pooled in a sterile beaker. The interior of the eggshell was rinsed with 20 mL of phosphate-buffered saline solution (PBS) [23] to eliminate residual egg whites. Eggshell and adhering membranes were crushed for one minute using a sterile glass rod in a sterile 50 mL centrifuge tube containing 20 mL of PBS. The sample was then divided in two sub-samples of 10 mL each for investigation of both pathogens. Buffered peptone water 2% (BPW) [24] was added for preenrichment of *Salmonella*, and 2X Bolton broth (BB) (with supplements and laked horse blood added) [24], for the selective enrichment of *Campylobacter*. The crushed shells and membranes were also

distributed in the two sub-samples. The pooled yolks were initially diluted with PBS (1:1 v/v), homogenized and divided in two sub-samples. The procedure was then the same as for eggshells. BPW samples were incubated at 37°C for 24 hours, and BB samples were incubated at 42°C for 24-48 hours under microaerobic conditions, using Ziploc bags filled with a microaerobic gas mixture (5% O2, 10% CO2, 85% N) [25]. Brilliant green sulfa agar plates (BGS) [26] supplemented with 200 ppm of nalidixic acid [23] were used for recovering *Salmonella* and modified cefoperazone charcoal deoxycholate agar (mCCDA) [24] plates for the selective detection of *Campylobacter*. Plates were incubated at 37°C for 24 hours and 42°C for 48 hours (under microaerobic conditions), respectively. Presumptive marker *Salmonella* colonies were confirmed by serology with *Salmonella* Poly O [27].

3.3.2. Hatch of Fertile Eggs

A total of 867 eggs, 675 from the IH flock and 192 from the CH flock (Set 1), were set in two different Jamesway incubators [28] at 37.5°C, and approximately 50% humidity. Eight days later, a second hatch was set with 450 eggs, 351 from IH and 99 from CH (Set 2). Transfer of the eggs was performed at day 24, and they remained in the hatchers until hatch on day 28. For both incubation groups, two different Jamesway hatchers were also used, both set at 36.9°C, with approximately 72% humidity. Hatchery trays containing eggshell residues and feces were swabbed at day of hatch, and pads with fecal dropping were also collected for analysis of *Salmonella* and *Campylobacter*. Eggs that did not hatch were identified, and the

day of death was estimated based on the degree of development of the embryo. Cracked, rotten and infertile eggs were also recorded.

3.3.3. Analysis of Semen and Testes of Toms and Reproductive Tract of Hens

Semen was collected from both groups of toms at 32 weeks (sampled 2 different days) and 33 weeks. Pooled semen from eight CT, and five and six IT, respectively, was collected by abdominal massage and analyzed for the presence of both bacteria. Three samples were analyzed for CT and six samples for IT. Enumeration of one sample per group (CT and IT) was performed in one occasion. For enumeration, 0.1 mL of semen was combined with 0.9 mL of buffered peptone water (BPW) 1% and homogenized, serial dilutions were plated (0.1 mL) onto mCCDA. The detection limit was 1.0×10^2 cfu/mL. For *Campylobacter* detection, a 0.1 mL aliquot of semen was directly spread plated onto mCCDA and incubated 48 hours at 42°C in microaerobic conditions. Another 0.1 mL were added into 6 mL of BB (with supplements and laked horse blood) for enrichment. After incubation in microaerobic conditions at 42°C for 48 hours, samples were streaked onto mCCDA. For *Salmonella* detection, 1 mL of semen was added into 6 mL of BPW for enrichment. BPW samples were incubated at 37 °C for 24 hours followed by a selective enrichment step, with 0.5 mL of the previously enriched solution added into Tetrathionate (TT) broth base and 0.1 mL into Rappaport-Vassiliadis (RV) broth [29]. Tubes were vortexed and incubated at 42°C for 24 hours. After incubation a loopful from each of the tubes was streaked onto BGS agar. Presumptive *Salmonella* colonies were stabbed and streaked in triple sugar iron agar and

lysine iron agar slants. Slants were incubated for 24 hours at 37°C. Isolates giving typical reactions were streaked onto nutrient agar (NA) plates [30] for purification, and confirmed by serology with *Salmonella* Poly O [27] and Poly H antiserum [24].

Birds were periodically euthanized by electrocution following the guidelines of the approved Institutional Use and Animal Care Committee's protocol at North Carolina State University. After examination of absence of corneal reflex and vital signs, exteriors were sprayed with 70% ethanol prior to opening the abdominal cavity. Testes were aseptically removed at weeks 33 and 38 and analyzed for the presence of both bacteria. Testes were weighed and macerated with a rubber mallet and BB (with supplements and laked horse blood added) or BPW 1% were added at a ratio of three times the weight of each sample for the enrichment of *Campylobacter* and *Salmonella,* respectively. Additional steps were the same as described for semen analysis. Reproductive tract of breeder hens was collected at 65 weeks and ovarian follicles and the upper segment of the tract (infundibulum, isthmus and magnum) were analyzed. Each sample was divided in two for analysis of each pathogen. Samples were processed as described for testes.

3.3.4. Offspring Monitoring

At day of hatch, 496 poults from inoculated hens (IP) and 126 poults from control hens (CP), were placed in 24 and 7 pens, respectively. Pens were located in opposite sides of a growout house. Fecal samples from both groups were analyzed weekly to determine the presence of both pathogens. Pooled fecal droppings from the different pens from each group

of poults were directly streaked onto mCCDA for detection of *Campylobacter*. Incubation conditions were the same as in previous sections. For *Salmonella* identification, feces were diluted in BPW 1% (1:10) and the following steps were as described in the previous section.

At one and five weeks, 20 poults were euthanized by cervical dislocation, following the guidelines of the approved Institutional Use and Animal Care Committee's protocol at North Carolina State University. Organs (spleen, liver and gallbladder) and gastrointestinal tracts were removed for analysis. Due to the small size of the poults, organs and gastrointestinal tracts were pooled as a composite of viscera, and treated as only one sample. Samples were initially processed the same as described for testes. Feed, pine wood shavings, and darkling beetle larvae collected in the offspring house were also analyzed (Table 3.2.).

3.3.5. Campylobacter and Salmonella Preservation

Campylobacter colonies were sub-cultured on Mueller-Hinton agar (MHA) [24] for purification, and nutrient agar was used for *Salmonella*. Isolates were preserved in cryovials containing brain heart infusion broth (BHI) with 20% glycerol at -80°C.

3.3.6. Campylobacter and Salmonella Subtyping

Campylobacter species were determined by multiplex polymerase chain reaction (PCR) using primers for amplifying the *C. jejuni* specific *hip* gene (hipF 5'-ATG ATG GCT TCT TCG GAT AG-3' and hipR 5'-GCT CCT ATG CTT ACA ACT GC-3'), and the *C. coli ceu*

gen*e* (ceuF 5’-ATG AAA AAA TCT TTA GTT TTT GCA-3' and ceuR 5'-GAT TTT ATT ATT TGT AGC AGC G-3') [31–33]. *Salmonella* isolates were serotyped by SMART, a multiplex PCR and capillary electrophoresis analysis [34].

3.3.7. Campylobacter Antibiotic Resistance Test

Campylobacter isolates were tested for resistance to a panel of antibiotics using an agar dilution method. Antibiotics and concentrations tested included kanamycin (25 μg/mL), nalidixic acid (20 μg/mL), ciprofloxacin (4 μg/mL), gentamicin (200 μg/mL), all four from Fisher [23], and tetracycline (16 μg/mL), erythromycin (10 μg/mL) and streptomycin (15 μg/mL) from Sigma [35]. Isolates were also grown on MHA plates to ensure viability and *C. jejuni* ATCC 33560 [36], sensitive to all the antibiotics tested, was included in the test as a quality control strain.

3.3.8. Pulsed-Field Gel Electrophoresis

Campylobacter isolates were analyzed by pulsed-field gel electrophoresis (PFGE) after DNA restriction with *Sma*I [37]. Genetic relatedness based on the on the PFGE banding patterns were analyzed using BioNumerics [38]. Dice coefficient and unweighted pair group method with arithmetic mean (UPGMA), with 1.5% optimization and 1.7% position tolerance were used.

3.3.9. Statistical Analysis

Two-sided Fisher's exact test was used to test independency between egg treatments. Fisher's test was performed using JMP 11 software [39]. In hatch experiments data sets were analyzed using the GLM procedure of SAS [40]. Significance of data was set at $P \leq 0.05$ in all cases.

3.4. RESULTS AND DISCUSSION

3.4.1. Artificial Insemination and Egg Analysis

Salmonella and *Campylobacter* were not detected in the first set of eggs analyzed prior to insemination using the inoculated semen. Eggs analyzed from hens inseminated with semen containing marker strains of *Salmonella* (*S.* Enteritidis NAL) and *Campylobacter* (*C. coli* GK), were positive for *S.* Enteritidis NAL in five (5.6%) of the 90 unwashed eggshells analyzed; in turn, *S.* Enteritidis NAL was not found in eggshells of washed eggs (Table 3.3.). Furthermore, *S.* Enteritidis NAL was not detected in any of the yolks analyzed. In eggs from CH, *S.* Enteritidis NAL (marker strain inoculated into IH through semen) was isolated in two (8.3%) of the 24 eggshells analyzed (Table 3.3.). The isolation of the marker *Salmonella* strain from control eggs was likely due to cross-contamination during egg collection or handling since CH and IH were placed in the same turkey house, although in different areas. The farm crew collected the eggs daily, and placed them in plastic racks in a cool room, separating the eggs from CH and IH. The eggs were then transported from the farm to the

laboratory for analysis. Environmental testing revealed that horizontal transmission could be also possible, via rodents or insects, which tested positive for *Salmonella* during the study (results showed in Chapter 2). *Campylobacter* was not detected in any of the eggs tested, either eggshells or yolks.

Two-sided Fisher's exact test showed no significant differences between the eggs from either IH or CH analyzed. The main limitation in this study was the small number of eggs analyzed, which led to a low statistical power. Besides, considering the low frequencies of transmission of *Salmonella* through the eggs in *Salmonella*-positive hens [9, 12], the number of eggs and egg contents tested should be increased in future studies. Nevertheless, the fact that *Salmonella* was not detected in any of the washed eggs may suggest a positive effect of washing and sanitizing the eggs on decreasing the incidence of *Salmonella* transmitted through the eggshell.

3.4.2. Hatch Experiments

Eggs from CH (n=192) hatched at 72.5% from first hatch, and 75% in the second hatch (n=99). Eggs from IH (n=675) hatched at 78.1% rate for the first set and 80.7% for the second set (n=351) (Table 3.4.). Significant differences in fertility were found between treatments (Control, Inoculated) (p=0.0023), with higher fertility of in eggs from IH, but not between trials (Set 1, Set 2) (Table 3.4.). The number of eggs used for the experiments was inferior to typical commercial numbers, which may affect the results. Also, the differences in numbers of eggs used per group of hens may have an effect. This limitation should be

addressed in future experiments, hatching a higher number of eggs to get a better representation of commercial conditions, as well as similar numbers of eggs from both groups of hens (CH, IH). Hatchability rates were significantly different between treatments (Control/ Inoculated) (p= 0.0056) with a higher hatching rate on eggs from the IH. However, for the hatchability of fertile eggs p value=0.0483 was close to the significance level. Differences in hatchability between groups can be attributed to slight oscillations detected in the temperature on each of the incubators used. Besides, there was no significant difference between the two sets of eggs from IH. *Salmonella* and *Campylobacter* were not detected in hatchery swabs with eggshell residues and fecal material, and paper pads with fecal droppings analyzed at day of hatch from both groups (CH and IH).

3.4.3. Semen, Testes and Reproductive Tract of Hens

Both species of *Campylobacter*, *C. jejuni* and *C. coli*, were identified in semen, but none of them were the marker strains initially oral-inoculated into the toms (*C. jejuni* TSKQ) or hens (*C. coli* GK) (Chapter 2). All samples analyzed (CT=3; IT=6), were positive for *Campylobacter*. Since samples were directly streaked in mCCDA, and also enriched, multiple colonies were analyzed per sample. A total of eight isolates were recovered for CT, and 11 isolates for IT. From the 19 isolates tested, 11 of them (57.9%) were *C. jejuni* resistant to tetracycline, 5 (26.3%) were *C. coli* resistant to kanamycin, and the remaining 3 (15.8%) were *C. jejuni* pan-sensitive. An analysis of the isolates detected by group is shown in figure 3.1. *C. jejuni* resistant to tetracycline was detected in four isolates from CT (50%),

and seven (63.6%) from IT. *C. coli* resistant to kanamycin was found in three (37.5%) of the isolates from CT, and two (18.2%) from IT; *C. jejuni* pan-sensitive was found in one (12.5%) isolate from CT, and two (18.2%) from IT (Fig. 3.1.). The enumeration of two semen samples showed *Campylobacter* levels of 6.0 x 10^2 cfu/mL in CT, and 9.0 x 10^2 cfu/mL in IT. Both, kanamycin resistant *C. coli* and tetracycline resistant *C. jejuni*, were previously detected in fecal samples of both groups of toms (CT, IT); however, pan-sensitive *C. jejuni* was not detected in feces from toms (Table A.3.). Furthermore, *S.* Agona was identified in one of the semen sample from CT. *S.* Agona was also isolated from fecal samples and cecum and jejunum of toms during the same project (Chapter 2). However, neither *Campylobacter* nor *Salmonella* were detected in testes. Negative testes, plus the lack of an aseptic semen collection technique suggested that semen was contaminated during contact with the cloaca.

In the case of reproductive tract of breeder hens, *S.* Enteritidis nalidixic acid resistant (marker strain) and *C. jejuni* pan-sensitive were detected in the upper sections of the reproductive tract (infundibulum, isthmus and magnum). However, neither of the pathogens was detected in ovaries and follicles. From the 20 hens analyzed of each group, *C. jejuni* (pan-sensitive) was detected in the upper segment of reproductive tract in two of the IH, and one of the CH. In the case of *Salmonella*, it was isolated from three of the upper segments in IH, but not detected in CH. All three *Salmonella* isolates were the marker strain (*S.* Enteritidis NAL^R). A detailed list of reproductive tissue analyzed, and *Campylobacter* and *Salmonella* isolates detected is presented in table A.5.

3.4.4. Offspring Monitoring

Feed and wood shavings analyzed were negative for both bacteria (Table 3.2.). At week 1 (February 2013), *S.* Typhimurium (nalidixic acid sensitive) was isolated from fecal samples on CP, however it was not detected in IP until week 10 (April 2013) (Table 3.5.). Furthermore, *S.* Typhimurium (nalidixic acid sensitive) was isolated from darkling beetle larvae (Tenebrionidae family) collected in the surrounding area of the pens of CP during week 5 (March 2013) (Table 3.2.). In spite of the presence of *Salmonella* in the environment and the potential horizontal vectors of transmission, *Salmonella* was not detected in IP until nine weeks later.

Campylobacter was not detected until week 12 (last week of April 2013) in IP, and week 13 (first week of May 2013) in CP (Table 3.5.). All *Campylobacter* isolates were *C. jejuni* pan-sensitive. These differences indicate a quicker spreading of *Campylobacter*, and a similar pattern was also observed during monitoring of the breeder parents of these poults (data not shown). Previous studies found that flocks were 100% colonized with some variation by day 7 to week 3 [41–43]. Colonization by *C. jejuni* at week 3 to 4 was thought to be related to the disappearance of maternal antibodies against *Campylobacter* in broiler chickens [44]. However, variation in colonization time, or even lack of colonization in turkey flocks have also been reported [42, 45, 46]. During monitoring of fecal samples of the breeder parents, naturally occurring strains of *Campylobacter* were detected at week 4 (second week of April) (Chapter 2). In both cases, other flocks were present in the houses prior to the placement of the poults (parent breeders and offspring). In the case of the parent

breeders the house was empty around three months prior to their placement. The house where the offspring was placed at day of hatch was empty around 4.5 months prior to the placement of the poults. Cleaning and sanitation in both houses was similar, however in the offspring house a disinfectant was not sprayed before placing the poults. Another difference could be the time of the year and temperature, parent breeders were placed in the house during the second half of March 2012 (March 20th), while the offspring was placed at the beginning of February 2013 (February 5th). In both cases, breeders and offspring, naturally occurring strains of *Campylobacter* were first detected in April. Environmental conditions such as temperature and humidity, and litter moisture, were not measured during the project, all these parameters may be implicated in the environmental spread of *Campylobacter* and *Salmonella,* and they can be taken into account in next studies. As observed for the breeder parents in Chapter 2, *Salmonella*, even though it was detected soon in CP (week 1), it was not detected in IP until 9 weeks later showing a not fast spreading. However, *Campylobacter* was detected later in fecal samples (week 12 and 13), but when it was detected in one group of poults (first in IP at week 12), it was detected during the next sampling (week 13) in the other group of poults (CP), showing a faster spreading ability.

During necropsy at 8 days, only one of 10 viscera composites from the CP was positive for *Salmonella* (Table 3.6.). However, *Salmonella* was not identified in IP. *Campylobacter* was not detected in viscera of any of the groups (CP, IP), thus, with the consequent lack of evidence of vertical transmission through semen. At 36 days, six of the 10 viscera composites on the CP were positive for *Salmonella*, showing an increase in the number of birds colonized in the same house area, even when they were in different pens. All samples from

IP were negative (Table 3.6.). *Campylobacter* was not detected in any of the groups. All the *Salmonella* isolates were characterized as *S.* Typhimurium (nalidixic acid sensitive) by multiplex PCR and capillary electrophoresis analysis [34]. As with *Campylobacter*, marker *Salmonella* strain inoculated through the semen was not isolated in fecal samples or intestinal samples in the poults.

3.4.5. Comparison of Campylobacter Isolates from Offspring Feces, Reproductive Tract of Hens, and Semen of Toms

PFGE was performed to the three pan-sensitive *C. jejuni* isolates from the upper segment of the reproductive tract of breeder hens, pan-sensitive *C. jejuni* isolates from fecal samples from the poults, and two pan-sensitive *C. jejuni* isolates from semen of toms (CT and IT), to determine whether these multiple pan-sensitive *C. jejuni* isolates were representatives of the same strain. PFGE profiles of the two isolates from IH reproductive tract (IH6 RT and IH7 RT) were undistinguishable (Fig. 3.2.). Furthermore, the same band pattern was observed in *C. jejuni* isolates from the reproductive tract of CH (CH4 RT), and fecal samples of CP (CP04) and IP (IP1 02) (Fig. 3.2.). The other group clustered *C. jejuni* pan-sensitive isolated from fecal samples of CP (CP03) and IP (IP05, IP06 and IP1 01), showing a band pattern similar to the one observed in reproductive tract of CH (CH4 RT) (Fig. 3.2.). Isolates from semen of breeder toms were different to the other pan-sensitive *C. jejuni* strains isolated from breeder hens (CH and IH) and from the offspring (CP and IP) (Fig. 3.2.). A hypothesis for this observation can be the possibility of different environmental origin or a different time of

entrance in the flock. Breeder toms were located in the same house during all the duration of the study, and semen samples were collected in weeks 32 and 33. However, the hens were moved to a different house for the lighting program prior to be inseminated, and they remained in this house for 12 weeks before returning to the initial breeder house, being exposed to new environments, and the collection and analysis of RT was performed at week 65. Besides, the offspring was initially placed in a different growout house where they grew until week 14. Grown poults were moved at week 14 to the breeder house were they remained until week 16. *Campylobacter* detection after several months in fecal samples of the grown poults (week 12 and 13 in IP and CP, respectively), suggested that the colonization of the second generation offspring was likely due to horizontal transmission rather than vertical. Another observation was that *Campylobacter* isolates from the reproductive tract of CH (CHC4 RT) and IH (IHC6 RT and IHC7 RT) had an undistinguishable band pattern with isolates from ceca from CH (CHC4 ceca) and IH (IHC6 ceca), respectively (Fig. 3.3.), suggesting a possible cross-contamination origin. Furthermore, the three different band patterns (A, B and C) observed for these pan-sensitive *C. jejuni* isolates (Fig. 3.3.), indicates diversity among these *C. jejuni* isolates, suggesting different environmental origins or times of entrance in the flock.

This study has given some insights for future projects that can assist poultry researchers and the poultry industry for trying to better understanding the uncertainties regarding the vertical transmission of *Campylobacter*, and reducing and eliminating *Salmonella* and from turkey fertile hatching eggs and breeder flocks. A limitation in this study was the small number of samples analyzed (eggs, reproductive tract tissues). If the frequency of occurrence

of vertical transmission is minimal, significantly larger numbers of animals, tissues, etc., will be required for detection of the pathogens in the offspring. Also, if the frequency of occurrence is extremely rare, it may not be possible to ever demonstrate this with studies such as this one. If this is the case, more sensitive methods such as qPCR or sequencing-based methodologies could be used.

The fitness of the marker strains used for the study can also affect the outcome of colonization of reproductive tissues. During monitoring of fecal and intestinal samples described in Chapter 2, naturally occurring strains seemed to outcompete marker strains and persist longer in the flock. In general, there would be important to replicate these experimental trials and compare the results.

3.5. CONCLUSIONS AND APLICATIONS

1. The presence of *Salmonella* and *Campylobacter* in semen during artificial insemination of the breeder hens did not show a negative effect on egg hatchability or fertility in this experiment.
2. The results of this study indicated that washing and sanitizing the eggs decreased the incidence of *Salmonella* transmitted through the eggshell.
3. Vertical transmission of either pathogen through contaminated semen could not be demonstrated.
4. Further study is needed to investigate this route of transmission.

3.6. ACKNOWLEDGMENTS

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Table 3.1. Eggs analyzed before and after insemination of IH with semen containing marker strains of *Salmonella* and *Campylobacter*.

Hen Group	**Pre-insemination with bacteria**	**Post-insemination with bacteria**				
	Set 1	Set 1	Set 2	Set 3	Set 4	Set 5
Control (unwashed)	6	6	6	6 (2)*	0	6
Inseminated (unwashed)	20	20	20 (2)*	20 (3)*	10	20
Inseminated (washed)	0	0	0	16	20	16

* Values in parenthesis indicate number of eggs (eggshells) positive for marker *Salmonella*.

Table 3.2. Vectors and other environmental samples analyzed.

Sampling Date	Sample	*Salmonella*	*Campylobacter*
Feb13	Feed (25g), wood shavings (whirl-pak bag), darkling beetle larvae (25-30)	-	-
Mar13	Darkling beetle larvae (25-30)	-	-
Mar13	Darkling beetle larvae (25-30)	+	-

Table 3.3. *Salmonella* detected on eggshells from Control (CH) or Inoculated (IH) hens.

	Eggshells positive for *Salmonella*/ total number of eggs analyzed (%)
CH (Unwashed)	2/24 (8.3)
IH (Unwashed)	5/90 (5.6)
IH (Washed)	0/52 (0.0)

CH, Control Hens; IH, Inoculated Hens.

Table 3.4. Hatch experiment. Poults hatched, early death rates and pips.

Set	Parent flock	Total eggs	Infertile eggs (%)	Poults hatched	% Hatched (total eggs /fertile eggs)	% Death rate first 7 days of incubation	% Internal pip (26 days)	% Pip (27 days)
1	Control	190	19 (10%)	124	65.3/72.5	0.09	1.58	9.47
1	Inoculated	675	40 (5.9)	496	73.5/78.1	0.08	0.89	8.74
2	Control	98	14 (14.3)	63	64.3/75.0	0.07	0	12.24
2	Inoculated	351	24 (6.8)	264	75.2/80.7	0.09	0	5.41

Table 3.5. *Salmonella* and *Campylobacter* detected in fecal samples from both groups of poults (CP and IP).

	Poults from CONTROL flock (CP)		**Poults from INOCULATED flock (IP)**	
Week	*Salmonella*	*Campylobacter*	*Salmonella*	*Campylobacter*
1	+	-	-	-
2	-	-	-	-
3	+	-	-	-
5	-	-	-	-
6	+	-	-	-
7	+	-	-	-
8	+	-	-	-
9	+	-	-	-
10	ND	-	+	-
11	+	-	-	-
12	+	-	-	+
13	ND	+	ND	+
15	-	+	-	+

ND, not determined.

Table 3.6. *Campylobacter* and *Salmonella* isolated from viscera composites of poults at day 8 and day 36. (Number of positive samples/number of birds analyzed).

	Control Parent Flock		**Inoculated Parent Flock**	
Poults age	*Campylobacter*	*Salmonella*	*Campylobacter*	*Salmonella*
8 days	0/10	1/10*	0/10	0/10
36 days	0/10	6/10*	0/10	0/10

*Further testing demonstrated that these isolates were not the marker strains inoculated through the semen into breeder hens.

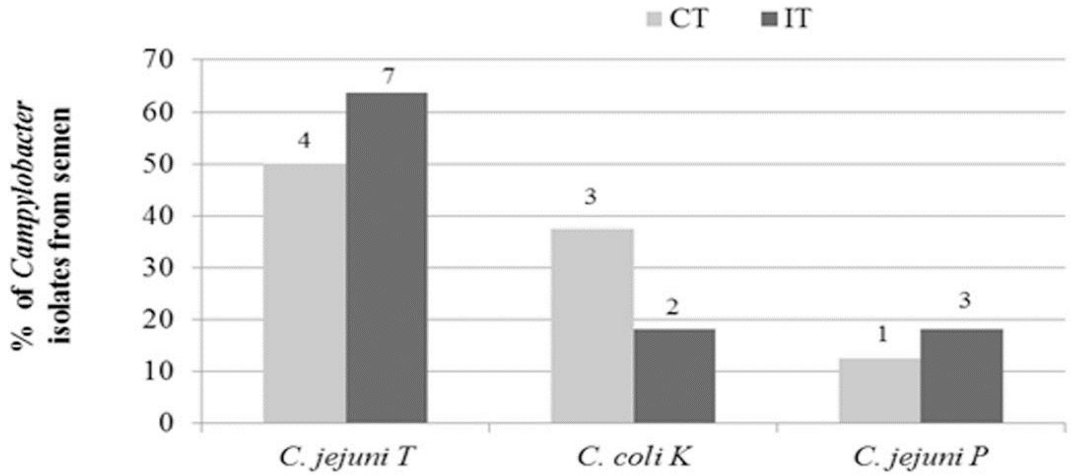

Fig. 3.1. Percentage of *Campylobacter* species and antibiotic resistance profile isolated from semen samples. T, tetracycline; K, kanamycin; P, Pan-sensitive. Frequencies were calculated based on total positive isolates per group (CT=8, IT=11). CT, Control Toms; IT, Inoculated Toms.

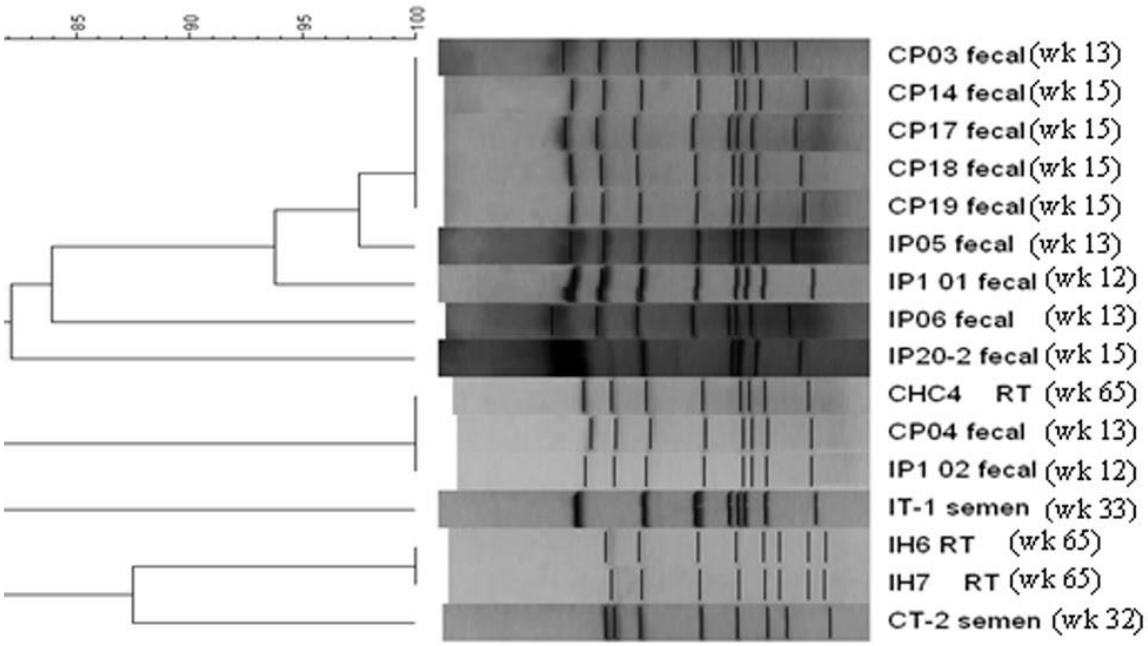

Fig. 3.2. PFGE profiles of pan-sensitive *C. jejuni* isolates detected in RT of hens, semen of toms (CT and IT), and fecal samples from both groups of the offspring (CP and IP).
The age of the turkeys at the moment of sampling is in parenthesis. CH, Control hens; IH, Inoculated hens; RT, Reproductive tract; IT, Inoculated Toms; CT, Control Toms; CP, Poults from Control Hens; IP, Poults from Inoculated Hens.

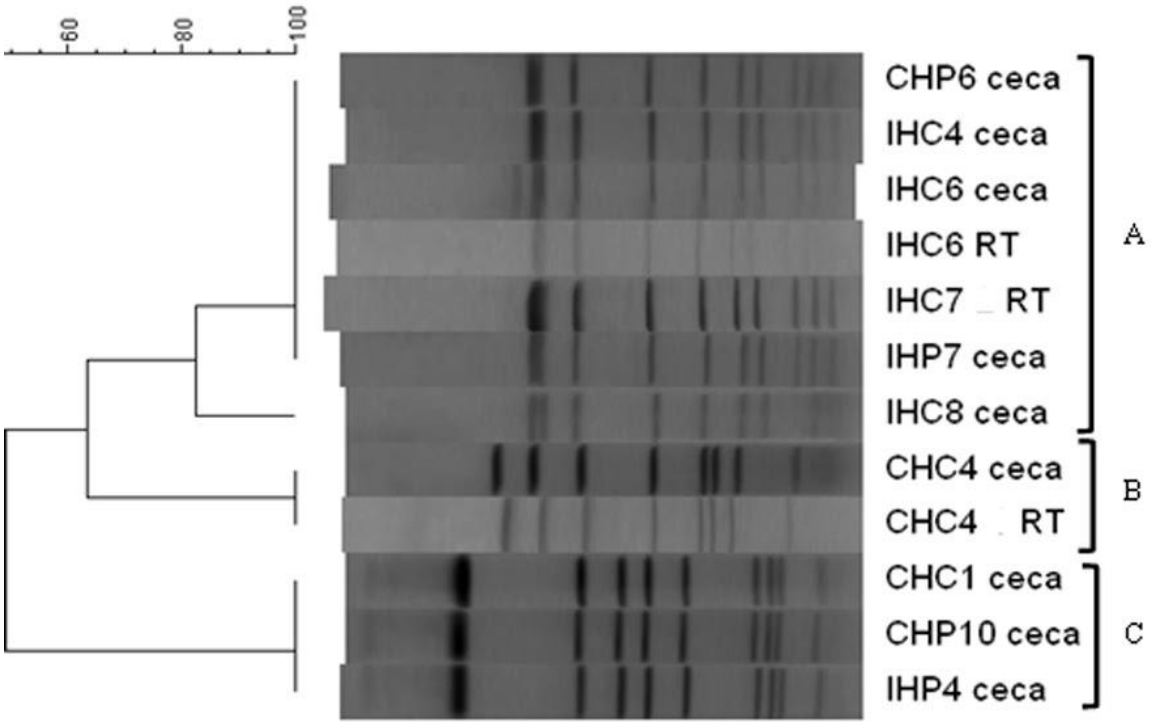

Fig. 3.3. PFGE profiles of pan-sensitive *C. jejuni* isolates recovered from the upper segment of reproductive tract (RT) and ceca of hens. CH, Control Hens; IH, Inoculated Hens; RT, Reproductive tract. A, B and C, indicate different band patterns.

3.7. REFERENCES AND NOTES

1. Buhr, R.J., N.A. Cox, N.J. Stern, M.T. Musgrove, J.L. Wilson, and K.L. Hiett. 2002. Recovery of *Campylobacter* from segments of the reproductive tract of broiler breeder hens. Avian Dis. 46: 919–924.

2. Cox, N.A., N.J. Stern, K.L. Hiett, and M.E. Berang. 2002. Identification of a new source of *Campylobacter* contamination in poultry: Transmission from breeder hens to broiler chickens. Avian Dis. 46: 535–541.

3. Newell, D.G., and C. Fearnley. 2003. Sources of *Campylobacter* colonization in broiler chickens. Appl. Environ. Microbiol. 69: 4343–4351.

4. Cole, K., A.M. Donoghue, P.J. Blore, and D.J. Donoghue. 2004. Isolation and prevalence of *Campylobacter* in the reproductive tracts and semen of commercial turkeys. Avian Dis. 48: 625–630.

5. Cox, N.A., L.J. Richardson, R.J. Buhr, and P.J. Fedorka-Cray. 2009. *Campylobacter* species occurrence within internal organs and tissues of commercial caged Leghorn laying hens. Poult. Sci. 88: 2449–2456.

6. Cox, N.A., N.J. Stern, J.L. Wilson, M.T. Musgrove, R.J. Buhr, and K.L. Hiett. 2002. Isolation of *Campylobacter* spp. from semen samples of commercial broiler breeder roosters. Avian Dis. 46: 717–720.

7. Donoghue, A., P. Blore, K. Cole, N. Loskutoff, and D. Donoghue. 2004. Detection of *Campylobacter* or *Salmonella* in turkey semen and the ability of poultry semen extenders to reduce their concentrations. Poult. Sci. 83: 1728–1733.

8. Fajardo, T.A., R.C. Anantheswaran, V.M. Puri, and S.J. Knabev. 1995. Penetration of *Salmonella enteritidis* and *Salmonella heidelberg* into egg yolks in an in vitro contamination model. J. Food Prot. 58: 473–477.

9. Gast, R.K., P.S. Holt, and T. Murase. 2005. Penetration of *Salmonella enteritidis* and *Salmonella heidelberg* into egg yolks in an in vitro contamination model. Poult. Sci. 84: 621–625.

10. Williams, J.E., L.H. Dillard, and G.O. Hall. 1968. The penetration patterns of *Salmonella* Typhimurium through the outer structures of chicken eggs. Avian Dis. 12: 445–466.

11. Buck, J. De, F. Van Immerseel, F. Haesebrouck, and R. Ducatelle. 2004. Colonization of the chicken reproductive tract and egg contamination by *Salmonella*. J. Appl. Microbiol. : 233–245.

12. Cox, N.A., M.E. Berrang, and J.A. Cason. 2000. *Salmonella* penetration of egg shells and proliferation in broiler hatching eggs. Poult. Sci. 79: 1571–1574.

13. Hiett, K.L., N.A. Cox, R.J. Buhr, and N.J. Stern. 2002. Genotype analyses of *Campylobacter* isolated from distinct segments of the reproductive tracts of broiler breeder hens. Curr. Microbiol. 45: 400–404.

14. Cox, N.A., L.J. Richardson, J.J. Maurer, M.E. Berrang, P.J. Fedorka-Cray, R.J. Buhr, J.A. Byrd, M.D. Lee, C.L. Hofacre, P.M. O'Kane, A.M. Lammerding, A.G. Clark, S.G. Thayer, and M.P. Doyle. 2012. Evidence for horizontal and vertical transmission in *Campylobacter* passage from hen to her progeny. J. Food Prot. 75: 1896–1902.

15. Shanker, S., A. Lee, and T.C. Sorrell. 1986. *Campylobacter jejuni* in broilers: The role of vertical transmission. J. Hyg. (Lond). 96: 153–159.

16. Sahin, O., P. Kobalka, and Q. Zhang. 2003. Detection and survival of *Campylobacter* in chicken eggs. J. Appl. Microbiol. 95: 1070–1079.

17. Callicott, K.A., V. Friethriksdóttir, J. Reiersen, R. Lowman, J. Bisaillon, E. Gunnarsson, E. Berndtson, K.L. Hiett, D.S. Needleman, and N.J. Stern. 2006. Lack of evidence for vertical transmission of *Campylobacter* spp. in chickens. Appl. Environ. Microbiol. 72: 5794–5798.

18. Cox, N.A., L.J. Richardson, M.E. Berrang, R.J. Fedorka-Cray, and R.J. Buhr. 2009. *Campylobacter coli* naturally resistant to elevated levels of gentamicin as a marker strain in poultry research. J. Food Prot. 72: 1288–1292.

19. Minnesota Turkey Growers Association, St. Paul, MN.

20. Continental Plastic Corp., Delavan, WI.

21. PI quat 20®, Preserve International, Zepher Cove, NV.

22. Musgrove, M.T., D.R. Jones, J.K. Northcutt, M.A. Harrison, N.A. Cox, K.D. Ingram, and A.J. Hinton. 2005. Recovery of *Salmonella* from commercial shell eggs by shell rinse and shell crush methodologies. Poult. Sci. 84: 1955–1958.

23. Fisher Biotech, Fair Lawn, NJ.

24. Oxoid Ltd, Hampshire, England.

25. Airgas, Durham, NC.

26. Acumedia, Neogen, Lansing, MI.

27. BD and company Sparks, MD.

28. Jamesway 52. Jamesway. The incubator company. Cambridge, ON, Canada.

29. Hardy Diagnostics, Santa Maria, CA.

30. Thermo Fisher Scientific, Remel Products, Lenexa, Kansas.

31. Houng, H.S., O. Sethabutr, W. Nirdnoy, D.E. Katz, and L.W. Pang. 2001. Development of a ceuE-based multiplex polymerase chain reaction (PCR) assay for direct detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in Thailand. Diagn. Microbiol. Infect. Dis. 40: 11–19.

32. Marshall, S.M., P.L. Melito, D.L. Woodward, W.M. Johnson, F.G. Rodgers, and M.R. Mulvey. 1999. Rapid idenntification of *Campylobacter*, *Arcobacter*, and *Helicobacter* isolates by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene. J. Clin. Microbiol. 37: 4158–4160.

33. Gonzalez, I., K.A. Grant, P.T. Richardson, S.F. Park, and M.D. Collins. 1997. Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the ceuE gene encoding a putative virulence determinant. J. Clin. Microbiol. 35: 759–763.

34. Leader, B.T., J.G. Frye, J. Hu, P.J. Fedorka-Cray, and D.S. Boyle. 2009. High-throughput molecular determination of *Salmonella enterica* serovars by use of multiplex PCR and capillary electrophoresis analysis. J. Clin. Microbiol. 47: 1290–1299.

35. Sigma Chemical Co., St. Louis, MO.

36. American Type Culture Collection.

37. New England BioLabs Inc.

38. BioNumerics, version 4.6, Applied Maths, Saint-Marten-Latem, Belgium.

39. JMP®, Version 11. SAS Institute Inc., Cary, NC.

40. SAS Institute. SAS/STAT User's Guide. Version 9.1 for Windows.2004. SAS Inst. Inc., Cary, NC.

41. Wallace, J.S., K.N. Stanley, and K. Jones. 1998. The colonization of turkeys by thermophilic campylobacters. J. Appl. Microbiol. 85: 224–230.

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

43. Jacobs-Reitsma, W.F., A.W. van de Giessen, N.M. Bolder, and R.W. Mulder. 1995. Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. Epidemiol. Infect. 114: 413–421.

44. Sahin, O., Q. Zhang, J.C. Meitzler, B.S. Harr, T.Y. Morishita, and R. Mohan. 2001. Prevalence, antigenic specificity, and bactericidal activity of poultry anti-*Campylobacter* maternal antibodies. Appl. Environ. Microbiol. 67: 3951–3957.

45. Thorsness, J.L., J.S. Sherwood, G.T. Danzeisen, C. Doetkott, and C.M. Logue. 2008. Baseline *Campylobacter* prevalence at a new turkey production facility in North Dakota. J. Food Prot. 71: 2295–2300.

46. Giacomelli, M., C. Salata, M. Martini, C. Montesissa, and A. Piccirillo. 2014. Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* from poultry in Italy. Microb. Drug Resist. 20: 181–8.

CHAPTER IV

PREVALENCE OF *CAMPYLOBACTER* AND *SALMONELLA* AT SLAUGHTER IN A SECOND GENERATION OFFSPRING OF TURKEYS

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4.1. SUMMARY

Salmonella and *Campylobacter* are pathogens of public health concern associated with poultry products. Pre-harvest control in breeder flocks and their offspring is crucial to reduce the transmission of these bacteria through the food chain.

This study was the last part of a survey covering the life of breeder turkeys from day of hatch until 65 weeks of age, including reproduction stages and monitoring of the flock's offspring. The breeder parents were orally inoculated by gavage with marker strains of *Salmonella* and *Campylobacter* at 10 days and 12 weeks, and then monitored by analyzing feces and intestinal samples (discussed in Chapter 2). During artificial insemination (weeks 39 to 43), the semen was also inoculated with marker strains of both pathogens, a nalidixic-acid-resistant strain of *S.* Enteritidis and a *C. coli* strain resistant to gentamicin (both strains were previously orally-inoculated into the hens), to investigate the possible vertical transmission through fertile eggs to the offspring (discussed in Chapter 3). A control group of breeder hens (CH) was inseminated without the pathogens present in semen. Poults hatched from control hens (CP) and from inoculated hens (IP), were placed in a growout house at day of hatch, and fecal samples and intestinal samples were monitored for *Salmonella* and *Campylobacter* through market age in both groups (Chapter 3). At 16 weeks, 20 turkeys from each group (CP and IP) were slaughtered to evaluate the prevalence of *Campylobacter* and *Salmonella* colonizing ceca, and contamination of carcasses was also evaluated by analysis of sponges taken from the skin surface. At 20 weeks, another 40 turkeys, all of them CP, were slaughtered and ceca were analyzed. Twenty turkeys of this group received a direct-fed

microbial (PrimaLac®) added into the water for two weeks prior to slaughter. The other twenty did not receive the probiotic. At slaughter (16 weeks), 80% of CP and 90% of CP ceca samples were *Campylobacter*-positive. A low prevalence (0-10%) of *Salmonella* was detected in ceca of IP and CP, respectively. At week 20, a significantly lower prevalence (p=0.0001) of *Campylobacter* was detected in ceca of both, PrimaLac (40%) and control (45%) groups. However, there was not a significant difference between birds receiving PrimaLac and controls.

4.2. DESCRIPTION OF PROBLEM

Salmonella and *Campylobacter* are two leading causes of gastrointestinal disease worldwide, very often associated with poultry meat [1–4]. The estimates of foodborne illness per year in the United States, published by the CDC in 2011, indicated that 1.0 million of the foodborne illness was caused by non typhoidal *Salmonella*, and 0.8 million by *Campylobacter spp.* [5]. Both pathogens are frequently found colonizing the intestinal tract of poultry. While *Salmonella* have been found to colonize the flock at early ages and decrease in prevalence at slaughter [6, 7], *Campylobacter* spp. have been commonly shown to persist during all the lifetime once colonizes the host [7–9]. A correlation between flock cecal colonization with *Salmonella* and/or *Campylobacter* and poultry carcass contamination have been reported by several authors [10–13]. Jeffrey *et al.* in 2001 reported a higher probability of skin contamination (35 times greater) during processing when broilers were colonized by *Campylobacter* [14]. *Campylobacter* colonization at the farm and transport

play an important role in carcass contamination at slaughter, furthermore, handling and other processing steps contribute to the diffusion of the bacteria [15, 16]. Contamination with feces, exiting the cloaca during defeathering, has also been associated with *Campylobacter* contamination of carcasses [13, 17, 18]. These observations reiterate the importance of hygiene practices during processing, and also the role of pre-harvest interventions for reducing colonization of turkeys at the farm level to reduce carcasses contamination. The turkey industry is growing in economic importance; however most of the studies regarding *Salmonella* and *Campylobacter* prevalence during processing, and application of strategies for decreasing the prevalence of these pathogens on farm, have been done in broilers. Furthermore, intervention strategies for reducing the prevalence of both pathogens that can be applied for short periods of time prior to slaughter could be of interest for the turkey industry. The objectives of this study were 1) to determine the prevalence of *Campylobacter* and *Salmonella* in turkeys at slaughter; 2) to evaluate skin contamination prior to evisceration; and, 3) to evaluate the effect of a probiotic (PrimaLac) administered two weeks prior to slaughter, in the prevalence of *Campylobacter* at slaughter.

4.3. MATERIALS AND METHODS

Poults hatched from two groups of breeder turkeys were used for this study. One group of poults (IP) hatched from breeder hens inoculated with marker strains of *Salmonella* and *Campylobacter* via semen through artificial insemination. The other group (CP) hatched from a control group of hens that was not inoculated with the bacteria.

4.3.1. Analysis of Fecal Samples

Pooled fecal samples from each pen (two pens of IP and seven pens of CP, with approx. 12-14 turkeys/pen) were collected at week 15. *Campylobacter* was evaluated by direct streaking onto modified cefoperazone charcoal deoxycholate agar (mCCDA) [19]. Enumeration was performed for *Campylobacter* in several fecal samples from CP. For enumeration, 1 gram of feces was combined with 9 mL of buffered peptone water (BPW) 1% and homogenized, serial dilutions were plated (0.1 mL) onto mCCDA. The detection limit was 1.0×10^2 cfu/g. All plates were incubated at 42°C for 48 hours under microaerobic conditions. One *Campylobacter* colony per plate was sub-cultured on MHA for purification and further characterization including antibiotic susceptibility test and species determination.

4.3.2. Analysis of Ceca and Skin Samples after Slaughtering

At 16 weeks, 40 turkeys, 20 from each of the IP and CP groups, were stunned, killed via exsanguination, scalded at 63°C for 60 seconds and defeathered in a pilot slaughter plant located at NCSU, following the guidelines of the approved Institutional Use and Animal Care Committee's protocol at North Carolina State University. Carcasses were tested for prevalence of *Salmonella* and *Campylobacter*. Immediately after defeathering, skin on the upper part of the back and the left thigh was swabbed with a sponge over a 100 cm^2 frame for *Campylobacter*. A second sponge was taken from skin of the lower part of the back and the right thigh for *Salmonella* analysis. Sponge samples were analyzed using the methodology

described by the USDA-FSIS-Office of Public Health Science guidebook for "Detection and enumeration method for *Campylobacter jejuni/coli* from poultry rinses and sponge samples" (MLG 41.01), and the "Isolation and identification of *Salmonella* from meat, poultry, pasteurized egg and catfish products" (MLG 4.04) [20, 21]. Cecal contents were directly streaked onto mCCDA plates for detection of *Campylobacter* and incubated with microaerobic conditions at 42°C for 48 hours. For *Salmonella* identification, samples were individually weighed and macerated with a rubber mallet, buffered peptone water 1% [19] was added to the bag in a ratio 1:10 and then stomached for 60 seconds; bags were then incubated at 37^{o}C for 24 hours. Next steps were as described by the USDA-FSIS-Office of Public Health Science guidebook "Isolation and identification of *Salmonella* from meat, poultry, pasteurized egg and catfish products" (MLG 4.04) [21]. At week 20, another 40 turkeys were slaughtered. At this time all turkeys were from the control group (CP). Processing was performed the same as for week 16, but sponge samples were not taken.

4.3.3. Probiotic Treatment Two Weeks Prior to Slaughter

A group of 20 turkeys, all of them originated from the control group of hens (CP), received PrimaLac [22] applied in the drinking water two weeks prior to be slaughter at week 20. Another group of 20 turkeys (CP) did not receive the probiotic (control group). Treated and untreated turkeys were distributed in eight pens (four pens each). Treatment and control birds were placed in alternative pens, with five turkeys per pen. A stock solution of three ounces of PrimaLac/gallon of water was initially prepared, and then one ounce of stock

solution per gallon of drinking water was administered to the turkeys for two consecutive weeks. After two weeks the birds were slaughtered and ceca were aseptically collected and analyzed for the presence of *Salmonella* and *Campylobacter*. Cecal contents were processed as in the previous section. Enumeration of *Campylobacter* in cecal contents was performed in two turkeys per group (control/PrimaLac).

4.3.4. Campylobacter and Salmonella Subtyping

Species of *Campylobacter* isolates were determined by multiplex polymerase chain reaction (PCR) using primers to amplify the *C. jejuni* specific *hip* gene (hipF 5'-ATG ATG GCT TCT TCG GAT AG-3' and hipR 5'-GCT CCT ATG CTT ACA ACT GC-3'), and the *C. coli ceu* gene (ceuF 5'-ATG AAA AAA TCT TTA GTT TTT GCA-3' and ceuR 5'-GAT TTT ATT ATT TGT AGC AGC G-3') [23–25]. *Salmonella* isolates were characterized by SMART, a multiplex PCR and capillary electrophoresis analysis [26].

4.3.5. Campylobacter Antibiotic Resistance Test

Campylobacter isolates were tested for resistance to a panel of antibiotics (Table 4.1.) by agar dilution method. All isolates were also grown on Mueller-Hinton agar [19] plates to ensure viability. *C. jejuni* ATCC 33560 [27], sensitive to all the antibiotics tested, was included in the test as a quality control strain.

4.3.6. Pulsed-Field Gel Electrophoresis

Genomic DNA fingerprinting of *Campylobacter* isolates recovered from ceca at week 16 were determined by Pulsed-field gel electrophoresis (PFGE) using *Sma*I [28].

4.3.7. Statistical Analysis

Frequencies of detection in ceca were compared between groups using two-tailed Fisher's exact test using JMP 11 software [29]. Significance was defined at $P \leq 0.05$. Clonal relationships of *Campylobacter* isolates based on PFGE banding patterns were analyzed using BioNumerics [30]. Dice coefficient and unweighted pair group method with arithmetic mean (UPGMA), with 1.5% optimization and 1.5% position tolerance were used.

4.4. RESULTS AND DISCUSSION

4.4.1. Fecal Analysis, Cecal Colonization at Slaughter and Skin Contamination of Carcasses

The monitoring of fecal and intestinal samples during the first 15 weeks of life of these poults was covered in Chapter 2. Briefly, *Salmonella* was detected at day seven in CP, and nine weeks later (April 2013) in fecal samples from IP. *Campylobacter* was first detected in feces at week 12 (last week of April) in IP, and week 13 (first week of May) in CP. All *Campylobacter* isolates in weeks 12 and 13 were pan-sensitive *C. jejuni*. Fecal samples from

all pens were positive for *Campylobacter* at week 15. Pan-sensitive *C. jejuni* was identified as in previous fecal samples (Chapter 3), and tetracycline resistant *C. jejuni* was also isolated from one pen. This antibiotic profile was previously detected in breeder hens and toms located in this house. Enumeration of *Campylobacter* in fecal samples from four pens of CP showed levels of 10^4 cfu/g feces (1.0×10^4, 2.5×10^4, 8.0×10^4, 1.8×10^4 cfu/g, respectively). These observations suggested that warmer temperatures may have an effect on *Campylobacter* colonization of the flock. In chickens, a higher prevalence of *Campylobacter* colonization in early spring and summer has been previously reported [31, 32]. However there is variation and controversy regarding to seasonality, and others have found higher rates of *Campylobacter* in carcasses at slaughter in cooler months, attributing the differences to a geographical effect [15].

Salmonella was not detected in feces of CP (seven pens tested) or IP (two pens tested) at week 15; however, it was isolated from ceca of two CP (10%) at week 16. One of the isolates was *S.* Enteritidis nalidixic acid resistant, the marker strain inoculated into the breeder hens (IH), and the other was a naturally occurring strain of *S.* Typhimurium (nalidixic acid sensitive). One explanation for the existence of marker strains in these birds was that these poults were moved to the breeder house at week 14 (Fig. 4.1). Although they were placed in the opposite side of the house, environmental cross-contamination could have been possible. Furthermore, CP pens were located closer to the breeder hen pens than IP (Fig. 4.1.).

At slaughter, in turkeys from control breeders (CP), *Campylobacter* was isolated from 16 of the 20 (80%) ceca samples and *Salmonella* was detected in two of the 20 ceca samples (10%) (Table 4.2.). In birds hatched from inoculated hens (IP), *Campylobacter* was isolated

from 18 of the 20 (90%) ceca samples and *Salmonella* was not detected in any of the 20 ceca samples (Table 4.2.). Two sided Fisher's exact test with a 5% significance level showed no significant differences between groups. Previous studies in turkeys have also found a higher prevalence of *Campylobacter* colonization as opposed to *Salmonella* in turkeys [7, 33]. Species and antibiotic susceptibility were determined for all the isolates except for one sample of IP that could not be purified and isolated. All the isolates characterized from both groups were pan-sensitive *C. jejuni*.

For each group, *Campylobacter* was identified in only one of the sponges taken from the skin (Table 4.2.), and *Salmonella* was not isolated from any of the sponge samples. For *Campylobacter*, the overgrowth of background microflora in the plates due to the use of enrichment media, may have led to false negative and thus inconclusive results. Future studies must address this limitation in the future, for example, including a direct plating of the samples prior to enrichment.

4.4.2. Cecal Colonization at Slaughter after Treatment with PrimaLac

At week 20, in turkeys receiving PrimaLac for two consecutive weeks, *Campylobacter* was detected in nine of the 20 (45%) ceca analyzed, and S*almonella* was not detected in any of the samples. In the control group (no PrimaLac), *Campylobacter* was detected in eight of the 20 (40%) ceca analyzed, and *Salmonella* was positive in one of 20 (5%) ceca (Table 4.3.). The prevalence of *Campylobacter* detection in ceca at week 20 was significantly inferior ($p=0.0001$) than the observed in the previous group at week 16. Fluctuations in carriage and

number of *Campylobacter* in ceca of turkeys and broilers have been previously described [31, 34], however there is little understanding about the dynamics of *Campylobacter* cecal colonization. Testing fecal samples from turkeys between weeks 5 to 14, Wright *et al.*(2008) found an increase in overall *Campylobacter* prevalence with aging, with most of the increase being mediated by *C. jejuni* [34]. Our results could be also affected by a lower concentration of campylobacters in ceca at this time point that could lead to a lack of detection by direct plating. Wallace *et al.* (1997) reported higher carriage rates with enrichment procedures than with direct plating [35]. Enumeration of *Campylobacter* from two cecal samples showed levels of approx. 4×10^6 cfu/g and $<1 \times 10^2$ cfu/g, respectively. Enumeration from cecal contents of two turkeys receiving PrimaLac showed *Campylobacter* levels of approx. 1×10^3 cfu/g and $<1 \times 10^2$ cfu/g, respectively. Both samples with *Campylobacter* numbers under detection limits were also negative by direct plating. However, enumeration to know the size of *Campylobacter* populations was not performed for all cecal samples. The use of more sensitive detection methods, such as enrichment, qPCR, or sequencing-based techniques could bring more accurate results. *Salmonella* was only detected in one cecal sample from the group that did not receive PrimaLac (Table 4.3.). Two sided Fisher's exact test with a 5% significance level showed no significant differences between *Salmonella* or *Campylobacter* cecal colonization in birds treated with PrimaLac and the control group at slaughter. Furthermore, differences by sex were not observed in any of the groups and weeks (Table 4.4.). Replication of these trials will be needed in future studies.

C. coli K and *C. jejuni* TSKQ were recovered only in breeder hens (Fig. 4.2). *C. jejuni* pan-sensitive was recovered in both breeder hens and offspring (Fig. 4.2). *C. jejuni* T was

recovered in two turkeys from the control group (without PrimaLac) at week 20 (Fig. 4.2). Although *C. jejuni* T was not detected in ceca of hens at 65 weeks, it was isolated from fecal samples, ceca, and jejuna of breeder hens at other time points (Chapter 2). Another factor that could contribute to these results was the placement of the progeny into the breeder house from week 14 to week 20 (Fig. 4.1.). Besides, only one *Campylobacter* colony per plate was sub-cultured and that could also affect the results.

4.4.3. PFGE Analysis of Cecal Campylobacter Isolates

All the isolates from ceca of CP (n=16), and 17 isolates from IP (n=18, one positive sample was not recovered), obtained at week 16 were characterized by PFGE. All these isolates were pan-sensitive *C. jejuni*. Several cecal isolates, also pan-sensitive *C. jejuni*, obtained from the parent breeder hens at week 65 were included in the analysis to check genetic relatedness. Isolates were clustered in two different groups (A and B). In group A, there is a similar band pattern in all the subgroups (A1 to A4). In group B, there were three different band patterns, B1 and B2 (same), B3 and B4 and B5 (same) (Fig. 4.3.).

The same strains of *C. jejuni* were present in both groups of turkeys, IP and CP, as shown in subgroup A2, A3, B1, B2, B4 and B5 (Fig. 4.3.). There were two isolates analyzed from the same turkey (IP13-1 and IP13-2) with different band patterns and clustered in the different groups, B1 and A2 respectively (Fig. 4.3.). This observation suggests that turkeys can be colonized by different strains even though they have the same phenotypic characteristics. Also, the analysis of only one isolate per plate may be underrepresenting the

variability of the population present as indicated in previous studies [36]. The subgroup B3 was exclusively formed by isolates from the parent breeder hens from both groups, CH and IH. However, one isolate from the IH (IHC8) was found to be the same strain isolated in CP (CP11) and IP (IP3), suggesting a common exposure (Fig. 4.3.). As previously mentioned, the progeny was grown on a different house as the breeder parents until week 13, but it was moved to the same house from week 14 to 20.

4.5. CONCLUSIONS AND APPLICATIONS

1. *Salmonella* colonized the flock early but decreased in prevalence by slaughter age. In turn, *Campylobacter* entered the flock later but spread rapidly and persisted until processing. This is similar to what occurs in broilers.
2. In this study, prevalence of *Salmonella* in ceca was lower than *Campylobacter*.
3. Antimicrobial susceptibility profiles alone were not a good indicator of the diversity of *Campylobacter* populations.

4.6. ACKNOWLEDGMENTS

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Table 4.1. Minimum inhibitory concentration of antibiotics tested for *Campylobacter* isolates.

Antibiotic	Concentration (µg/mL)
Kanamycin[1]	25
Tetracycline[2]	16
Erythromycin[2]	10
Streptomycin[2]	15
Nalidixic Acid[1]	20
Ciprofloxacin[1]	4
Gentamicin[1]	200

[1] [37]; [2] [38].

Table 4.2. *Campylobacter* and *Salmonella* isolated from ceca and skin (sponge swabs) samples of progeny (CP and IP) at week 16.

	CONTROL PARENT FLOCK (CP)		**INOCULATED PARENT FLOCK (IP)**	
Sample	***Campylobacter* (%)**	***Salmonella* (%)**	***Campylobacter* (%)**	***Salmonella* (%)**
Ceca	16/20 (80)	2/20 (10)	18/20 (90)	0/20 (0)
Skin	1/10 (10)	0/20 (0)	1/20 (10)	0/20 (0)

Table 4.3. *Campylobacter* and *Salmonella* isolated from ceca of progeny slaughtered at week 20. Twenty turkeys received PrimaLac for two weeks prior to slaughter, the other 20 turkeys did not received the treatment (control).

Treatment	**Turkeys slaughtered per group**	**Week of slaughter**	***Campylobacter* (%)**	***Salmonella* (%)**
PrimaLac	20	20	8/20 (40)	0/20 (0)
Control	20	20	9/20 (45)	1/20 (5)

Table 4.4. *Campylobacter* and *Salmonella* isolated from ceca of progeny (hens and toms) hatched from control breeders (CP) and inoculated breeders (IP), at weeks 16 and 20.

		TOMS		**HENS**	
Parent Flock	**Week of slaughter**	***Campylobacter* (%)**	***Salmonella* (%)**	***Campylobacter* (%)**	***Salmonella* (%)**
Control (CP)	16	7/11 (63.6)	0/11 (0)	8/9 (88.8)	2/9 (22.2)
Inoculated (IP)	16	8/9 (88.8)	0/9 (0)	10/11(90.9)	0/11 (0)
Control (CP)[1]	20	4/7 (57.1)	0/7 (0)	5/13 (38.5)	1/13 (7.7)
Inoculated (CP)[2]	20	3/8 (37.5)	0/8 (0)	5/12 (41.7)	0/12 (0)

[1] PrimaLac treatment was applied for 2 weeks prior to slaughter.
[2] Control group, PrimaLac was not applied.

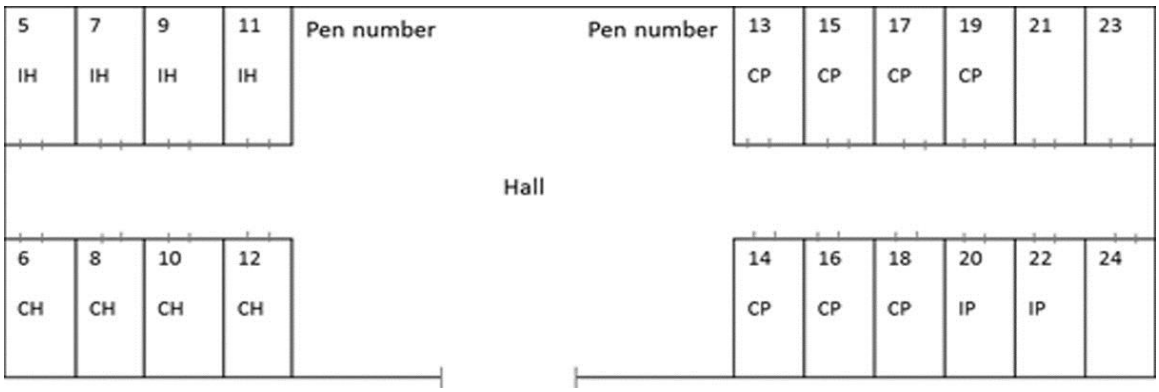

Fig. 4.1. Distribution of pens of poults hatched from control hens (CP) and inoculated hens (IP) in the breeder house at week 15. CH, Control Hens; IH, Inoculated Hens.

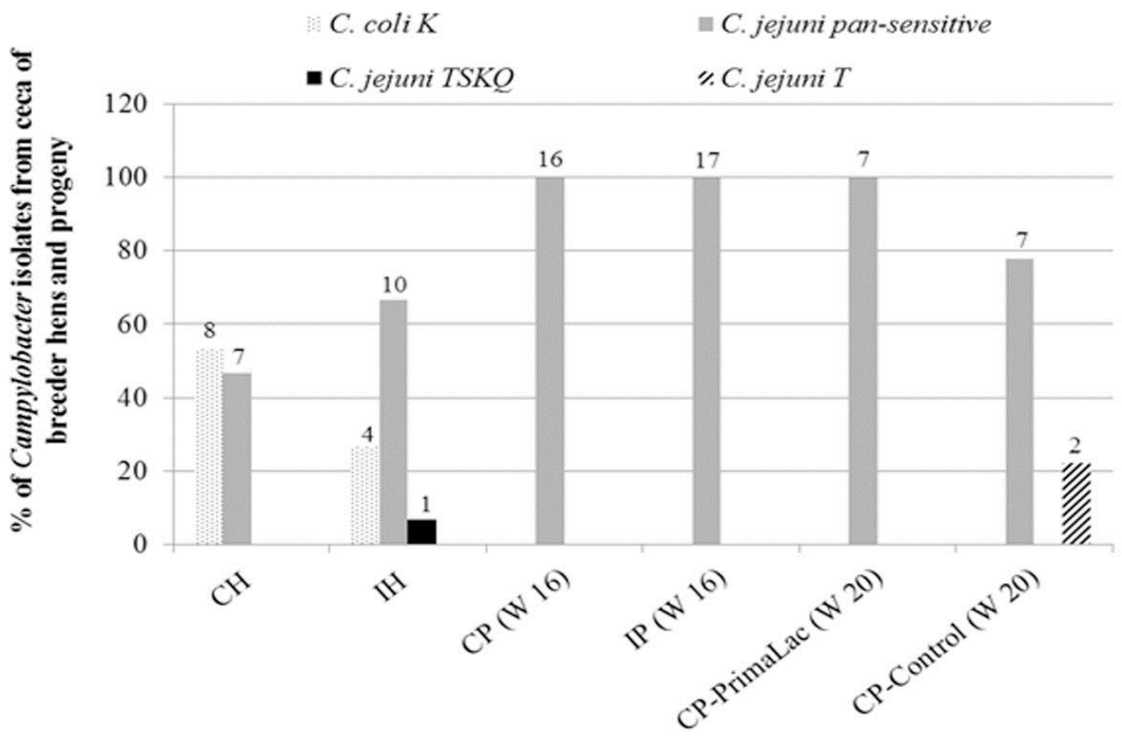

Fig. 4.2. Percentage of *Campylobacter* species and antibiotic susceptibility isolated from ceca of breeder hens at week 65 (week 19 of progeny), and progeny at week 16 and 20. Total *Campylobacter* isolates analyzed per group: CH=15, IH=15, CP (w16)=16, IP (w16)=17, CP-PrimaLac (w20)=7, CP-Control (w20)=9. CH, Control Hens; IH, Inoculated Hens; CP, Poults hatched from Control Hens; IP, Poults hatched from Inoculated Hens. T, Tetracycline; S, Streptomycin; K, Kanamycin; Q, Quinolones (Nalidixic acid and Ciprofloxacin).

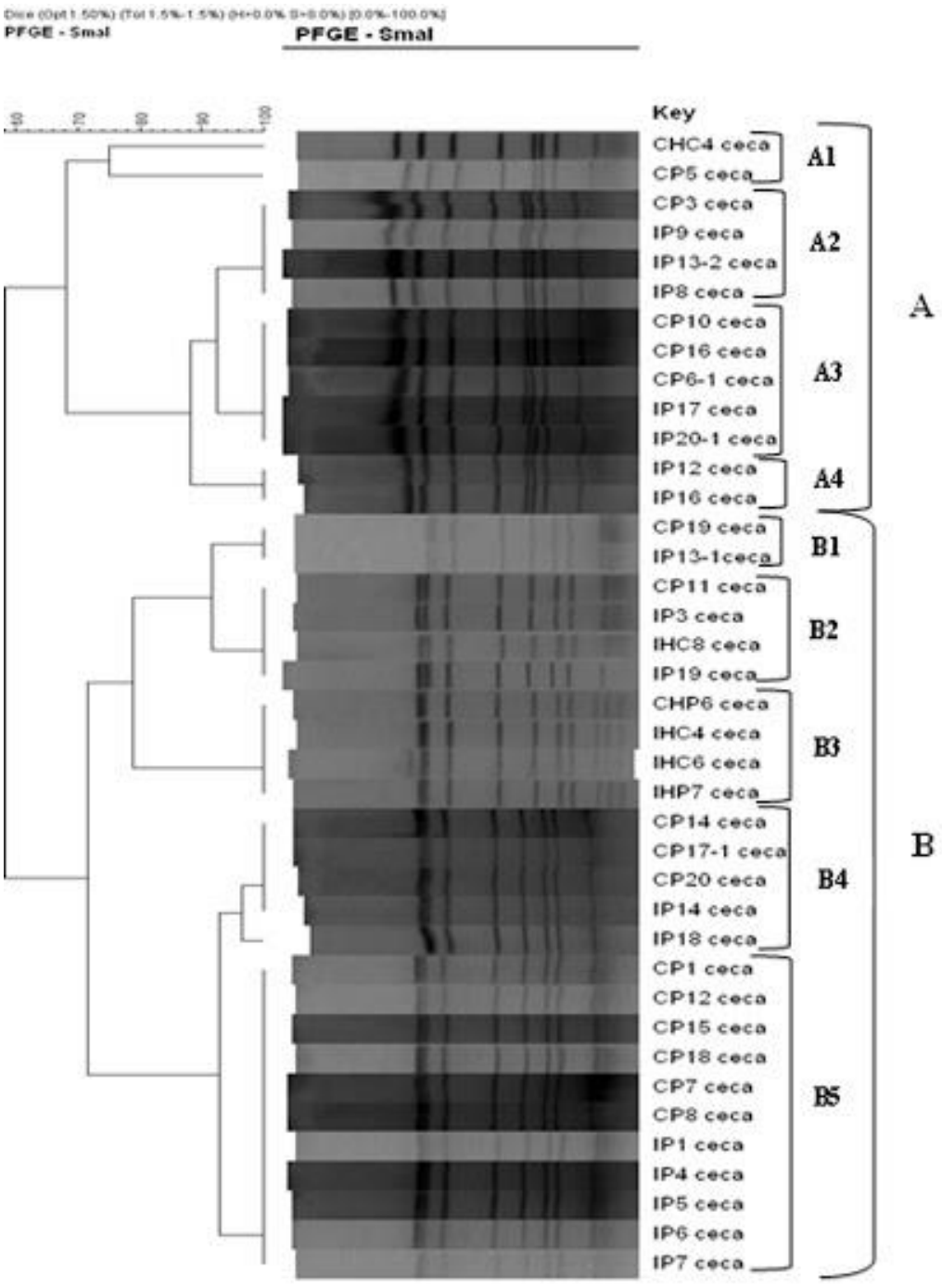

Fig. 4.3. PFGE band patterns of fecal and cecal isolates from both groups of poults (IP and CP), and ceca from breeder hens slaughter at 65 weeks. All isolates were pan-sensitive *C. jejuni*. Key refers to the identification (ID) name of the isolate. First two letters on the "key" identification refer to their origin: CH, Control Hens; IH, Inoculated Hens, CP, Poults hatched from Control Hens; IP, Poults hatched from Inoculated Hens.

4.7. REFERENCES AND NOTES

1. Domingues, A.R., S.M. Pires, T. Halasa, and T. Hald. 2012. Source attribution of human campylobacteriosis using a meta-analysis of case-control studies of sporadic infections. Epidemiol. Infect. 140: 970–981.

2. Lindmark, H., S. Boqvist, M. Ljungström, P. Agren, B. Björkholm, and L. Engstrand. 2009. Risk factors for campylobacteriosis: An epidemiological surveillance study of patients and retail poultry. J. Clin. Microbiol. 47: 2616–2619.

3. Kusumaningrum, H.D., E.D. van Asselt, R.R. Beumer, and M.H. Zwietering. 2004. A quantitative analysis of cross-contamination of *Salmonella* and *Campylobacter* spp. via domestic kitchen surfaces. J. Food Prot. 67: 1892–1903.

4. Luber, P., S. Brynestad, D. Topsch, K. Scherer, and E. Bartelt. 2006. Quantification of *Campylobacter* species cross-contamination during handling of contaminated fresh chicken parts in kitchens. Appl. Environ. Microbiol. 72: 66–70.

5. Scallan, E., R.M. Hoekstra, F.J. Angulo, R. V. Tauxe, M.A. Widdowson, S.L. Roy, J.L. Jones, and P.M. Griffin. 2011. Foodborne illness acquired in the United States — Major pathogens. Emerg. Infect. Dis. 17: 1–21.

6. Danguy des Deserts, J., R.H. Davies, K. Vaughan, I. Mclaren, P. Canning, A. Wintrip, D. Mueller-Doblies, and J.J. Carrique-Mas. 2011. A longitudinal study of *Salmonella* infection in different types of turkey flocks in Great Britain. Zoonoses Public Health. 58: 200–208.

7. Wesley, I. V., and W.T. Muraoka. 2009. Time of entry of *Salmonella* and *Campylobacter* into the turkey brooder house. Food Bioprocess Technol. 4: 616–623.

8. Jacobs-Reitsma, W.F., A.W. van de Giessen, N.M. Bolder, and R.W. Mulder. 1995. Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. Epidemiol. Infect. 114: 413–421.

9. Berrang, M.E., D.P. Smith, W.R. Windham, and P.W. Feldner. 2004. Effect of intestinal content contamination on broiler carcass *Campylobacter* counts. J. Food Prot. 67: 235–238.

10. Hue, O., V. Allain, M.J. Laisney, S. Le Bouquin, F. Lalande, I. Petetin, S. Rouxel, S. Quesne, P.-Y. Gloaguen, M. Picherot, J. Santolini, S. Bougeard, G. Salvat, and M. Chemaly. 2011. *Campylobacter* contamination of broiler caeca and carcasses at the slaughterhouse and correlation with *Salmonella* contamination. Food Microbiol. 28: 862–868.

11. Rivoal, K., M. Denis, G. Salvat, P. Colin, and G. Ermel. 1999. Molecular characterization of the diversity of *Campylobacter* spp. isolates collected from a poultry slaughterhouse: Analysis of cross-contamination. Lett. Appl. Microbiol. 29: 370–374.

12. Arsenault, J., A. Letellier, S. Quessy, V. Normand, and M. Boulianne. 2007. Prevalence and risk factors for *Salmonella* spp. and *Campylobacter* spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. Prev. Vet. Med. 81: 250–264.

13. Reich, F., V. Atanassova, E. Haunhorst, and G. Klein. 2008. The effects of *Campylobacter* numbers in caeca on the contamination of broiler carcasses with *Campylobacter*. Int. J. Food Microbiol. 127: 116–120.

14. Jeffrey, J.S., K.H. Tonooka, and J. Lozanot. 2001. Prevalence of *Campylobacter* spp. from skin, crop, and intestine of commercial broiler chicken carcasses at processing. Poult. Sci. 80: 1390–1392.

15. Logue, C.M., J.S. Sherwood, L.M. Elijah, P.A. Olah, and M.R. Dockter. 2003. The incidence of *Campylobacter* spp. on processed turkey from processing plants in the midwestern United States. J. Appl. Microbiol. 95: 234–241.

16. Stern, N.J., M.R. Clavero, J.S. Bailey, N.A. Cox, and M.C. Robach. 1995. *Campylobacter* spp. in broilers on the farm and after transport. Poult. Sci. 74: 937–941.

17. Berrang, M.E., R.J. Buhr, J.A. Cason, and J.A. Dickens. 2001. Broiler carcass contamination with *Campylobacter* from feces during defeathering. J. Food Prot. 64: 2063–2066.

18. Wempe, J.M., C.A. Genigeorgis, T.B. Farver, and H.I. Yusufu. 1983. Prevalence of *Campylobacter jejuni* in two California chicken processing plants. Appl. Environ. Microbiol. 45: 355–359.

19. Oxoid Ltd, Hampshire, England.

20. USDA-FSIS. Microbiology laboratory guidebook. Detection and enumeration method for *Campylobacter jejuni/coli* from poultry rinses and sponge samples (MLG 41.01). http://www.fsis.usda.gov/shared/PDF/MLG_41_01.pdf.

21. USDA-FSIS. Microbiology laboratory guidebook. Isolation and identification of *Salmonella* from meat, poultry, pasteurized egg and catfish products. http://www.fsis.usda.gov/shared/PDF/MLG_4_05.pdf.

22. Direct-fed microbials PrimaLac® Poultry W/S- Star labs. PO box 77- Clarksdale, MO.

23. Houng, H.S., O. Sethabutr, W. Nirdnoy, D.E. Katz, and L.W. Pang. 2001. Development of a ceuE-based multiplex polymerase chain reaction (PCR) assay for direct detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in Thailand. Diagn. Microbiol. Infect. Dis. 40: 11–19.

24. Marshall, S.M., P.L. Melito, D.L. Woodward, W.M. Johnson, F.G. Rodgers, and M.R. Mulvey. 1999. Rapid idenntification of *Campylobacter*, *Arcobacter*, and *Helicobacter* isolates by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene. J. Clin. Microbiol. 37: 4158–4160.

25. Gonzalez, I., K.A. Grant, P.T. Richardson, S.F. Park, and M.D. Collins. 1997. Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the ceuE gene encoding a putative virulence determinant. J. Clin. Microbiol. 35: 759–763.

26. Leader, B.T., J.G. Frye, J. Hu, P.J. Fedorka-Cray, and D.S. Boyle. 2009. High-throughput molecular determination of *Salmonella enterica* serovars by use of multiplex PCR and capillary electrophoresis analysis. J. Clin. Microbiol. 47: 1290–1299.

27. American Type Culture Collection.

28. New England BioLabs Inc.

29. JMP®, Version 11. SAS Institute Inc., Cary, NC.

30. BioNumerics, version 4.6, Applied Maths, Saint-Marten-Latem, Belgium.

31. Wallace, J.S., K.N. Stanley, J.E. Currie, P.J. Diggle, and K. Jones. 1997. Seasonality of thermophilic *Campylobacter* populations in chickens. J. Appl. Microbiol. 82: 219–224.

32. Chowdhury, S., G.E. Themudo, M. Sandberg, and A.K. Ersboll. 2012. Spatio-temporal patterns of *Campylobacter* colonization in Danish broilers. Epidemiol. Infect. : 1–12.

33. Cox, N.A., N.J. Stern, S.E. Craven, M.E. Berrang, and M.T. Musgrove. 2000. Prevalence of *Campylobacter* and *Salmonella* in the cecal droppings of turkeys during production. J. Appl. Poult. Res. 9: 542–545.

34. Wright, S.L., D.K. Carver, R.M. Siletzky, S. Romine, W.E.M. Morrow, and S. Kathariou. 2008. Longitudinal study of prevalence of *Campylobacter jejuni* and *Campylobacter coli* from turkeys and swine grown in close proximity. J. Food Prot. 71: 1791–1796.

35. Wallace, J.S., K.N. Stanley, and K. Jones. 1998. The colonization of turkeys by thermophilic campylobacters. J. Appl. Microbiol. 85: 224–230.

36. Jordan, J.S. 2013. Diversity of *Campylobacter jejuni* and *Campylobacter coli* in the ceca of individual turkeys. Thesis. North Carolina State University.

37. Fisher Biotech, Fair Lawn, NJ.

38. Sigma Chemical Co., St. Louis, MO.

APPENDICES

Appendix A

Table A.1. *Campylobacter* and *Salmonella* detection in fecal samples per group by week.

		Campylobacter				***Salmonella***			
Group/ Week		**IT**	**IH**	**CT**	**CH**	**IT**	**IH**	**CT**	**CH**
1		-	-	-	-	-	-	-	-
2	Weeks after Gavage	**Gavage with *Campylobacter* and *Salmonella* marker strains**							
3	1	Cj TSKQ	Cc GK	-	-	NAL^R	NAL^R	-	NO
4	2	Cj TSKQ	Cc GK	Cc K	Cc K	-	NAL^R	NA	NA
4	3	Cj TSKQ Cc GK	Cj TSKQ Cc GK	Cc GK	Cj TSKQ Cc GK	NAL^R	NAL^R	-	NO
5	4	Cj TSKQ Cc GK	Cc GK	Cj TSKQ Cc GK	Cj TSKQ	NAL^R	-	-	NO
6	5	Cj TSKQ	Cc GK	Cj TSKQ Cc GK	Cj TSKQ	NAL^R	NAL^R	-	NO
7	6	-	Cj TSKQ Cc GK Cj T	Cj TSKQ	+ (NC)	-	NO	-	NO
9	7	$-^{(BG)}$	$-^{(BG)}$	+ (NC)	+ (NC)	NAL^R	NO	NO	NO

Table A.1. Continued

10	8	- (BG)	- (BG)	+ (NC)	Cc K	NAL^R NO	NO	NO	NO
11	9	- (BG)	Cj TSKQ Cj T Cc K	-	Cc K	-	NO	NO	NO
11	10	- (BG)	Cj TSKQ	- (BG)	- (BG)	NAL^R	NO	NO	NO
12	**11**	**Gavage with *Campylobacter* and *Salmonella* marker strains**							
13	12	- (BG)	Cj TSKQ	- (BG)	- (BG)	NAL^R	NAL^R NO	NO	NO
14	13	- (BG)	Cj TSKQ	- (BG)	- (BG)	NAL^R	NAL^R	NO	NO
15	14	Cj TSKQ	Cj TSKQ	- (BG)	- (BG)	NAL^R	NAL^R	NO	NO
17	16	-	Cj TSKQ	- (BG)	- (BG)	NAL^R	NAL^R	NO	NO
19	18	Cj TSKQ Cj T	Cj TSKQ Cj T Cc K Cj P	Cc K	Cj T Cc K	NAL^R	NAL^R	NO	NO
21	20	Cj TSKQ Cc K	Cc K	Cc K	Cj P	-	NO	NO	-
23	22	Cj T	Cj T	+ (NC)	Cj T	-	-	NO	-

Table A.1. Continued

27	26	Cc K	Cc K	Cj T Cc K	Cc K	-	-	NO	-
32	30	Cj T Cc K	Cj T Cc K	Cj T	Cj T Cc K	-	-	NO	-
39	37	-	Cj T Cc K Cj P	-	+	-	-	+	-
61	60	NA	Cj T Cc K	NA	Cj T	NA	NAL^R	NA	-

IT, Inoculated Toms; IH, Inoculated Hens; CT, Control Toms; CH, Control Hens.
Cc, *C. coli;* Cj, *C. jejuni.* T, Tetracycline; S, Streptomycin; K, Kanamycin; Q, Quinolones (Nalidixic acid and Ciprofloxacin); G, Gentamicin; P, pan-sensitive; BG, Background microflora. NAL^R, Nalidixic acid resistant (marker *Salmonella* strains detected at least in one pen); NO, Naturally occurring; NA, Not analyzed; NC, Not characterized.

Table A.2. Fecal samples analyzed for *Salmonella*. Marker strains and naturally occurring serotypes detected.

				Salmo-nella	Marker strains NAL^R	Naturally occurring serotypes			
	Group	Source	Date	Pos (+) Neg (-)	Marker	*S.* Agona	*S.* Typhimu-rium	*S.* Liver-pool	NC
1	CH	Fecal	4/4/2012	+	-	+			
2	CH	Fecal	4/5/2012	+	-	+			
3	CH	Fecal	4/5/2012	+	-	+			
4	CH	Fecal	4/16/2012	+	-				+
5	CH	Fecal	4/23/2012	+	-				+
6	CH	Fecal	4/30/2012	+	-	+			
7	CH	Fecal	5/8/2012	+	-	+			
8	CH	Fecal	5/16/2012	+	-				+
9	CH	Fecal	5/25/2012	+	-	+			
10	CH	Fecal	5/30/2012	+	-				+
11	CH	Fecal	6/4/2012	+	-				+
12	CH	Fecal	6/18/2012	+	-	+			
13	CH	Fecal	6/18/2012	+	-	+			
14	CH	Fecal	6/25/2012	+	-				+
15	CH	Fecal	6/25/2012	+	-				+
16	CH	Fecal	7/2/2012	+	-				+
17	CH	Fecal	7/2/2012	+	-				+
18	CH	Fecal	7/16/2012	+	-				+
19	CH	Fecal	7/16/2012	+	-				+
20	CH	Fecal	7/31/2012	+	-				+
21	CH	Fecal	7/31/2012	+	-				+
22	CH	Fecal	8/14/2012	-	-				
23	CH	Fecal	8/27/2012	-	-				
24	CH	Fecal	8/27/2012	-	-				
25	CH	Fecal	9/25/2012	-	-				
26	CH	Fecal	9/25/2012	-	-				

Table A.2. Continued

27	CH	Fecal	10/26/2012	-	-				
28	CH	Fecal	10/26/2012	-	-				
29	CH	Fecal	12/14/2012	-	-				
30	CH	Fecal	12/14/2012	-	-				
31	CH	Fecal	2/12/2013	-	-				
32	CH	Fecal	2/12/2013	-	-				
33	CH	Fecal	2/12/2013	-	-				
34	CH	Fecal	5/28/2013	-	-				
35	CH	Fecal	5/28/2013	-	-				
36	CH	Fecal	5/28/2013	-	-				
1	CT	Fecal	4/4/2012	-	-				
2	CT	Fecal	4/16/2012	-	-				
3	CT	Fecal	4/23/2012	-	-				
4	CT	Fecal	4/30/2012	-	-				
5	CT	Fecal	5/8/2012	-	-				
6	CT	Fecal	5/16/2012	+	-	+			
7	CT	Fecal	5/25/2012	+	-	+			
8	CT	Fecal	5/30/2012	+	-				+
9	CT	Fecal	6/4/2012	+	-				+
10	CT	Fecal	6/18/2012	+	-	+			
11	CT	Fecal	6/25/2012	+	-				+
12	CT	Fecal	7/2/2012	+	-				+
13	CT	Fecal	7/16/2012	+	-				+
14	CT	Fecal	7/31/2012	+	-				+
15	CT	Fecal	8/14/2012	+	-	+			
16	CT	Fecal	8/27/2012	+	-	+			
17	CT	Fecal	9/25/2012	+	-	+			
18	CT	Fecal	10/26/2012	+	-	+			
19	CT	Fecal	12/14/2012	+	-				+
20	CT	Fecal	2/12/2013	-	-				
1	IH	Fecal	4/4/2012	+	S.E.				

Table A.2. Continued

2	IH	Fecal	4/4/2012	+	S.E.				
3	IH	Fecal	4/4/2012	+	S.E.				
4	IH	Fecal	4/11/2012	+	S.E.				
5	IH	Fecal	4/11/2012	+	S.E.				
6	IH	Fecal	4/11/2012	+	S.E.				
7	IH	Fecal	4/16/2012	+	S.E.				
8	IH	Fecal	4/16/2012	-	-				
9	IH	Fecal	4/16/2012	-	-				
10	IH	Fecal	4/23/2012	-	-				
11	IH	Fecal	4/23/2012	-	-				
12	IH	Fecal	4/23/2012	-	-				
13	IH	Fecal	4/30/2012	+	S.E.				
14	IH	Fecal	4/30/2012	+	S.E.				
15	IH	Fecal	4/30/2012	-	-				
16	IH	Fecal	5/8/2012	+	-	+			
17	IH	Fecal	5/8/2012	+	-	+			
18	IH	Fecal	5/8/2012	-	-				
19	IH	Fecal	5/16/2012	+	-				+
20	IH	Fecal	5/16/2012	+	-				+
21	IH	Fecal	5/16/2012	-	-				
22	IH	Fecal	5/25/2012	+	-	+			
23	IH	Fecal	5/25/2012	+	-	+			
24	IH	Fecal	5/25/2012	+	-	+			
25	IH	Fecal	5/25/2012	+	-			+	
26	IH	Fecal	5/30/2012	+	-				+
27	IH	Fecal	5/30/2012	+	-				+
28	IH	Fecal	5/30/2012	+	-				+
29	IH	Fecal	6/4/2012	+	-				+
30	IH	Fecal	6/4/2012	+	-				+
31	IH	Fecal	6/4/2012	-	-				
32	IH	Fecal	6/18/2012	+	S.E.				

Table A.2. Continued

33	IH	Fecal	6/18/2012	+	-	+			
34	IH	Fecal	6/18/2012	+	S.E.				
35	IH	Fecal	6/18/2012	+	S.E.				
36	IH	Fecal	6/18/2012	+	-	+			
37	IH	Fecal	6/18/2012	+	S.E.				
38	IH	Fecal	6/18/2012	+	-	+			
39	IH	Fecal	6/18/2012	+	S.E.				
40	IH	Fecal	6/18/2012	+	-	+			
41	IH	Fecal	6/18/2012	+	S.E.				
42	IH	Fecal	6/18/2012	+	-	+			
43	IH	Fecal	6/25/2012	+	S.E.				
44	IH	Fecal	6/25/2012	+	S.E.				
45	IH	Fecal	6/25/2012	+	S.E.				
46	IH	Fecal	6/25/2012	+	S.E.				
47	IH	Fecal	6/25/2012	+	S.E.				
48	IH	Fecal	6/25/2012	+	S.E.				
49	IH	Fecal	7/2/2012	+	S.E.				
50	IH	Fecal	7/2/2012	+	S.E.				
51	IH	Fecal	7/2/2012	+	S.E.				
52	IH	Fecal	7/2/2012	+	S.E.				
53	IH	Fecal	7/2/2012	+	S.E.				
54	IH	Fecal	7/2/2012	+	S.E.				
55	IH	Fecal	7/16/2012	-	-				
56	IH	Fecal	7/16/2012	+	S.E.				
57	IH	Fecal	7/16/2012	-	-				
58	IH	Fecal	7/16/2012	-	-				
59	IH	Fecal	7/16/2012	+	S.E.				
60	IH	Fecal	7/16/2012	-	-				
61	IH	Fecal	7/31/2012	-	-				
62	IH	Fecal	7/31/2012	-	-				
63	IH	Fecal	7/31/2012	+	S.E.				

Table A.2. Continued

64	IH	Fecal	7/31/2012	-	-				
65	IH	Fecal	7/31/2012	-	-				
66	IH	Fecal	7/31/2012	-	-				
67	IH	Fecal	8/14/2012	-	-				
68	IH	Fecal	8/14/2012	-	-				
69	IH	Fecal	8/14/2012	+	-				+
70	IH	Fecal	8/27/2012	-	-				
71	IH	Fecal	8/27/2012	-	-				
72	IH	Fecal	8/27/2012	-	-				
73	IH	Fecal	8/27/2012	-	-				
74	IH	Fecal	9/25/2012	-	-				
75	IH	Fecal	9/25/2012	-	-				
76	IH	Fecal	9/25/2012	-	-				
77	IH	Fecal	10/26/2012	-	-				
78	IH	Fecal	10/26/2012	-	-				
79	IH	Fecal	12/14/2012	-	-				
80	IH	Fecal	12/14/2012	-	-				
81	IH	Fecal	12/14/2012	-	-				
82	IH	Fecal	2/12/2013	-	-				
83	IH	Fecal	2/12/2013	-	-				
84	IH	Fecal	2/12/2013	-	-				
85	IH	Fecal	2/12/2013	-	-				
86	IH	Fecal	2/12/2013	-	-				
87	IH	Fecal	2/12/2013	-	-				
88	IH	Fecal	2/12/2013	-	-				
89	IH	Fecal	2/12/2013	-	-				
90	IH	Fecal	2/12/2013	-	-				
91	IH	Fecal	2/12/2013	-	-				
92	IH	Fecal	2/12/2013	-	-				
93	IH	Fecal	5/28/2013	+	S.E.				
94	IH	Fecal	5/28/2013	-	-				

Table A.2. Continued

95	IH	Fecal	5/28/2013	-	-				
96	IH	Fecal	5/28/2013	-	-				
1	IT	Fecal	4/4/2012	-	-				
2	IT	Fecal	4/5/2012	+	S.T.				
3	IT	Fecal	4/11/2012	-	-				
4	IT	Fecal	4/16/2012	+	S.T.				
5	IT	Fecal	4/23/2012	+	S.T.				
6	IT	Fecal	4/30/2012	+	S.T.				
7	IT	Fecal	5/8/2012	-	-				
8	IT	Fecal	5/16/2012	+	S.T.				
9	IT	Fecal	5/25/2012	+	S.T.				
10	IT	Fecal	5/25/2012	+	-	+			
11	IT	Fecal	5/30/2012	-	-				
12	IT	Fecal	6/4/2012	+	S.T.				
13	IT	Fecal	6/18/2012	+	-				
14	IT	Fecal	6/18/2012	+	-				
15	IT	Fecal	6/25/2012	+	S.T.				
16	IT	Fecal	6/25/2012	+	S.T.		+		
17	IT	Fecal	7/2/2012	+	S.T.		+		
18	IT	Fecal	7/2/2012	+	S.T.				
19	IT	Fecal	7/16/2012	+	S.T.				
20	IT	Fecal	7/16/2012	+	S.T.				

CH, Control Hens; CT, Control Toms; IH, Inoculated Hens; IT, Inoculated Toms.
S.E., *Salmonella* Enteritidis; S.T., *Salmonella* Typhimurium.

Table A.3. Fecal samples analyzed for *Campylobacter*. *Campylobacter* species and antibiotic resistance profile of isolates detected.

				Campylobacter			
	Group	Source	Date	Pos (+) Neg (-)	*C. coli*	*C. jejuni*	Antibiotic resistance profile
1	CH	Fecal	4/4/2012	-	-	-	
2	CH	Fecal	4/11/2012	+	+	-	K
3	CH	Fecal	4/16/2012	+	-	+	TSKQ
4	CH	Fecal	4/16/2012	+	+	-	GK
5	CH	Fecal	4/23/2012	+	-	+	TSKQ
6	CH	Fecal	4/30/2012	+	-	+	TSKQ
7	CH	Fecal	5/8/2012	+	NC	NC	NO
8	CH	Fecal	5/16/2012	+	NC	NC	NC
9	CH	Fecal	5/25/2012	+	+	-	K
10	CH	Fecal	5/30/2012	+	+	-	K
11	CH	Fecal	6/4/2012	-	-	-	
12	CH	Fecal	6/18/2012	-	-	-	
13	CH	Fecal	6/18/2012	-	-	-	
14	CH	Fecal	6/25/2012	-	-	-	
15	CH	Fecal	6/25/2012	-	-	-	
16	CH	Fecal	7/2/2012	-	-	-	
17	CH	Fecal	7/2/2012	-	-	-	
18	CH	Fecal	7/16/2012	-	-	-	
19	CH	Fecal	7/16/2012	-	-	-	
20	CH	Fecal	7/31/2012	+	-	+	T
21	CH	Fecal	7/31/2012	+	-	+	T
22	CH	Fecal	7/31/2012	+	+	-	K
23	CH	Fecal	7/31/2012	+	+	-	K
24	CH	Fecal	8/14/2012	+	-	+	P
25	CH	Fecal	8/27/2012	+	-	+	T
26	CH	Fecal	8/27/2012	+	-	+	T
27	CH	Fecal	9/25/2012	+	+	-	K

Table A.3. Continued

28	CH	Fecal	9/25/2012	+	-	+	T
29	CH	Fecal	10/26/2012	+	+	-	K
30	CH	Fecal	10/26/2012	+	-	+	T
31	CH	Fecal	12/14/2012	+	NC	NC	NC
32	CH	Fecal	12/14/2012	+	NC	NC	NC
33	CH	Fecal	2/12/2013	+	-	+	T
34	CH	Fecal	2/12/2013	+	+	-	K
35	CH	Fecal	2/12/2013	+	-	+	T
36	CH	Fecal	5/20/2013	+	-	+	T
37	CH	Fecal	5/20/2013	-	-	-	
38	CH	Fecal	5/20/2013	+	-	+	P
1	CT	Fecal	4/4/2012	-	-	-	
2	CT	Fecal	4/11/2012	+	+	-	K
3	CT	Fecal	4/16/2012	+	+	-	GK
4	CT	Fecal	4/16/2012	+	+	-	GK
5	CT	Fecal	4/23/2012	+	-	+	TSKQ
6	CT	Fecal	4/23/2012	+	+	-	GK
7	CT	Fecal	4/30/2012	+	-	+	TSKQ
8	CT	Fecal	4/30/2012	+	+	-	GK
9	CT	Fecal	5/8/2012	+	-	+	TSKQ
10	CT	Fecal	5/16/2012	+	NC	NC	NC
11	CT	Fecal	5/25/2012	+	NC	NC	NC
12	CT	Fecal	5/30/2012	-	-	-	
13	CT	Fecal	6/4/2012	-	-	-	
14	CT	Fecal	6/18/2012	-	-	-	
15	CT	Fecal	6/25/2012	-	-	-	
16	CT	Fecal	7/2/2012	-	-	-	
17	CT	Fecal	7/16/2012	-	-	-	
18	CT	Fecal	7/31/2012	+	+	-	K
19	CT	Fecal	7/31/2012	+	+	-	K
20	CT	Fecal	7/31/2012	+	+	-	K

Table A.3. Continued

21	CT	Fecal	8/14/2012	+	+	-	K
22	CT	Fecal	8/27/2012	+	NC	NC	NC
23	CT	Fecal	9/25/2012	+	+	-	K
24	CT	Fecal	9/25/2012	+	-	+	T
25	CT	Fecal	10/26/2012	+	-	+	T
26	CT	Fecal	12/14/2012	-	-	-	
27	CT	Fecal	2/12/2013	+	+	-	K
1	IH	Fecal	4/4/2012	+	+	-	GK
2	IH	Fecal	4/4/2012	+	+	-	GK
3	IH	Fecal	4/4/2012	+	+	-	GK
4	IH	Fecal	4/11/2012	+	+	-	GK
5	IH	Fecal	4/11/2012	+	+	-	GK
6	IH	Fecal	4/11/2012	+	+	-	GK
7	IH	Fecal	4/16/2012	+	+	-	GK
8	IH	Fecal	4/16/2012	+	+	-	GK
9	IH	Fecal	4/16/2012	+	+	-	GK
10	IH	Fecal	4/16/2012	+	-	+	TSKQ
11	IH	Fecal	4/16/2012	+	-	+	TSKQ
12	IH	Fecal	4/16/2012	+	-	+	TSKQ
13	IH	Fecal	4/23/2012	+	+	-	GK
14	IH	Fecal	4/23/2012	+	+	-	GK
15	IH	Fecal	4/23/2012	+	+	-	GK
16	IH	Fecal	4/30/2012	+	+	-	GK
17	IH	Fecal	4/30/2012	-	-	-	
18	IH	Fecal	4/30/2012	-	-	-	
19	IH	Fecal	5/8/2012	+	-	+	TSKQ
20	IH	Fecal	5/8/2012	+	+	-	GK
21	IH	Fecal	5/8/2012	+	-	+	T
22	IH	Fecal	5/8/2012	+	-	+	TSKQ
23	IH	Fecal	5/16/2012	-	-	-	
24	IH	Fecal	5/16/2012	-	-	-	

Table A.3. Continued

25	IH	Fecal	5/16/2012	-	-	-	
26	IH	Fecal	5/25/2012	-	-	-	
27	IH	Fecal	5/25/2012	-	-	-	
28	IH	Fecal	5/25/2012	-	-	-	
29	IH	Fecal	5/30/2012	+	+	-	K
30	IH	Fecal	5/30/2012	+	-	+	T
31	IH	Fecal	5/30/2012	+	-	+	TSKQ
32	IH	Fecal	6/4/2012	-	-	-	
33	IH	Fecal	6/4/2012	+	NC	NC	NO
34	IH	Fecal	6/4/2012	+	-	+	TSKQ
35	IH	Fecal	6/18/2012	-	-	-	
36	IH	Fecal	6/18/2012	-	-	-	
37	IH	Fecal	6/18/2012	-	-	-	
38	IH	Fecal	6/18/2012	-	-	-	
39	IH	Fecal	6/18/2012	+	-	+	TSKQ
40	IH	Fecal	6/18/2012	+	-	+	TSKQ
41	IH	Fecal	6/25/2012	-	-	-	
42	IH	Fecal	6/25/2012	-	-	-	
43	IH	Fecal	6/25/2012	-	-	-	
44	IH	Fecal	6/25/2012	-	-	-	
45	IH	Fecal	6/25/2012	+	-	+	TSKQ
46	IH	Fecal	6/25/2012	+	-	+	TSKQ
47	IH	Fecal	7/2/2012	+	-	+	TSKQ
48	IH	Fecal	7/2/2012	+	-	+	TSKQ
49	IH	Fecal	7/2/2012	+	-	+	TSKQ
50	IH	Fecal	7/2/2012	+	-	+	TSKQ
51	IH	Fecal	7/2/2012	+	-	+	TSKQ
52	IH	Fecal	7/2/2012	+	-	+	TSKQ
53	IH	Fecal	7/16/2012	-	-	-	
54	IH	Fecal	7/16/2012	-	-	-	
55	IH	Fecal	7/16/2012	-	-	-	

Table A.3. Continued

56	IH	Fecal	7/16/2012	-	-	-	
57	IH	Fecal	7/16/2012	+	-	+	TSKQ
58	IH	Fecal	7/16/2012	-	-	-	
59	IH	Fecal	7/31/2012	+	+	-	K
60	IH	Fecal	7/31/2012	+	-	+	P
61	IH	Fecal	7/31/2012	+	-	+	T
62	IH	Fecal	7/31/2012	+	-	+	T
63	IH	Fecal	7/31/2012	+	-	+	T
64	IH	Fecal	7/31/2012	+	-	+	T
65	IH	Fecal	7/31/2012	+	-	+	TSKQ
66	IH	Fecal	7/31/2012	+	-	+	T
67	IH	Fecal	7/31/2012	+	-	+	TSKQ
68	IH	Fecal	8/14/2012	+	+	-	K
69	IH	Fecal	8/14/2012	+	+	-	K
70	IH	Fecal	8/14/2012	+	+	-	K
71	IH	Fecal	8/27/2012	+	-	+	NC
72	IH	Fecal	8/27/2012	+	-	+	NC
73	IH	Fecal	8/27/2012	+	NC	NC	NC
74	IH	Fecal	8/27/2012	+	NC	NC	NC
75	IH	Fecal	9/25/2012	+	+	-	K
76	IH	Fecal	9/25/2012	+	+	-	K
77	IH	Fecal	9/25/2012	+	+	-	K
78	IH	Fecal	10/26/2012	+	+	-	K
79	IH	Fecal	10/26/2012	+	-	+	T
80	IH	Fecal	12/14/2012	+	+	-	K
81	IH	Fecal	12/14/2012	+	-	+	T
82	IH	Fecal	12/14/2012	+	-	+	P
83	IH	Fecal	12/14/2012	+	+	-	K
84	IH	Fecal	12/14/2012	+	-	+	P
85	IH	Fecal	12/14/2012	+	-	+	T
86	IH	Fecal	2/12/2013	+	NC	NC	NC

Table A.3. Continued

87	IH	Fecal	2/12/2013	+	-	+	T
88	IH	Fecal	2/12/2013	+	+	-	K
89	IH	Fecal	2/12/2013	+	+	-	K
90	IH	Fecal	2/12/2013	+	-	+	T
91	IH	Fecal	2/12/2013	+	NC	NC	NC
92	IH	Fecal	2/12/2013	+	NC	NC	NC
93	IH	Fecal	2/12/2013	+	+	-	K
94	IH	Fecal	2/12/2013	+	NC	NC	NC
95	IH	Fecal	2/12/2013	+	+	-	K
96	IH	Fecal	5/20/2013	-	-	-	
97	IH	Fecal	5/20/2013	+	+	-	K
98	IH	Fecal	5/20/2013	-	-	-	
99	IH	Fecal	5/20/2013	+	-	+	T
1	IT	Fecal	4/4/2012	+	-	+	TSKQ
2	IT	Fecal	4/11/2012	+	-	+	TSKQ
3	IT	Fecal	4/16/2012	+	-	+	TSKQ
4	IT	Fecal	4/16/2012	+	+	-	GK
5	IT	Fecal	4/23/2012	+	+	-	GK
6	IT	Fecal	4/23/2012	+	-	+	TSKQ
7	IT	Fecal	4/30/2012	+	-	+	TSKQ
8	IT	Fecal	5/8/2012	-	-	-	
9	IT	Fecal	5/16/2012	-	-	-	
10	IT	Fecal	5/25/2012	-	-	-	
11	IT	Fecal	5/30/2012	-	-	-	
12	IT	Fecal	6/4/2012	-	-	-	
13	IT	Fecal	6/18/2012	-	-	-	
14	IT	Fecal	6/18/2012	-	-	-	
15	IT	Fecal	6/25/2012	-	-	-	
16	IT	Fecal	6/25/2012	-	-	-	
17	IT	Fecal	7/2/2012	+	-	+	TSKQ
18	IT	Fecal	7/2/2012	+	-	+	TSKQ

Table A.3. Continued

19	IT	Fecal	7/16/2012	-	-	-	
20	IT	Fecal	7/16/2012	-	-	-	
21	IT	Fecal	7/31/2012	+	-	+	T
22	IT	Fecal	7/31/2012	+	-	+	T
23	IT	Fecal	7/31/2012	+	-	+	TSKQ
24	IT	Fecal	7/31/2012	+	-	+	TSKQ
25	IT	Fecal	7/31/2012	+	-	+	TSKQ
26	IT	Fecal	7/31/2012	+	-	+	TSKQ
27	IT	Fecal	8/14/2012	+	-	+	TSKQ
28	IT	Fecal	8/14/2012	+	+	-	K
29	IT	Fecal	8/27/2012	+	-	+	T
30	IT	Fecal	8/27/2012	-	-	-	
31	IT	Fecal	9/25/2012	+	+	-	K
32	IT	Fecal	9/25/2012	+	+	-	K
33	IT	Fecal	10/26/2012	+	+	-	K
34	IT	Fecal	10/26/2012	+	-	+	T
35	IT	Fecal	12/14/2012	-	-	-	
36	IT	Fecal	2/12/2013	+	+	-	K

IT, Inoculated Toms; IH, Inoculated Hens; CT, Control Toms; CH, Control Hens.
T, Tetracycline; S, Streptomycin; K, Kanamycin; Q, Quinolones (Nalidixic acid and Ciprofloxacin); G, Gentamicin; P, Pan-sensitive; NC, Not characterized

Table A.4. *Campylobacter* and *Salmonella* detected in blood, intestines (cecum, jejunum) and organs (spleen, liver and gallbladder). *Campylobacter* species and antibiotic resistance profile of isolates detected.

				Campylobacter				***Salmonella***
	Group	Source	Date	Pos (+) Neg (-)	*C. coli*	*C. jejuni*	Antibiotic resistance profile	Pos (+) Neg (-)
1	IH 1	Blood	6/4/12	-	-	-		-
2	IH 2	Blood	6/4/12	-	-	-		-
3	IH 3	Blood	6/4/12	-	-	-		-
4	IH 4	Blood	6/4/12	-	-	-		-
5	IH 5	Blood	6/4/12	-	-	-		-
6	IH 6	Blood	6/4/12	-	-	-		-
7	IH 7	Blood	6/4/12	-	-	-		-
8	IH 8	Blood	6/4/12	-	-	-		-
9	IH 9	Blood	6/4/12	-	-	-		-
10	IH 1	Cecum	6/4/12	-	-	-		+[1]
11	IH 2	Cecum	6/4/12	-	-	-		-
12	IH 3	Cecum	6/4/12	-	-	-		-
13	IH 1	Jejunum	6/4/12	-	-	-		-
14	IH 2	Jejunum	6/4/12	-	-	-		-
15	IH 3	Jejunum	6/4/12	-	-	-		-
16	IH 2	Spleen	6/4/12	-	-	-		-
17	IH 3	Spleen	6/4/12	-	-	-		-
18	IH 1	Liver	6/4/12	-	-	-		-
19	IH 2	Liver	6/4/12	-	-	-		-
20	IH 3	Liver	6/4/12	-	-	-		-
21	IH 1	Blood	8/14/12	-	-	-		-
22	IH 1	Cecum	8/14/12	-	-	-		-
23	IH 1	Jejunum	8/14/12	-	-	-		-
24	IH 1	Spleen	8/14/12	-	-	-		-
25	IH 1	Liver	8/14/12	-	-	-		-

Table A.4. Continued

26	IH 1	Blood	8/27/12	-	-	-		-
27	IH 1	Cecum	8/27/12	+	+	-	K	-
28	IH 1	Cecum	8/27/12	+	+	-	K	-
29	IH 1	Jejunum	8/27/12	+	-	+	P	-
30	IH 1	Spleen	8/27/12	-	-	-		-
31	IH 1	Liver	8/27/12	-	-	-		-
32	IH 13	Cecum	3/28/13	+	+	-	K	+[2]
33	IH 13	Jejunum	3/28/13	-	-	-		-
34	IH 13	Liver	3/28/13	-	-	-		-
35	IH 14	Cecum	3/28/13	+	-	+	T	-
36	IH 14	Jejunum	3/28/13	+	-	+	T	-
37	IH 14	Liver	3/28/13	-	-	-		-
38	IH 15	Cecum	3/28/13	+	+	-	K	-
39	IH 15	Jejunum	3/28/13	-	-	-		-
40	IH 15	Liver	3/28/13	-	-	-		-
41	IH 16	Cecum	3/28/13	+	-	+	T	-
42	IH 16	Jejunum	3/28/13	-	-	-		-
43	IH 16	Liver	3/28/13	-	-	-		-
44	IH 17	Cecum	3/28/13	+	+	-	K	-
45	IH 17	Jejunum	3/28/13	-	-	-		-
46	IH 17	Liver	3/28/13	-	-	-		-
47	IH 1-1	Cecum	6/18/13	+	-	+	P	+[2]
48	IH 1-2	Cecum	6/19/13	+	-	+	P	-
49	IH 2	Cecum	6/18/13	-	-	-		+[2]
50	IH 3-1	Cecum	6/18/13	+	-	+	P	-
51	IH 3-2	Cecum	6/19/13	+	-	+	P	-
52	IH 4	Cecum	6/18/13	+	-	+	P	-
53	IH 5	Cecum	6/18/13	+	+	-	K	-
54	IH 6	Cecum	6/18/13	+	-	+	NC	-
55	IH 7	Cecum	6/18/13	+	-	+	TSKQ	-
56	IH 8	Cecum	6/18/13	+		+	P	-

Table A.4. Continued

57	IH 9-1	Cecum	6/18/13	+	+	-	K	-
58	IH 9-2	Cecum	6/19/13	+	+	-	K	-
59	IH 10	Cecum	6/18/13	+	-	+	P	-
60	IH 11	Cecum	6/18/13	+	+	-	K	-
61	IH 12	Cecum	6/18/13	-	-	-		-
62	IH 13	Cecum	6/18/13	-	-	-		-
63	IH 14	Cecum	6/18/13	+	+	-	K	-
64	IH 15	Cecum	6/18/13	+	-	+	P	-
65	IH 16	Cecum	6/18/13	+	-	+	P	-
66	IH 17	Cecum	6/18/13	+	-	+	P	-
67	IH 18	Cecum	6/18/13	-	-	-		-
68	IH 19	Cecum	6/18/13	-	-	-		-
69	IH 20	Cecum	6/18/13	+	-	+	P	-
1	IT 1	Blood	6/4/12	-	-	-		-
2	IT 2	Blood	6/4/12	-	-	-		-
3	IT 3	Blood	6/4/12	-	-	-		-
4	IT 1	Cecum	6/4/12	-	-	-		-
5	IT 1	Jejunum	6/4/12	-	-	-		-
6	IT 1	Spleen	6/4/12	-	-	-		-
7	IT 1	Liver	6/4/12	-	-	-		-
8	IT 1	Cecum	9/11/12	+	+	-	K	-
9	IT 1	Jejunum	9/11/12	+	-	+	T	-
10	IT 1	Spleen	9/11/12	-	-	-		-
11	IT 1	Liver	9/11/12	-	-	-		-
12	IT 1	Cecum	9/25/12	+	+	-	K	-
13	IT 1	Cecum	9/25/12	+	+	-	K	-
14	IT 1	Jejunum	9/25/12	+	-	+	T	-
15	IT 1	Jejunum	9/25/12	+	-	+	T	-
16	IT 1	Spleen	9/25/12	-	-	-		-
17	IT 1	Liver	9/25/12	-	-	-		-
18	IT 1	Cecum	11/8/12	+	+	-	K	-

Table A.4. Continued

19	IT 1	Cecum	11/8/12	+	+	-	K	-
20	IT 1	Cecum	11/8/12	+	+	-	K	-
21	IT 1	Jejunum	11/8/12	+	+	-	K	-
22	IT 1	Spleen	11/8/12	-	-	-		-
23	IT 1	Liver	11/8/12	-	-	-		-
24	IT 2	Cecum	11/8/12	+	+	-	K	-
25	IT 2	Jejunum	11/8/12	-	-	-		-
26	IT 2	Spleen	11/8/12	-	-	-		-
27	IT 2	Liver	11/8/12	-	-	-		-
28	IT 60	Cecum	12/14/12	+	+	-	K	-
29	IT 60	Jejunum	12/14/12	-	-	-		-
30	IT 60	Liver	12/14/12	-	-	-		-
31	IT 60	Spleen	12/14/12	-	-	-		-
32	IT 61	Cecum	12/14/12	+	NC	NC	NC	-
33	IT 61	Jejunum	12/14/12	-	-	-		-
34	IT 61	Liver	12/14/12	-	-	-		-
35	IT 61	Spleen	12/14/12	-	-	-		-
36	IT 65	Cecum	3/6/13	+	+	-	K	+[1]
37	IT 65	Jejunum	3/6/13	-	-	-		-
38	IT 68	Cecum	3/6/13	+	+	-	K	-
39	IT 68	Jejunum	3/6/13	-	-	-		-
1	CH 1	Blood	8/14/12	-	-	-		-
2	CH 1	Cecum	8/14/12	-	-	-		-
3	CH 1	Jejunum	8/14/12	-	-	-		-
4	CH 1	Liver	8/14/12	+	-	+	T	-
5	CH 1	Blood	8/27/12	-	-	-		-
6	CH 1	Cecum	8/27/12	+	+	-	K	-
7	CH 1	Cecum	8/27/12	+	+	-	K	-
8	CH 1	Jejunum	8/27/12	+	-	+	T	-
9	CH 1	Jejunum	8/27/12	+	-	+	T	-
10	CH 1	Spleen	8/27/12	-	-	-		-

Table A.4. Continued

11	CH 1	Liver	8/27/12	-	-	-		-
12	CH 1	Cecum	6/18/13	+	+	-	K	-
13	CH 2	Cecum	6/18/13	+	+	-	K	-
14	CH 3	Cecum	6/18/13	-	-	-		-
15	CH 4	Cecum	6/18/13	+	-	+	P	-
16	CH 5	Cecum	6/18/13	+	-	+	P	-
17	CH 6	Cecum	6/18/13	-	-	-		-
18	CH 7	Cecum	6/18/13	+	-	+	P	-
19	CH 8	Cecum	6/18/13	+	+	-	K	-
20	CH 9	Cecum	6/18/13	+	+	-	K	-
21	CH 10	Cecum	6/18/13	+	-	+	P	-
22	CH 11	Cecum	6/18/13	+	-	+	P	-
23	CH 12	Cecum	6/18/13	-	-	-		-
24	CH 13	Cecum	6/18/13	-	-	-		-
25	CH 14	Cecum	6/18/13	+	+	-	K	-
26	CH 15-1	Cecum	6/18/13	+	+	-	K	-
27	CH 15-2	Cecum	6/19/13	+	+	-	K	-
28	CH 16	Cecum	6/18/13	+	-	+	P	-
29	CH 17	Cecum	6/18/13	+	-	+	P	-
30	CH 18	Cecum	6/18/13	-	-	-		-
31	CH 19	Cecum	6/18/13	+	-	+	P	-
32	CH 20	Cecum	6/18/13	+	+	-	K	-
1	CT 1	Cecum	11/8/12	+	+	-	K	-
2	CT 1	Jejunum	11/8/12	+	-	+	T	-
3	CT 1	Spleen	11/8/12	-	-	-		-
4	CT 1	Liver	11/8/12	-	-	-		-
5	CT 3	Cecum	12/14/12	+	+	-	NC	-
6	CT 3	Cecum	12/14/12	+	+	-	NC	-
7	CT 3	Jejunum	12/14/12	-	-	-		+[1]
8	CT 3	Liver	12/14/12	-	-	-		-
9	CT 3	Spleen	12/14/12	-	-	-		-

Table A.4. Continued

10	CT 4	Cecum	12/14/12	+	NC	NC	NC	-
11	CT 4	Jejunum	12/14/12	-	-	-		-
12	CT 4	Liver	12/14/12	-	-	-		-
13	CT 4	Spleen	12/14/12	-	-	-		-
14	CT 8	Cecum	12/14/12	+	NC	NC	NC	-
15	CT 8	Jejunum	12/14/12	-	-	-		-
16	CT 8	Liver	12/14/12	-	-	-		-
17	CT 8	Spleen	12/14/12	-	-	-		-
18	CT 2	Cecum	3/6/13	+	+	-	K	+[1]
19	CT 2	Jejunum	3/6/13	-	-	-		-
20	CT 5	Cecum	3/6/13	+	+	-	K	+[1]
21	CT 5	Jejunum	3/6/13	-	-	-		-

[1] *S.* Agona
[2] *S.* Enteritidis NAL^R (marker)
IT, Inoculated Toms; IH, Inoculated Hens; CT, Control Toms; CH, Control Hens. Numbers indicate tag number, pen number, or order in which turkeys were processed. T, Tetracycline; S, Streptomycin; K, Kanamycin; Q, Quinolones (Nalidixic acid and Ciprofloxacin); G, Gentamicin; P, Pan-sensitive; NC, Not characterized.

Table A.5. *Campylobacter* and *Salmonella* detected in reproductive tissue. Testes from toms and reproductive tract of hens (Follicles and ovary, and upper segment from the infundibulum to the isthmus).

					Campylobacter			***Salmonella***
	Group	Source	Date	Pos (+) Neg (-)	*C. coli*	*C. jejuni*	Antibiotic resistance profile	Pos (+) Neg (-)
1	CT1	Testes	11/8/12	-	-	-	-	-
2	IT1	Testes	11/8/12	-	-	-		-
3	IT2	Testes	11/8/12	-	-	-		-
4	CT3	Testes	12/14/12	-	-	-		-
5	CT8	Testes	12/14/12	-	-	-		-
6	CT8	Testes	12/14/12	-	-	-		-
7	IT60	Testes	12/14/12	-	-	-		-
8	IT61	Testes	12/14/12	-	-	-		-
9	IH 1	Follicles	8/14/12	-	-	-		-
10	IH 1	R.T. Upper	6/18/13	-	-	-		-
11	IH 2	R.T. Upper	6/18/13	-	-	-		-
12	IH 3	R.T. Upper	6/18/13	-	-	-		-
13	IH 4	R.T. Upper	6/18/13	-	-	-		-
14	IH 5	R.T. Upper	6/18/13	-	-	-		-
15	IH 6	R.T. Upper	6/18/13	+	-	+	P	-
16	IH 7-1	R.T. Upper	6/18/13	+	-	+	P	-
17	IH 7-2	R.T. Upper	6/18/13	+	-	+	P	-
18	IH 8	R.T. Upper	6/18/13	-	-	-		-
19	IH 10	R.T. Upper	6/18/13	-	-	-		-
20	IH 1	Follicles	6/18/13	-	-	-		-
21	IH 2	Follicles	6/18/13	-	-	-		-
22	IH 3	Follicles	6/18/13	-	-	-		-
23	IH 4	Follicles	6/18/13	-	-	-		+[1]
24	IH 5	Follicles	6/18/13	-	-	-		-
25	IH 6	Follicles	6/18/13	-	-	-		-

Table A.5. Continued

26	IH 7	Follicles	6/18/13	-	-	-		+[1]
27	IH 8	Follicles	6/18/13	-	-	-		+[1]
28	IH 9	Follicles	6/18/13	-	-	-		-
29	IH 10	Follicles	6/18/13	-	-	-		-
30	CH 1	R.T. Upper	6/18/13	-	-	-		-
31	CH 2	R.T. Upper	6/18/13	-	-	-		-
32	CH 3	R.T. Upper	6/18/13	-	-	-		-
33	CH 4-1	R.T. Upper	6/18/13	+	-	+	P	-
34	CH 4-2	R.T. Upper	6/19/13	+	-	+	P	-
35	CH 5	R.T. Upper	6/18/13	-	-	-		-
36	CH 6	R.T. Upper	6/18/13	-	-	-		-
37	CH 7	R.T. Upper	6/18/13	-	-	-		-
38	CH 8	R.T. Upper	6/18/13	-	-	-		-
39	CH 9	R.T. Upper	6/18/13	-	-	-		-
40	CH 10	R.T. Upper	6/18/13	-	-	-		-
41	CH 1	Follicles	6/18/13	-	-	-		-
42	CH 2	Follicles	6/18/13	-	-	-		-
43	CH 3	Follicles	6/18/13	-	-	-		-
44	CH 4	Follicles	6/18/13	-	-	-		-
45	CH 5	Follicles	6/18/13	-	-	-		-
46	CH 6	Follicles	6/18/13	-	-	-		-
47	CH 7	Follicles	6/18/13	-	-	-		-
48	CH 8	Follicles	6/18/13	-	-	-		-
49	CH 9	Follicles	6/18/13	-	-	-		-
50	CH 10	Follicles	6/18/13	-	-	-		-

[1] *S.* Enteritidis NAL^R (marker strain)
CT, Control Toms; IT, Inoculated Toms; CH, Control Hens; IH, Inoculated Hens.
Numbers indicate tag number or order in which turkeys were processed. P, Pan-sensitive.