GENETIC DIVERSITY OF *CAMPYLOBACTER* ON BROILER CARCASSES

by

SHAYLA HUNTER

(Under the Direction of Mark E. Berrang)

ABSTRACT

Campylobacter jejuni and *C. coli* are the most important human enteropathogens among the campylobacters. The objective of this study was to determine how diversity in *Campylobacter* found on chicken carcasses collected from re-hang and post chill sites at 17 poultry processing plants in the United States is impacted during processing by sequencing the Short Variable Region of the flaA locus. Seventy percent of carcasses had one *flaA*-SVR type detected. *Campylobacter* genetic diversity decreased as carcasses proceeded through processing; carcasses sampled at re-hang had significantly more genetic diversity in *Campylobacter* populations than carcasses sampled at post chill. There was more diversity in C*ampylobacter* on carcasses collected during winter than spring, summer, or fall. There were certain types that were present at re-hang that were not present at post chill, and vice versa, suggesting that there are certain types that are prone to perish during processing while others may survive or persist in the stressful processing environments.

INDEX WORDS: *Campylobacter*, genetic diversity, *fla* typing, *flaA*-SVR

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CHAPTER 1

INTRODUCTION

Campylobacter jejuni and *C. coli* are spiral, curved, or s-shaped rods that are 0.2 to 0.8 μm in width and 0.5 to 5 μm in length and the most important human enteropathogens among the campylobacters, affecting an estimated 2.4 million cases each year in the U.S. alone (*2, 10*). In the U.S., there were approximately 5,712 laboratory-confirmed cases of campylobacteriosis in 2006, according to the Centers for Disease Control and Prevention; second only to *Salmonella* infections (*2*). Although *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* are all capable of causing sickness in humans, most cases of campylobacteriosis are caused by *C. jejuni* (85%) with the majority of the remaining caused by *C. coli* (*2, 9, 10*). Risk factors for contracting campylobacteriosis include consumption and handling of raw and undercooked poultry, most commonly chicken and turkey products, cross contamination with other foods, and contaminated milk and water (*1, 4, 11, 15, 8)*.

Both *C. coli* and *C. jejuni* colonize the intestines of food-producing animals and humans (*16*). The favored environment appears to be the intestines of avians, which may be due the body temperature of 42˚ C, which is the optimal growth temperature for *Campylobacter*. There have been many investigations into the possible sources of contamination of poultry flocks with *Campylobacter*, but no definitive factor has been determined to explain its incidence (*4, 8*). Since *Campylobacter* is ubiquitous, it is easy for the flock to be exposed to many potential sources of contamination; current sampling

and culture methods may not be adequate to recover all bacterial cells present. In avians, *Campylobacter* is a commensal that colonizes the gut. After one member of a flock has become contaminated, *Campylobacter* is transmitted from that animal to other animals vertically and horizontally (*5, 7*).

C. jejuni and *C. coli* can be found on 90% of poultry in the U.S. (*17*), and from 18% to >90% of poultry in Europe, varying from country to country (*12*). Carry over contamination from a positive flock to a negative one is a source for contamination in processing (*3, 12*). Cross contamination in the slaughter plant due to contaminated equipment and processing water, is very difficult to control. Finished products that are heavily contaminated with high numbers of microbes are considered undesirable from a food safety and quality point of view. There are various steps in processing designed to eliminate or control these microbes, such as multiple washes and chilling. In some instances, processing aids are used to further reduce the microbial load on chicken carcasses by removing surface contamination.

Measures taken to reduce or eliminate the prevalence of *Campylobacter* are being researched and applied in the food industry. Prevalence in foodstuffs is likely to have a huge impact on the health care industry, as *Campylobacter*-related illness and hospitalization costs are $8 billion in the United States alone (*6*). The underlying principle for reduction of *Campylobacter* infection in humans is to prevent colonization of poultry, or to reduce the prevalence on carcasses during processing.

Information relative to the diversity of *Campylobacter* and nature of infection helps in the investigations of adaptation. Molecular subtyping is an important tool for epidemiological studies; it helps in tracing sources and routes of transmission of human

infection, identifying and monitoring specific strains over time and different regions with important characteristics. Molecular subtyping also contributes to the development of strategies to control transmission, elucidate sources, and determine possible routes of contamination in the food chain (*13, 14, 19*).

The objective of this study were

1. Determine the genetic diversity of *Campylobacter* collected from rehang and post-chill sites by sequencing the Short Variable Region (SVR) of the flagellin locus in *Campylobacter*
2. Determine how diversity in *Campylobacter* found on chicken carcasses is impacted by processing

References

1. 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.

2. Anonymous. 2007. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 states, 2006. The Morbidity and Mortality Weekly Report, United States Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

3. Atterbury, R. J., P. L. Connterton, C. E. R. Dodd, C. E. D. Rees and I. F. Connerton. 2003. Isolation and characterization of *Campylobacter* bacteriophages from retail poultry. *Appl Environ Microbiol*. 69: 4511-4518.

4. Barrios, P.R., J. Reirsen, R. Lowman, J.R. Bisaillon, P. Michael, V. Fridriksdóttir, E. Gunnarsson, N. Stern, O. Berke, S. McEwena, and W. Martin. 2006. Risk factors for *Campylobacter* spp. colonization in Iceland. *Prev Vet Med*. 74:264-278.

5. 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 hens. *Avian Dis* 46:919-924.

6. Buzby, J. C., B. M. Allos, and T. Roberts. 1997. The economic burden of *Campylobacter* associated Guillian-Barrè Syndrome. *J Infect Dis*. 176 (Suppl 2):S192-S197.

7. Cortez, A. L. L., A. C. F. B. Carvahlo, E. Scarcelli, S. Miyashiro, A. Vidal-Martins and K. Burger. 2007. Survey of chicken abattoir for the presence of *Campylobacter jejuni* and *Campylobacter coli*. *Rev Inst Med trop S Paulo* 48:307-310.

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

9. Fricker, C. R., and R. W. A. Park. 1989. A two-year study of the distribution of 'thermophilic' campylobacters in human, environmental, and food samples from the Reading area with particular reference to toxin production and heat-stable serotype. *J Appl Bacteriol*. 66:477-490.

10. Friedman, C. R., J. Neiman, H. C. Wegener, and R. V. Tauxe. 2000. Epidemiology of *Campylobacter* infections in the United States and other industrialized nations, p. 121-138. *In* I. Nachamkin and M. Blaser (ed.) *Campylobacter*. ASM Press, Washington, DC.

11. Miller, W.G. and R.C. Mandrell. 2005. Prevalence of *Campylobacter* in the food and water supply, p. 101-164. *In* J.M. Ketley and M.E. Konkel (eds.) *Campylobacter* Molecular and Cellular Biology. Horizon Bioscience, Norfolk, NR UK.

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

13. Newell, D.G. and J. A. Wagenaar. 2000. Poultry infections and their control at the farm level. p. 497-509. *In* I. Nachamkin and M. Blaser (eds.) C*ampylobacter*. ASM Press, Washington, DC.

14. Nielsen, E. M., J. Engberg, and V. Fussing. 2001. Genotypic and serotypic stability of *Campylobacter jejuni* strains during in vitro and in vivo passage. *Int J Med Microbiol*. 291:379-385.

15. Skirrow, M. B. 1982. *Campylobacter* enteritis—the first five years. *J Hyg*. 89:175-184.

16. Skirrow, M. B. 1994. Diseases due to *Campylobacter*, *Helicobacter*, and related organisms. *J Comp Pathol*. 111:113-149.

17. Stern, N. J.; P. Fedorka-Cray, J. S. Bailey, N. A. Cox, S. E. Craven, K. L. Hiett, M. T. Musgrove, S. Ladely, D. Cosby, and G. C. Mead. 2001. Distribution of *Campylobacter* spp. in slected U.S. poultry production and processing operations. *J Food Prot*.64:1705-1710.

18. Tauxe, R. V., M. S. Demming, and P. A. Blake. 1985. *C. jejuni* infections on college campuses: a national survey. *Am J Public Health*. 75:659-660.

19. Wassenaar, T. M., and D. G. Newell. 2000. Genotyping of *Campylobacter* spp. *Appl Environ Microbiol*. 66:1-9.

CHAPTER 2

LITERATURE REVIEW

History

Campylobacter was first observed in 1886 by Theodore Escherich (*34*), who described it as a non culturable *Vibrio* organism found in the stool of infants with diarrhea. Before the second half of the 20th century, it was more recognized as a veterinary disease than a human pathogen. John Macfayden and Stewart Stockman (*125*) were pioneers in veterinary *Campylobacter* research. They were responsible for determining its role in animal husbandry as the etiologic agent responsible for veterinary deaths and stillbirths. Until 1987, campylobacters were classified as *Vibrio* species. Vibrionic abortion was a major cause of abortion in sheep and cattle in the entire world. "V. fetus," now known as *C. fetus*, was found to be the cause (*126*). It was also first found to cause diarrhea and enteric disease in animals.

Bacteria that currently belong to the *Campylobacter* species were originally difficult to classify and were assigned to a *Vibiro* species due to cell morphology. *V. jejuni* was classified in 1927, while *V. coli* was classified in 1944. *V. jejuni* was associated with bovine dysentery, human gastroenteritis, and aborted sheep fetuses and *V. coli* was associated with pigs with diarrhea (*30*). However, there were differences between these vibrionic organisms and those in the *Vibrio* family, including microaerophilic growth requirements, and non-fermentative metabolism (*139*). In 1973, Vèron and Chatelain (*140*) performed a comprehensive study on the taxonomy of these

microaerophilic, vibrionic organisms, and added *C. coli, C. jejuni,* and *C. sputorum* to the genus *Campylobacter* that was created in 1963 by Sebald and Vèron. During the 1980's there was an explosion of C*ampylobacter* research, where *C. conscious* (*131*), *C. sputorum* subsp. *mucosalis* (*67, C. nitrofigilis* (*74*), *C. hyointestilalis* (*44*), *C. lari* (*9*), *C. pylori* (*73*), *C. cryaerophila* (*85*), *C.cinaedi* (*136*), *C. fennelliae* (*136*), and *C. mustelae* (*39*) were isolated and classified. In rRNA homology studies of a portion of the 16S rRNA sequence, results showed that *Campylobacter* species belongs to three major phylogenetic clusters: the first one includes *C. fetus, C. hyointestinalis, C. sputorum, C. jejuni, C. coli, C. lari, C. upsaliensis, C. conscious* and *C. mucosalis*. The second includes *C. pylori, C. fennelliae,* and *Wolinella succinogenes.* And the third includes *C. nitrofigilis,* and *C. cryaerophila* (*134*). Revision of *Campylobacter* taxonomy was initiated in 1989 by Goodwin. The genus *Helicobacter* was created to include *C. pylori* and *C. mustelae*. *C. cinaedi* and *C. fenellia* was changed to *H. cinaedi* and *H. fennelia* (*45*). Organisms in the second cluster of campylobacters were given the name *Arcobacter* (*139*). The family *Campylobacteriaceae* is now comprised of *Campylobacter*, *Arcobacter*, *Helicobacter*, *Sulfurospirillum* and *Bacteroides ureorlyticus* (*139*).

General Characteristics

Bacterial cells in the genus *Campylobacter* are spiral, curved, or s-shaped rods that are 0.2 to 0.8 μm in width and 0.5 to 5 μm in length (*139*). Old or abused cultures form coccoid bodies, that may not grow by standard microbiological techniques, which is termed the viable but not culturable (VBNC) state (*53*). *Campylobacter* are non-sporeforming, microaerophilic rods with atmospheric requirements of an O_2

concentration of 3 to 15%, a CO_2 concentration of 3 to 5%, and the balance nitrogen. Some campylobacters require H_2 in its microaerophilic environment and growth may be enhanced due to its addition. Mobility and chemotaxis are due to an unsheathed flagella at one or both ends of the cell. Metabolism is respiratory and chemoorganic (*139*). *C. gracilis* is non motile and *C. showae* has multiple flagella (*139*). Typical biochemical reactions that help to identify this organism are reduction of fumarate to succinate, negative methyl red reaction, and acetoin and indole production (*139*). *C. jejuni* and *C. coli* are commonly referred to as thermophilic campylobacters, which grow best at 37 to 42 C with a genome of about 1600 to 1700 kb (*34*). *C. jejuni* can be differentiated from *C. coli* by its ability to hydrolyze hippurate. (*52*)

Isolation

In early *Campylobacter* research, culture and isolation were problematic. Originally, selective filtration, using the small cell size and motility, was the method used to separate *C. jejuni* from fecal samples (*18*, 27). It is difficult to isolate *Campylobacter spp.* from food samples because of their low numbers and the presence of damaged cells. Isolation from food may require an additional pre-enrichment step, where damaged cells are not inhibited, with a reduced temperature of 37˚C to promote growth and survival. After a short enrichment step, usually 12 hours, the sample is then plated onto selective media. *Campylobacter* is resistant to bacitracin, novobiocin, polymixin B, cephalothin, vancomycin, vitampicin (*17*, *103*), and these antibiotics are commonly added to media to aid in selection for campylobacters. However, with the addition of some antibiotics, such as cephalothin, some strains of *C. jejuni*, *C. coli*, and *C. lari* are missed due to sensitivity (*14*). Media generally includes antibiotics, as well as ingredients to neutralize possible

toxic effects from oxygen and light, such as lysed blood, charcoal, a combination of ferrous sulphate, sodium metabisulphite and sodium pyruvate; also haemin or haematin.

Campylobacter and Human Infection

C. jejuni and *C. coli* are the most important human enteropathogens among the campylobacters, causing an estimated 2.4 million cases each year in the U.S. alone (*5,41*). *C. jejuni* is the predominant species in poultry and *C. coli* is commonly found swine. In the U.S., there were approximately 5,712 laboratory-confirmed cases of campylobacteriosis in 2006, according to the CDC; second only to *Salmonella* infections (*5*). The incidence of infection has decreased by 30% from baseline studies, conducted from 1996 to 1998; most of the decline occurred by 2001, due to implementation of HACCP programs in 1996 (*4*).

Risks for contracting campylobacteriosis are consumption and handling of raw and undercooked poultry, most commonly chicken and turkey products, cross contamination with other foods, and contaminated milk and water (*3, 8, 79, 121, 132*). *C. jejuni* and *C. coli* can be found on 90% of poultry in the U.S.(*129*), and from 18% to >90% of poultry in Europe, varying from country to country (*87*). Nordic countries typically have lower prevalence than southern countries (*87*). Although *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* are all capable of causing sickness in humans, most cases of campylobacteriosis are caused by *C. jejuni* (85%) and the majority of the remaining caused by *C. coli* (*5, 40, 41*). Most cases occur as isolated, sporadic events, not as part of large outbreaks (23). Active surveillance through FoodNet indicates about 12.71 cases are diagnosed each year for every 100,000 persons on the U.S. population.

Symptoms of the disease are fever, abdominal cramps, and diarrhea (*23*). Illness lasts about a week, and the innate immune response is responsible for termination of colonization and disease. There are an estimated 124 fatal cases a year, in the U.S., usually in the immunocompromised (*23*). About one in every 1000 diagnosed cases can result in chronic sequeleae, which includes reactive arthritis and Gillian-Barrè Syndrome (GBS).

Worldwide, there are estimated to be about 400 million cases of campylobacteriosis per year (*41*). In the U.S., common source outbreaks are due to the consumption of raw milk, contaminated food sources, and contaminated drinking water. Between 1978 and 1987, consumption of raw milk accounted for over half of all outbreak-associated cases of campylobacteriosis. Between 1988 and 1996, consumption of contaminated food sources accounted for 83% of outbreaks (*41*). Asymptomatic human carriers in susceptible populations are uncommon, but not impossible.

Campylobacter is known to behave differently depending on the host population. In susceptible populations, exposure to *Campylobacter* is infrequent. The host's immune response is responsible for ending disease and colonization. The immune response may also provide short-term protection against future infection. Endemic populations encounter *Campylobacter* from different sources which is evidenced by different strains and species present (*120*). The organism is present, probably transiently, since no colonization is evident.

Guillian-Barrè Syndrome (GBS)

GBS is an acute post infectious autoimmune-mediated disorder affecting the peripheral nervous system, clinically defined by flaccid paralysis, areflexia and

albuminocytological dissociation in the spinal fluid (*48*). Ascending paralysis evolves rapidly and weakness of limbs and respiratory muscles is common (*20*). There are no specific tests to provide a confirmatory diagnosis of GBS. Known antecedents associated with the development of GBS are infections with *C. jejuni*, *Mycoplasma pneumoniae*, cytomegalovirus, Epstein-Barr virus, varicella-zoster virus and some vaccines (rabies, swine influenza) (*57*). GBS incidence is from 0.4 to 4.0 cases/100,000 (*13, 57*). The first confirmed link between infection with *C. jejuni* and GBS was reported by Rhodes and Tattersfield in 1982. *C. jejuni* has been isolated from approximately 15% of patients with GBS (*84*). The risk of developing GBS after *Campylobacter* infection is 1/1000 and for the serotype, about 1 per 200 (*19*). *C. jejuni* may trigger GBS through molecular mimicry between lipooligosachharides (LOS) in the bacterial cell wall and gangliosides in peripheral nerve tissue (*124*). Prior *Campylobacter* infection is associated with GBS, however, proof of infection is not needed to diagnose the condition (*31*).

Pathogenesis

Both *C. coli* and *C. jejuni* colonize the intestines of food-producing animals and humans (*122*). The favored environment appears to be the intestines of birds, which may be due the body temperature of 42˚ C, which is the optimal growth temperature for *Campylobacter*. In some birds, particularly poultry, *Campylobacter* is a commensal. In humans, *Campylobacter* infection causes sickness. Pathogenesis of *C. jejuni* is characterized by motility, chemotaxis, translocation across host cell membranes, adherence, invasion and toxin production. Motility, conferred to *C. jejuni* and *C. coli* by its polar flagella, is important in colonization of the host (*82, 97*). The flagellar filament protein is comprised of flaA and flaB, which are coded by the adjacent *flaA* and *flaB*

genes. Mutants with reduced expression of *flaA* of *flaB* can have truncated flagella and reduced invasiveness (*47, 143*). Both proteins are needed for full motility and virulence. Chemotaxis is important for bacterial cells to respond to stimulus. Non chemotactic cells showed a threefold increase in adherence and invasion of INT 407 cells, compared to wild-type isolate, but was unable to colonize mice or cause symptoms (*151*).

Translocation may permit bacteria access to underlying tissues and could promote their dissemination throughout the host. In 1992, Everest et al. noted that 86% of *Campylobacter* isolates from individuals with colitis were able to translocate across polarized Caco-2 cells versus 48% of strains isolated from individual with non-inflammatory disease. The consensus among investigators is that after *C. jejuni* is ingested and passed through the stomach, it initially colonizes the jejunum and ileum, and then the colon (*1*, *124*). After the bacterial cell adheres to the receptor site on the host cell, a subpopulation is then able to invade the host cell and cause mucosal damage and inflammation (*35*). A number of *Campylobacter* species, including strains of *C. jejuni*, *C. coli*, *C. lari*, *C. fetus*, and *C. upsaliensis*, produce cytolethal distending toxin (CDT) (*61, 81*). The production of CDT by *Campylobacter* isolates was first reported by Johnson and Lior in 1988. CDT causes cell distension evident through elongation, swelling, and enlargement of the nuclei of host cells. CDT ultimately disintegrates and kills cells. CDT is encoded by three genes, *cdtA*, *cdtB*, and *cdtC* (*102*). CDT is heat labile and trypsin sensitive (*61*). All three components of the toxin are necessary for full activity (*66*).

Campylobacter and Poultry Rearing

There have been many investigations into the possible sources of contamination of poultry flocks with *Campylobacter*, but no definitive factor has been determined to explain its incidence (*8, 26*). Since *Campylobacter* is ubiquitous, it is possible for the flock to be exposed to many potential sources of contamination; current sampling and culture methods may not be adequate to recover all bacterial cells present. Studies on the prevalence of *Campylobacter* in poultry have indicated that *C. jejuni* is more common than *C. coli* (*32, 62*). Colonization usually occurs in chicks around 2-3 weeks of age, and the rapid spread from one broiler to the entire grow out house of 10,000 to 30,000 birds may be due to a low infective dose, which may be as low as 35-40 cells (*23, 128*).

Vertical and Horizontal Transmission

After one member of a flock has become contaminated, *Campylobacter* is transmitted from that animal to other animals vertically and horizontally (*15, 26, 106*). Vertical transmission, a controversial topic among scientists, is passage of bacteria from hen to chick through contaminated eggs. There has been much research done to determine the role vertical transmission plays in *Campylobacter* transmission, with contrasting results (*21, 26) Campylobacter* and other pathogens can colonize the reproductive tract, as the egg passes through the tract it can become contaminated with pathogens that were a part of the flora of the hen (*15*). Breeder hens are usually colonized by multiple strains of *C. jejuni* (*58*). *C. jejuni* has also been isolated from rooster semen (*87*, showing that *Campylobacter* may also be transmitted sexually in poultry. Intact eggshells are permeable to *C. jejuni*. When immersed in an infectious organism, 4% of eggshells were experimentally infected (*26*). Eggs infected by

immersion can be colonized inside the shell or on the shell. An egg can become contaminated through the feces from the infected hen in the nest. Although Cox (*26*) have shown vertical transmission can occur, others believe it is rare (*58, 62,, 99, 109*).

Horizontal transmission is from bird to bird, generally within a flock. Risk factors for horizontal transmission include bird age, flock size, lack of biosecurity, carry over from a previous flock, contaminated air, soil, and water (*87*). Most scientists agree that feed, feed additives, and fresh litter are not sources of contamination (*10*). *C. jejuni* has been isolated from drinking water lines and reservoirs in chicken houses. The same phenotype and genotype in water reservoirs has been found in chicken feces (*87, 100*). *C. jejuni* is known to survive in water under favorable conditions, however, because of antimicrobial usage and water flow, water lines and reservoirs may not be a *Campylobacter*-friendly environment. Overall, Newell (*87*) suggested that water contaminated with *C. jejuni* constitutes a low risk. In countries where litter is not reused and the grow-out house is cleaned between flocks, contamination is sporadic and unpredictable (*118*. In the U.K., 15% of sequential flocks in 100 houses showed evidence of genotypically identical strains, which suggests strains were being carried over between flocks (*117*. However, Nordic studies (*87*) show that there is variance of strains from one flock to another, suggesting disinfection can be successful in eliminating *C. jejuni*.

Poultry are coprophagic; consumption of contaminated feces and cecal droppings is an important characteristic of C*ampylobacter* spread in a grow-out house. Line and Hiett (*70*) estimated the colonization dose 50% (CD_{50}) to be 316 CFU when coprophagia was eliminated. Chickens exposed to the outside environment are likely to encounter

more than one strain or species of *Campylobacter* capable of establishing a persistent commensal relationship during the lifetime of the bird (*16*). Barn-reared broiler chicken flocks are likely to be exposed to a somewhat more limited set of *Campylobacter* types (*90*) but these flocks have also been reported to exhibit the dominance of one *Campylobacter* type only to be replaced with another type later in rearing (*16*). Currently the factors that enable one species or strain to become the dominant type in the chicken gut are largely unknown but it appears to be more complicated than being the first strain to establish colonization. Shreeve (*118*) also noticed in some cases a flock may have several genotypes present, yet subsequent flock may have only one of the genotypes found in the first flock, suggesting some types are more successful at surviving transmission. It is also common to isolate more than one species or subtype from the same bird (*16, 114, 118*).

Campylobacters can be carried into broiler houses on clothing, boots, hands, and equipment (*87*). Biosecurity measures are taken but may not be that effective; the level needed prevent colonization is not known. When birds are restricted in their movement, those who are close to doors without hygiene barriers are the first ones to become colonized (*87*).

Campylobacter prevalence in poultry flocks is higher in summer than in winter, and it varies with latitude; number of cecal organisms per bird and strain types present varies between seasons (*56, 142*). There is a peak in human *Campylobacter*-related sickness and prevalence in warmer months (*8, 60*). This may be due to migratory birds or insects (*8, 59*). In a 2006 study (*8*), 95% of *Campylobacter*-positive flocks were detected in the April-September period, and there was a peak in July to September. However, it

was found that after controlling for the season and farm, the seasonal prevalence did not change during the study period.

Campylobacter and Poultry Processing

Carry-over contamination from a positive flock to a negative one can be a source for contamination in processing (*7, 87*). Cross contamination in the slaughter plant due to contaminated equipment and processing water, is very difficult to control. The presence of *C. jejuni* and *C. coli* in the intestine and liver may be important for the transmission of this bacterium to poultry meat (*33, 129*). Barrios (*8*) and Miwa (*80*) showed that efforts to improve sanitation at slaughterhouses have been difficult since *C. jejuni* are spread from intestinal contents.

Poultry processing is comprised of several steps—slaughter, scalding, defeathering, evisceration, and chilling. Damage to the intestines during the slaughter and evisceration processes can lead to direct contamination of carcasses and other organs. Berrang and Dickens (11) studied the presence and level of *Campylobacter* on broiler carcasses throughout the processing plant. In that study, samples were collected immediately before scald, post-scald, post-pick before transfer to the evisceration line, immediately after the removal of the viscera, after the final washer, and post-chill.

Scald

The scalding process is meant to loosen feathers in the follicles; *Campylobacter* has been recovered from scald water (*126*). Miwa (80) found that at the beginning of a processing day, scald and evisceration water is *Campylobacter*-negative, and the same *flaA*-RAPD profile was recovered from carcasses after defeathering and evisceration when negative flocks were processed after positive ones. Berrang and Dickens (11)

found prevalence was highest pre-scald and counts dropped significantly, after carcasses were scalded.

Defeathering

The defeathering process involves automated pickers to remove feathers (*11, 80*). These pickers may cause inter-flock cross-contamination, due to finger-like projections applying pressure to carcasses that may cause leakage of intestinal contents onto the surface of the pickers. *Campylobacter*-negative flocks are indirectly contaminated by *Campylobacter*-positive flocks during defeathering (*146*). Berrang and Dickens (*11*) concluded that *Campylobacter* populations and the percentage of positive carcasses increase significantly during the picking process.

Evisceration

During evisceration, there is a high chance for bacterial contamination, if the removal of the viscera leads to rupture of the intestines and leakage of fecal material. *Campylobacter* can be spread from the intestines to the outside of the carcass.

Chilling

Chilling is an important critical control point for microbiological contamination. Despite chilling water being a source for cross contamination in processing (*111*), this step also washes surface *Campylobacter* off and post-chill carcasses have lower bacterial loads than anywhere else on the line (*11, 107*). There are certain parameters that must be met in order for this critical control point to be effective—the temperature must be correct, organic matter should be kept at a minimum, pH must be the optimum range for chlorine to be effective, and concentration of antimicrobials must be monitored (*108*).

The post-chill area of the processing line has been found to have the least prevalence of *Campylobacter* (*11*).

Overall, *Campylobacter* prevalence drops as flocks move through the plant. However, *Campylobacter* counts detected on positive carcasses leaving the chill tank were not significantly different from those detected on positive carcasses leaving the scald tank (*11*). Defeathering and evisceration are the two points in processing with the highest microbial load.

Campylobacter g*enetic diversity in poultry processing*

There is a direct correlation between high *Campylobacter* prevalence and high genetic diversity on a carcass (*68*). Changes in *fla* type distribution occur during processing (*89*). These changes in *fla* type suggests that some strains are more resistant to the stressors encountered during processing. The substantial genetic diversity of *Campylobacter* is evidenced by strain-to-strain variability in virulence and tolerance to certain environmental stressors (*95*). Subtypes that are more robust may contaminate the abattoir environment, surviving through carcass chilling, and carry over into subsequent flocks (*89*).

Processing Aids

Finished poultry products that are heavily contaminated with high numbers of microbes are considered undesirable from a food safety and quality point of view. There are various steps in processing designed to eliminate or control these microbes, such as chilling and multiple washes. In some instances, processing aids are used to further reduce the microbial load on chicken carcasses by removing surface contamination. Common chemical processing aids include trisodium phosphate, acidified sodium

chlorate, food-grade acids, peroxyacids, and chlorine. These aids not only are effective at washing and killing unattached bacterial cells from the surface of poultry meat, they are also effective in removing and killing attached cells.

Trisodium phosphate (TSP)

TSP is a white, free flowing crystalline material with a pH of 11.8 at a concentration of 12% (*115*). When residual TSP on carcasses enters the chill tank, a rise in pH results, rendering chlorinated chill water ineffective in reducing microbial loads (*108*). TSP is more effective against gram-negative bacteria. Mechanisms of action include destruction effect due to the high pH (11), removal of non-attached bacteria, removal of surface fat, which by default removes bacteria, and an effect on the bacterial cell wall (*63*). It is effective in reducing the bacterial load up to 2 log CFU/ml when the concentration is between 10 and 12% (*28, 36, 127*). Del Rio (28) found that after dipping for 15 mins and storage at 3˚C, 12% TSP reduced natural microflora and spoilage organisms 0.5 to 2 log CFU/ml. Arritt (*6*) found that there was a more than one log reduction in bacterial counts when 10% TSP was applied before or after immersion in bacterial suspension. TSP has been shown to not alter the sensory characteristics detrimentally (*125*).

Acidified sodium chlorite (ASC)

ASC is a solution of sodium chloride acidified with an organic acid to form chlorus acid. ASC operates at a pH of 2.3-3.2 (*63*). It is a broad-spectrum disinfectant, which oxidizes the microbial cell wall, and attacks sulfide and disulfide linkages in proteins (*63*). It attaches to the amino acid component of the cell membrane. It can be administered in a spray or dip, usually at concentrations of 1000 ppm. It is effective

against bacteria, viruses, fungi, yeasts, molds and some protozoa (*63*). It was found to reduce *Campylobacter* prevalence on broiler carcasses from 73.2% positive to 49.1% (*63*). Application of 0.1% ASC resulted in a greater than 1 log reduction in *Campylobacter* when applied before and after inoculation (*6*). Sinhamahapatra (*119*) found treatment with 1200 ppm ASC spray, was equivalent to a 70˚C hot water spray in the ability to lower coliform counts. Del Rios (*28*) showed that 1200 ppm ASC lowered mold and yeasts counts by 1.45 log CFU/g and did not detrimentally effect sensory characteristics.

Food-grade acids (FGAs)

Food-grade acids include citric acid, lactic acid, succinic acid, and propionic acid. They are soluble in water and GRAS substances approved for use in the food industry. They work by disrupting the cellular membrane and acidify cellular contents (*63*). They are stable in the presence of organic material, but they can corrode processing instruments and can cause off flavors, odors and colors in end products (*63*). 0.1% acetic acid added to scald water reduced *Salmonella* and *Campylobacter* counts by 0.5 to 1.5 CFU/ml (*93*). Sinhamahapatra (*119*) found that a lactic acid carcass dip was effective in reducing total plate counts by 1.36 log CFU/ml. Berrang (*12*) added organic acids to the cloaca prior to scalding and recorded a statistically significant reduction in *Campylobacter* numbers when compared to control groups. Zhao (152) found that carcass dips in 2% acetic acid at 4˚C reduced populations of *Campylobacter* by 1.4 log CFU.

Peroxyacids

Peroxyacids are a combination of acetic acid, octanoic acid, and hydrogen peroxide, they are commonly clear colorless liquid; oxidation/reduction reactions take place in water they generate water, acetic acid, octanoic acid. When solubilized in water the acids act by lowering pH, disrupting the cellular membrane and acidifying cellular contents. Spraying poultry carcasses for 15 sec with a 200mg/l peroxyacid solution reduces *C. jejuni* by 2 log, which was a greater effect than water alone (*116*).

Reduction/Elimination of *Campylobacter* in poultry

Campylobacter is a worldwide threat to human health and measures taken to reduce or eliminate its prevalence are being researched and applied in the food industry. Reduction of prevalence in foodstuffs is likely to have a huge impact on the health care industry, as *Campylobacter*-related illness and hospitalization costs were $8 billion in the United States alone (*19*). The underlying principle for reduction of *Campylobacter* infection in humans is to prevent colonization of poultry, or to reduce the prevalence on carcasses during processing. Methods that are currently employed to prevent or treat colonization by *Campylobacter* include vaccination, competitive exclusion, phage therapy, antimicrobial therapy, and alternative feed formulation.

Vaccination

The goal of vaccination is to prevent infection and colonization of poultry by pathogens. There are some main characteristics a perfect vaccine should have. Since *Campylobacter* is quickly spread to thousands of chicks within a matter of days, a quick protective response is beneficial. The vaccine should be cost-effective and easy to administer as there are upwards of 30,000 birds in one flock. The vaccine should not be

harmful or deleterious to animals or humans. The vaccine should either prevent colonization or provide a 2-3 log reduction in bacterial loads in colonized animals. A risk assessment by Rosenquist (*107*) showed that a 2-log reduction in chickens at the time of slaughter is sufficient to have an impact on human health. A vaccine of choice should also provide cross-protection between *C. coli* and *C. jejuni*, since both species colonize in poultry. Currently, there is no vaccine that possesses all these characteristics, but there are some that have potential to become very useful in the poultry industry. Some vaccines that are currently employed that are effective against *Campylobacter* are experimental colonization with wild-type *C. jejuni* administered orally, which produces about a 1-log reduction upon challenge (*23*). Formalin inactivated *C. jejuni* with and without *E. coli* heat labile toxin administered orally produced a 1.5 log reduction in bacterial numbers upon challenge (*105*). Native-flagellin vaccine administered with heat killed *C.jejuni* offers a 1-2 log reduction upon challenge (*147, 148*) and attenuated *Salmonella* expressing CjaA offers greater than a 6-log reduction upon challenge (*150*). The main difficulty of formulating a vaccine for *Campylobacter* is due to the genotypic and phenotypic instability of the bacterium. A conserved protective antigen is difficult to pinpoint, and until it is found, one vaccine may not be suitable for use in poultry for *Campylobacter*. Another obstacle to the development of an effective vaccine is the apparent change in a dominant colonizing type observed by Bull (*16*) which affects the usefulness of a particular vaccine.

Competitive exclusion

Competitive exclusion (CE) in chickens is the introduction of bacteria from the gut of mature birds to newly hatched chicks in order to rapidly develop the microflora of

chicks to protect against colonization by pathogens, particularly *Salmonella*. It is solely meant to be prophylactic, not treatment after colonization. CE is independent of breed, strain, sex of birds, but there is a difference in protective capability. It is usually administered in the drinking water or as a spray in the hatchery. Problems include uneven distribution of the protective effect across the flock and reduced viability of anaerobic organisms over time (*113*). CE is also used to reestablish intestinal microflora after antibiotic therapy. BroilAct®, a CE product established by the Orion Company in Finland, consisting of mixed culture derived from the cecal contents of a single hen from 1987. It included 32 different types of bacteria, including 22 strictly anaerobic rods and cocci, 10 facultatively anaerobic rods and cocci, and no spore-formers (*113*). BroilAct® has a protective effect not only for *Salmonella*, but also for *Campylobacter jejuni* and *E. coli* (*49, 50)*.

Campylobacter *phage*

Campylobacter-specific bacteriophages are ubiquitous in the environment and have been isolated from sewage, broiler chickens, and porcine manure (*7,46, 64, 110*). Bacteriophages are self-replicating, and self-limiting, and have host specificity that is not seen in broad antimicrobials and they do not disrupt the gut bacterial flora (*140)* . *Campylobacter* phage have been used with limited success as a treatment to reduce the numbers of *Campylobacter* in poultry microflora (*7, 141*). Since they are commonly isolated from poultry excrement, their use will not introduce any new biological entity into the food chain. Wagenaar (*141*) found that there is a 3-log reduction in *C. jejuni* when phage is administered after colonization. When phage was administered before colonization, it was delayed, but not prevented. Atterbury (*7*) found *Campylobacter*

phage on retail meat, but only when *Campylobacter* was present. Phage also has the ability to overcome the genetic and phenotypic instability of *Campylobacter*. They are constantly adapting to mutating host defenses, thus maintaining an evolutionary balance. Successful administration of phage therapy must be carefully timed to have maximum effect. Phage therapy may be used as a biocontrol agent, but is best used in combination with other biocontrol measures.

Antimicrobials

There are many concerns with the use of antimicrobials in food production including their effect on human health, and the rise of antibiotic resistant bacteria; this has led to the ban or restriction in uses in food production. The EU has banned the use of many antibiotics, especially for use as growth promoters (*7, 113, 138*). In the U.S., the use of antibiotics has also come under examination. The use of bacteriophages may help fill the void left by the ultimate discontinuation of antibiotics.

Feed formulation

Feed formulations can alter the microenvironment of the chicken gut, making it unsuitable for certain bacteria to flourish. Mucin composition, pH, free volatile fatty acid content, non-soluble polysaccharide content, and gas production are dependent on feed formulation and can have an effect on the ability of certain pathogens to colonize the gut (*137*). However, since *Campylobacter* is not considered a poultry pathogen, but commensal, feed formulations have had a limited effect on its colonizing capabilities. A study by Line (*69*) showed that supplementing poultry feed with *Saccharomyces boulardii* reduced *Salmonella* colonization from 70% to 20 and 5% in the 1x and 100x yeast treated birds, respectively. However, the yeast supplemented feed had no effect on

colonization of *Campylobacter*. Udayamputoor (*137*) found that protein source had an effect on colonization by *Campylobacter*. Experimental groups fed a diet with plant protein had significantly lower cecal colonization, when compared to experimental groups fed a diet containing animal protein or a mixture of plant and animal proteins.

Molecular Subtyping

Clarification of the diversity and nature of *Campylobacter* infections helps in the investigation of disease. Molecular subtyping is an important tool for epidemiological studies; it helps in tracing sources and routes of transmission of human infection, identifying and monitoring specific strains over time and different regions with important characteristics. Molecular subtyping also lends itself to the development of strategies to control transmission, sources and possible contamination in the food chain (*88, 91, 144*). Methods for subtyping must be evaluated in the context in which they are used and must be able to give reliable, reproducible data (*91*). Discriminatory index is important for distinguishing results and choosing the correct typing method for the sample (*78*). In addition, discriminatory index is used to compare discriminatory power (DP) of typing method. A method with high DP may be better suited for a particular goal; to attain a greater DP two methods can be combined. Reproducibility is important between and within labs to be able to compare results and draw meaningful conclusions. Molecular typing methods must be standardized so that more information can be ascertained from results. It is possible for results to differ due to primers used, temperatures for PCR, culture methods and conditions, restriction enzymes used, number of restriction enzymes, etc. Efforts for standardization include databases with electrophoretic profiles and sequences, and standard primers.

Typing methods

There are three major types of typing schemes—phenotypic, genotypic and nucleic acid analysis. In phenotypic methods, observable physical or biochemical characteristics of an organism, which are influenced by genetic make up and environmental conditions, are used as the basis of separation. Major phenotypic methods include serotyping, phage typing, and biotyping. In genotypic methods, the genetic makeup of the organism, apart from the physical attributes is used as the basis of separation. Common genotypic methods include random amplified polymorphic (RAPD) DNA-PCR, restriction fragment length polymorphism (RFLP)-PCR, pulse-field gel electrophoresis (PFGE), *fla* typing, and ribotyping. With heterogeneous groups of strains, PFGE, and *fla* typing have greater discriminatory power than RAPD analysis or ribotyping (*151*). In nucleic acid analysis, the genome or a particular gene sequence is used for classification. Among the three major methods, nucleic acid analysis is the most reproducible. Those methods are fast, eliminate experimental variation, and facilitate interlaboratory comparisons (*78*).

Phenotypic methods

Serotyping

Serotyping separates bacteria based on antibody-antigen reactions. *Campylobacter* serotyping was developed in Canada in the 1980's by Penner and Lior (*149*). Penner schemes are based on the heat stable antigens using passive hemagglutinations (*101*) and Lior is based on the heat labile antigens using bacterial agglutination (*71*). The Penner scheme is used as the basis for the typing scheme in the Laboratory of Enteric Pathogens (LEP), Public Health Laboratory Service in the United

Kingdom (*42*). Advantages of serotyping include the wide acceptance as a valid typing method (*144*). Disadvantages of serotyping include a high level of untypeable strains, in human and veterinary samples (up to 20% in some instances), culturing conditions may render an isolate untypeable, ambiguous results, transient antigen expression, and cross-reactivity between certain antigens (*38*). The method requires a panel of antisera that is costly to maintain, is laborious and requires at least 5 to 7 days to complete (*38, 96, 112*), considering the need to repeatedly subculture isolates before testing. Using the LEP scheme, up to 40% of poultry isolates are untypeable.

Phagetyping

Phagetyping uses viruses as markers to identify microorganisms. Since bacteriophages are host-specific, identification to subspecies is possible. *Campylobacter* phage typing has many different schemes. The primary typing scheme was developed in the U.S. and uses common poultry phage. The scheme was adapted by other countries, with the basic or main phages remaining the same, but other phages added that were isolated from *Campylobacter* positive birds in that particular country. Disadvantages of phagetyping include the appearance of a unique phage pattern of a strain that Reacts with phage but Does Not Conform to a designated type (RDNC) (*65*). An advantage of phagetyping is the host specificity of phages. Host specificity reduces the chance of phages interacting with the wrong bacteria. Another advantage of phagetyping is its application as a complement to serotyping. Serotyping classifies bacteria into 20 broad groupings and phagetyping can further classify 6 to 29 subgroups from each of those 20 serogroups, thereby enhancing the discriminatory power of this epidemiological tool (*42*). There are 66 known serotypes and 76 known phage types for a total of 5016 possible

combinations (*88*) In Frost's study, (*42*) 336 phage-serotype combinations were identified among a sample of 2407 isolates, which may be indicative of the relationship between phage and serotypes.

Biotyping

Biotyping is used to distinguish isolates according to biochemical reactions and metabolic activities. Differentiation is based upon the results of the biochemical tests. The discriminatory index depends on the number of tests administered (*133*). There are many types of tests or combinations of tests that can be used in order to discriminate, which is an advantage of biotyping. The discriminatory power may be increased as the number of tests administered. Biotyping is very common and widely accepted as a separatory method. For example, Skirrow (123) developed a typing scheme based on hippurate hydrolysis, a rapid H_2S test in iron-containing media, and resistance to nalidixic acid for the differentiation of campylobacters into *C. jejuni*, *C. coli*, and a third group, the nalidixic acid-resistant thermophilic campylobacters. Disadvantages of biotyping include cost in supplies and labor. Cost and labor increases as discriminatory power increases, due to the increased number of tests performed. However, recent developments have made it possible to automate and perform many tests at one time. Biotypes may be dependent upon growth conditions and therefore reproducibility may be difficult, inter and intralaboratory.

Genotypic methods

Polymerase Chain Reaction (PCR)

PCR is used as the basis of many genotypic typing schemes. PCR is an automated, rapid, sensitive way to amplify DNA. PCR is comprised of three basic

steps—denaturation, annealing, and extension. The original double stranded DNA is denatured to form a single strand to which a primer can anneal. After annealing, a complimentary strand is formed. In theory, a single piece of DNA is enough to begin a reaction that multiplies exponentially to contain over one million pieces of DNA, depending on the number of cycles performed by the thermal cycler. PCR may amplify the whole genome or a specific gene; it is dependent upon the primers used in the reaction. Primer selection is depends on the target gene and reaction conditions. Specificity of amplification reaction depends on selectivity of primers, enzymes used, condition of primers and enzymes, and reaction conditions. PCR has become increasingly automated and more complex. Examples of this include real time PCR, reverse transcriptase PCR, and hot start-cold end PCR. PCR is also used in combination with other methods to increase discriminatory power. Limitations in PCR include inhibitors present in the sample matrix, condition of the primers and enzymes, and product assumption. Some samples are taken from a food matrix, especially in the case of *Campylobacter*, without enrichment. Some matrix constituents may have an inhibitory effect on the PCR efficiency. Advantages of PCR include automation of procedure, quick turnaround time, usefulness in detection. Disadvantages include high capital input for equipment, and cell viability is not known, as DNA can be amplified from dead or living cells.

Random Amplified Polymorphic DNA (RAPD)

RAPD is a whole genome typing method in which a single oligonucleotide is used to amplify a gene. The primer has to have the ability to bind in both forward and reverse directions. When performing RAPD, annealing temperatures must be taken into account.

Primer selection is the greatest determinant of the number of bands, since the primer acts on DNA in random places, random bands are produced. RAPD can have high discriminatory potential due to its ability to determine polymorphism in the entire genome (*144*). However, RAPD can have low reproducibility due to random digestion. Low reproducibility is also due to the ratio of DNA to primer concentration, model of thermal cycler, magnesium concentration or brand of *taq* DNA polymerase, which all effect banding patterns.

A combination of *fla* and RAPD typing have successfully been applied to *Campylobacter* (*2, 82, 83, 98*). However, disadvantages such as minor differences in band patterns and weak band patterns make RAPD's discriminatory capacity somewhat poor, which may lead to subjective interpretation of results (*72, 92, 144*).

PCR-Restriction fragment length polymorphism (RFLP)

PCR-RFLP is a method in which a particular sequence is amplified and enzymes are used to digest genomic DNA at certain sites. The results are precise band lengths, which produce identifiable electrophoretic patterns that can theoretically be reproduced because of the specificity of restriction enzymes used. Advantages of RFLP are simple setup, low maintenance costs, and rapid processing of samples. The level of discrimination can be improved with multiple restriction enzymes. Disadvantages of RFLP are difficulty in choosing an appropriate target for amplification; prior knowledge of genome is needed, and a small section of genome is examined. RAPD analysis has been compared to *fla* typing in a study, and it was found that *fla* typing is more discriminatory when analyzing broiler liver and intestine samples for *C. jejuni* and *C. coli* (*33*).

Pulsed-field gel electrophoresis (PFGE)

PFGE begins with whole cells that are lysed in agarose blocks to reduce contamination and shearing of DNA. The genomic DNA is washed to remove contaminants and extra cellular materials. The plug containing DNA is digested with restriction enzymes, that are chosen for optimum typing of each pathogen, which cleave the DNA into large fragments. The fragments are separated by multi-directional pulsed electric fields. PFGE is the method that is currently employed by epidemiologists at the U.S. Centers for Disease Control and Prevention as the standard for PulseNet (*130*). PulseNet is a national network of public health and safety agencies that exchange standardized PFGE patterns on food-borne pathogenic bacteria. Regulation and standardization of methods, especially through CDC's PulseNet has led to high reproducibility, rapid exchange of information between various laboratories nationally and internationally. Currently, PFGE is regarded as the 'gold standard' for subtyping methods, being the most discriminatory among commonly applied subtyping methods (*78*). Yet, without standardization of enzymes and other experimental conditions, reproducibility is questionable, and results are sometimes open to personal interpretation.

fla typing

Flagella are responsible for motility, chemotaxis and virulence in bacteria. Flagella of gram-negative cells are composed of a filament, basal body, and hook. Filament is comprised of protein subunits encoded by a gene called *fla* (*38, 51, 76*). The genes that code for these protein subunits are what are analyzed in *fla* typing. Campylobacters have two encoding genes, *flaA* and *flaB*. In *C. jejuni* the two genes are roughly 1.73 kb, arranged in tandem, and are separated by a segment of about 0.2 kb in

length *(37, 51*). Expression of *flaA* and *flaB* are differentially regulated, with each gene containing its own distinct promoter, σ^{28} for *flaA* and $\sigma^{54}$for *flaB* (*47, 54*). There are conserved regions and variable regions, which makes this locus suitable for typing. The flagellin B gene (*flaB*) is, in contrast to *flaA*, not essential for motility, or pathogenicity, and is thought to be a genetic reservoir for *flaA*. However, both gene products are required for fully functional flagella (*47*). *flaB* may serve as a gene donor, in which parts can be introduced into *flaA* through homologous recombination to compensate for deletions, mutations or to increase the immunogenic repertoire of a gene (*78*). Because both highly conserved and variable regions are present (*75*), this locus is suitable for RFLP, and RAPD analysis of PCR products. The conserved regions in the *flaA* locus are partially conserved in other species; *fla* typing is useful for the majority of *C. coli* strains, some strains of *C. lari*, and *C. helveticus* (*94*). Despite its simple and quick applications, disadvantages for the *fla* typing scheme include difficulty in interlaboratory comparisons, and genetic instability. There are many techniques used in *fla* typing and there is no standard for what methods should be used for comparison. Difficulty encountered in comparing results may be overcome by standardization of restriction enzymes and electrophoretic conditions.

Ribotyping

Ribotyping is based on the presence of conserved copies of the rRNA coding for the 23S and 16S genes. *Campylobacter* has three ribosomal gene copies, which make this method less discriminatory than others. Ribotyping cannot discriminate between *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* (*29*). The choice of restriction enzyme is very important. The combination of enzymes used in ribotyping increases the

discriminatory power. This method samples the entire genome, and is not largely affected by small polymorphisms unless at the restriction site. Ribotyping can be fully automated and is reproducible. However, it is costly and only a limited number of samples can be analyzed at one time.

Nucleotide analysis

Direct nucleotide sequencing (with or without PCR amplification) is becoming increasingly automated and is an alternative method for genotyping bacterial isolates. The advantage of sequence analysis is that it is highly reproducible and the results are easy to interpret. However, interpretation is highly dependent on computerized comparison programs and the parameters set by the software packages used (*144*) . Sequence analysis has been applied to the flagellin locus in several studies (*51, 75*).

Sequencing SVR of flaA locus

Sequence analysis of the flagellin locus has been used successfully as an epidemiologic typing method by Meinersmann (*75, 77*), Mellman (*78*), Fitzgerald (*38*), and Harrington (51). There is up to 30% variability among *C. jejuni* isolates, over two regions in the *flaA* gene, the first, a shorter one, from sequence positions 450 to 600 and a longer one, from sequence position 700 to 1450. The shorter variable region, termed the SVR is commonly used as a locus for *fla* typing. Meinersmann (*75*) found analysis using the SVR has similar discriminatory power as analysis of the entire *fla* gene. Fitzgerald (*38*) found that SVR analysis was able to differentiate between outbreak strains of *Campylobacter jejuni* and sporadic strains. Mellmann (*78*) found that analysis of the SVR provides adequate discrimination in short term epidemiology. An advantage of analysis of the SVR is that it can be sequenced with a twofold redundancy over the entire

region with a single pair of forward and reverse primers that bind to conserved flanking sequences, while analysis of the whole gene or a long variable region requires many more primers (*75*). Fitzgerald (*38*) compared various methods of subtyping outbreak isolates and found analysis of the SVR to be more discriminatory than serotyping and PCR-RFLP of the entire *flaA* gene, but less discriminatory than PFGE. When using the SVR as an epidemiological tool, care must be taken since the entire genome is not represented.

Genomic recombination of *C. jejuni*

Campylobacter is a genus of organisms that is known to participate in genetic recombination. Genetic instability can be defined as an event leading to a change in the genetic organization of a bacterial strain. Mechanisms leading to instability can be summarized as point mutations as a result the activity of mobile elements, i.e., *Campylobacter* phage; random or programmed recombinations of DNA segments; high frequency mutations; incorporation of foreign DNA after natural transformation (145).

Some genotypic and phenotypic subtyping methods are susceptible to genetic instability. Genetic recombination commonly occurs at the flagellin locus, and this can affect the applicability of many typing schemes, PFGE and *fla* typing, in particular. Harrington showed direct evidence for intragenomic flagellin recombination in a non mutant (*C. jejuni* TGH9011) strain and evidenced intergenomic recombination within natural populations of *C. jejuni* (*51*),.

Typing can be affected by recombination through difficulty in identifying clones, or methods may differentiate strains that are of the same origin that may have been subject to recombination (*88*) Recombination may occur in *Campylobacter fla* genes to give this bacterium a biological advantage over other pathogenic species. *flaB* can serve

as a genetic reservoir for *flaA* and during recombination introduction of *flaB* allows for *Campylobacter* to retain full motility (*2*). Recombination makes *fla* typing unsuitable for long-term surveillance of bacteria.

Phenotypic vs. genotypic methods

It is very difficult to compare genotypic and phenotypic methods for subtyping due to a lack of standard strains for comparison. Some serogroups have a variation in their phenotypic and genetic heterogenicity. Some groups are very closely related while others are not. Having a standard set of strains will eliminate variation and difficulty comparing genotypic and phenotypic methods. It is generally accepted that phenotypic characteristics can be unstable. For example, loss of toxin production by a *Campylobacter* does not necessarily alter the strain genetic identity. Generally, genotypic methods have greater discriminatory power than phenotypic methods. However, combining a genotypic method with serotyping or combining two independent genetic methods usually results in greater discriminatory power and more complete strain identification than a single genotyping method alone. The decision of which method to use is dependent upon the objectives of research and the desired discriminatory power.

References

1. Allos, B. M., and M. B. Blaser. 1995. *Campylobacter jejuni* and the expanding spectrum of related infections. *Clin Infect Dis* 20:1092-1099.

2. Alm, R. A., P. Guerry, and T. J. Trust. 1993 Significance of duplicated flagellin genes in *Campylobacter*. *J Mol Biol* 230:359-363.

3. 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.

4. Anonymous. 2003. Preliminary FoodNet Data on the Incidence of Foodborne Illness---selected sites, United States, 2002. Morb Mortal Wkly Rep, United States Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

5. Anonymous. 2007. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food--10 States, 2006. Morb Mortal Wkly Rep, United States Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

6. Arritt, F. M., J. D. Eiffert, M. D. Pierson, and S. S. Summer. 2002. Efficacy of antimicrobials against *Campylobacter jejuni* on chicken breast skin. *J Appl Poutl Res* 11:358-366.

7. Atterbury, R. J., P. L. Connterton, C. E. R. Dodd, C. E. D. Rees and I. F. Connerton. Isolation and Characterization of Campylobacter Bacteriophages from Retail Poultry. *Appl Environ Microbiol*. 69: 4511-4518.

8. Barrios, P.R., J. Reirsen, R. Lowman, J.R. Bisaillon, P. Michael, V. Fridriksdóttir, E. Gunnarsson, N. Stern, O. Berke, S. McEwena, and W. Martin. 2006. Risk factors for *Campylobacter* spp. Colonization in Iceland. *Prev Vet Med*. 74:264-278.

9. Benjamin, J., S. Leaper, R. J. Owen, and M. B. Skirrow. 1983. Description of *Campylobacter laridis*, a new species comprising the nalidixic acid resistant thermophilic *Campylobacter* (NARTC) group. *Curr Microbiol* 8:231-238.

10. Berndtson, E., U. Emmanuelson, A.Engvall, and M. L. Danielsson-Tham. 1996. A 1-year epidemiological study of campylobacters in 18 Swedish chicken farms. *Prev Vet Med* 26:167-185.

11. Berrang, M. E., and J. A. Dickens. 2000. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant *J Appl Poult Res*. 9:43-47.

12. Berrang, M. E., D. P. Smith, and A. Hinton, Jr. 2006. Organic acids placed into the cloaca to reduce *Campylobacter* contamination of broiler skin during defeathering. *J Appl Poult Res* 15:287-291.

13. Black, R. E., M. M. Levine, M. L. Clements, T. P. Hughes, and M. B. Blaser. 1988. Experimental *Campylobacter jejuni* infections in humans. *J Infect Dis* 157:472-480.

14. Brooks, B. W., M. M. Garcia, A. D. E. Frazer, H. Lior, R. B. Stewart, and A. Lammerding. 1986. Isolation and characterization of cephalothin-

susceptible *Campylobacter coli* from slaughtered cattle. *J Clin Microbiol* 24:591-595.

15. 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 hens. *Avian Dis* 46:919-924.

16. Bull, S. A., V. M. Allen, G. Domingue, F. Jorgensen, 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* 72:645-352.

17. Butzler, J.P., Dekeyser, P., and T. LaFontaine. 1974. Susceptibility of related vibrios and *Vibrio fetus* to twelve antibiotics. *Antimicrob Agents Chemother* 5:86-89.

18. Butzler, J.P., and M.B. Skirrow. 1979. *Campylobacter* enteritis. *J Clin Gastroenterol* 8:737-765.

19. Buzby, J. C., B. M. Allos, and T. Roberts. 1997. The economic burden of *Campylobacter* associated Gillian-Barrè Syndrome. *J Infect Dis* 176 (Suppl 2):S192-S197.

20. Buzby, J.C., Roberts, T., and Allos, B.M. 1997. Estimated annual costs of *Campylobacter*-associated Gillian-Barrè Syndrome. Agricultural Economics Report No (AER 756). U.S. Department of Agriculture, Washington, DC.

21. Callicott, K. A., V. Friðriksdóttir, J. Reiersen, R. Lowman, R. R. Bisaillon, E. Gunnarsson, E. Berndtson, K. L. Hiett, Needleman, D. S., and N. J. Stern.

2006. Lack of evidence for vertical transmission of *Campylobacter* spp. in chickens. *Appl Environ Microbiol*. 72:5794-5798.

22. Cawthraw, S., R. Ayling, P. Nuijten, T. M. Wassenaar, and D. G. Newell. 1994. Isotype, specificity and kinetics of systemic and mucosal antibodies to *Campylobacter jejuni* antigens including flagellin, during experimental oral infections in chickens. *Avian Dis* 38:341-349.

23. Centers for Disease Control and Prevention. *Campylobacter* Infections. Division of Bacterial and Mycotic Diseases. Available at: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/campylobacter_g.htm. Accessed 20 October 2007.

24. Chang, N., and D.E. Taylor. 1990. Use of pulsed-field agarose gel electrophoresis to size genomes of *Campylobacter* species and to construct a SalI map of *Campylobacter jejuni* UA 580. *J Bacteriol* 172:5211-5217.

25. Cortez, A. L. L., A. C. F. B. Carvahlo, E. Scarcelli, S. Miyashiro, A. Vidal-Martins and K. Burger. 2007. Survey of chicken abattoir for the presence of *Campylobacter jejuni* and *Campylobacter coli*. *Rev Inst Med trop S Paulo* 48:307-310.

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

27. Dekeyser, P., M. Gossuin-Detrain, J. P. Butzler, and J. Sternon. 1972. Acute enteritis due to related vibrio: first positive stool cultures. *J Infect Disease* 125:390-392.

28. del Rio, E., R. Muriente, M. Prieto, Alonso-Calleja, C., and R. Capita. 2007. Effectiveness of trisodium phosphate, acidified sodium chlorite, citric acid, and peroxyacids against pathogenic bacteria on poultry during refrigerated storage. *J Food Prot* 10:2063-2071.

29. Denes, A. S., C. L. Lutze-Wallace, M. L. Cornier, and M. M. Garcia. 1997. DNA fingerprinting of *Campylobacter fetus* using cloned constructs of ribosomal RNA and surface array protein genes. *Vet Microbiol* 54:185-193

30. Doyle, LP. . 1944. A vibrio associated with swine dysentery. *Am J Vet Res* 5:3-5.

31. Engberg, J., I. Nahcamkin, V. Fussing, G. M. McKhann, J. W. Griffin, and J. C. Piffaretti. 2001. Absence of clonality of *Campylobacter jejuni* in serotypes other that HS:19 associated with Gillian-Barrè Syndrome and gastro enteritis. *J Infect Dis* 14:605-613.

32. Engvall, A., A. Bergqvist, K. Sandstedt, and M. L. Danielsson-Tham. 1986. Colonisation of broilers with *Campylobacter* in conventional broiler-chicken flocks. *Acta Vet Scand* 27:540-547.

33. Ertas, H., B. Cetinkaya, A. Muz, and H. Ongor. 2004. Genotyping of Broiler-originated *Campylobacter jejuni* and *Campylobacter coli* using *fla* typing and random amplified polymorphic DNA methods. *Int J Food Microbiol* 9:203-209.

34. Escherich, T. 1886. Articles adding to the knowledge of intestinal bacteria, III. On the existence of vibrios in the intestines and feces of babies. *Munch Med Wochenschr* 33:815-817.

35. Everest, P., H. Goosens, J. P. Butzler, D. Lloyd, S. Knutton, J. M. Ketley, and P. H. Williams. 1992. Differentiated Caco-2 cells as a model for enteric invasion by *Campylobacter jejuni* and *C. coli*. *J Med Microbiol* 37:319-325.

36. Federighi, M., J. M. Cappelier, A. Rossero, P. Coppen, and J. C. Denis. 1995. An evaluation of the effects of decontamination treatments with poultry carcasses on thermophilic *Campylobacter*. *Sci Aliment* 15:393-401.

37. Fischer, S.H., and I. Nachamkin. 1991. Common and variable domains of the flagellin gene *flaA* in *Campylobacter jejuni*. *Molec Microbiol* 5:1151-1158.

38. Fitzgerald, C., L. T. Helsel, M. A. Nicholson, S. J. Olsen, D. L. Swerdlow, R. Flahart, J. Sexton, and P. I. Fields. 2001. Evaluations of methods for subtyping Campylobacter jejuni during an outbreak involving a food handler. *J Clin Microbiol* 39:2386-2390.

39. Fox, J. G., T. Chilvers, C.S. Goodwin, N. S. Taylor, P. Edmonds, L. I. Sly, and D.J. Brenner. 1989. *Campylobacter mustelae*, a new species resulting form the elevation of *Campylobacter pylori* subsp. *mustelae* to species status. *Int J Syst Bacteriol* 39:301-303.

40. Fricker, C. R. and R.. W. Park. 1989. A two-year study of the distribution of 'thermophilic' campylobacters in human, environmental, and food samples from the Reading area with particular reference to toxin production and heat-stable serotype. *J Appl Bacteriol* 66:477-490.

41. Friedman,C. J., J. Neiman, H. C. Wegener, and R. V. Tauxe. 2000. Epidemiology of *Campylobacter* infections in the United States and other industrialized nations. p. 121-138. *In* Nachamkin I, and M.Blaser (eds.) *Campylobacter*. ASM Press, Washingotn, D.C.

42. Frost, J. A., J. M. Kramer, and S. A. Gillandrs. 1999. Phage typing of *Campylobacter jejuni* and *Campylobacter coli* and its use as an adjunct to serotyping. *Epidemiol Infect* 1:47-55.

43. Frost, J. A., A. N. Oza, R. T. Thwaites, and B.Rowe. 1998.Serotyping scheme for *Campylobacter jejuni* and *Campylobacter coli* based on direct agglutination of heat-stable antigens. *J Clin Microbiol* 36:335-339.

44. Gebhart, C. J., G. E. Ward, K. Chang, and H. J. Kurtz. 1983. *Campylobacter hyointestinalis* (new species) isolated from swine with lesions or proliferative iletis. *Am J Vet Res* 44:361-367.

45. Goodwin, C. S., J. A. Armstrong, T. Chilvers, M. Peter, M. Collins, L. I. Sly, W. McConnell, and W. E. S. Harper. 1989. Transfer of *Campylobacter pylori and Campylobacter mustelae* to *Helicobacter* gen. nov. as *helicobacter pylori* comb. nov. and *Helicobacter mustelae* nov., respectively. *Int J Syst Bacteriol* 39:397-405.

46. Grawjewski, B. A., J. W. Kusek, and H. M. Gelfand. 1985. Development of a bacteriophage typing scheme for *Campylobacter jejuni* and *Campylobacter coli*. *Epidemiol. Infect* 104:403-414.

47. Guerry, P., R. A. Alm, M. E. Power, S. M. Logan, and T. J. Trust. 1991. Role of two flagellin genes in *Campylobacter* motility. *J Bacteriol* 173:4757-4764.

48. Guillain, G., G. Barrè, and A. Strohl. 1916. Sur un syndrome de radiculonebrite avec hyperalbuminose du liquide cephalo-rachidien sans reaction cellulaire. Remarques sur les caracters cliniques et graphiiques des reflexes tendineux. *Bull Soc Med Hop Paris* 40:1462.

49. Hakkinen, M., and C. Schneitz. 1996. Efficacy of a commercial competitive exclusion product against chicken pathogenic Escherichia coli and E. coli O157:H7. *Vet.Rec*. 139:139-141.

50. Hakkinen, M., and C. Schneitz. 1999. Efficacy of a commercial competitive exclusion product against *Campylobacter jejuni*. *Br Poult Sci*. 40:619-621.

51. Harrington, C., F. Thomson-Carter, and P. Carter. 1997. Evidence for recombination in the flagellin locus of *Campylobacter jejuni*: Implications for the flagellin gene typing scheme. *J Clin Micobiol* 35:2386-2392.

52. Harvey, S. M. 1980. Hippurate hydrolysis by *Campylobacter fetus*. *J Clin Microbiol* 11:435-437.

53. Harvey, P. and S. Leach. 1998. Analysis of coccal cell formation by *Campylobacter jejuni* using continuous culture techniques, and the importance of oxidative stress. *J Appl Microbiol* 85:398-404.

54. Hendrixson, D. R., B. J. Akerly, and V. J. DiRita. 2001. Transposon mutagenesis of *Campylobacter jejuni* identifies a bipartite energy taxis system required for motility. *Mol Microbiol* 40: 214-224.

55. Hinton Jr, A., J. A. Cason, and K. Ingram. 2004. Tracking spoilage bacteria in commercial poultry processiing and refrigerated storage of poultry carcasses. *Int J Food Microbiol* 91:155-165.

56. Hudson, J. A., C. Nicol, J. Wright, R. Whyte, and S. K. Hasell. 1999. Seasonal variation of C*ampylobacter* types from human cases, raw chicken, milk, and water. *J Appl Microbiol* 87:115-124.

57. Hughes, R. A. C. and J. H. Rees. 1997. Clinical and epidemiologic features of Gillian-Barrè Syndrome. *J Infect Dis* 176:92-98.

58. Jacobs-Reitsma, W. F. 1995. *Campylobacter* bacteria in breeder flocks. *Avian Dis* 39:355-359.

59. Jacobs-Reitsma, W. F. 1997. Aspects of epidemiology of *Campylobacter* in poultry. *Vet Q* 19:113-117.

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

61. Johnson, W. M., and H. Lior. 1988. A new heat-labile cytolethal distending toxin (CLDT) produced by *Campylobacter* spp. *Microbiol Path* 4:115-126.

62. Kazwala, R. R., J. D. Collins, J. Hannan, R. A. Crinion, and H. O'Mahony. 1990. Factors responsible for the introduction and spread of *Campylobacter jejuni* infection in commercial poultry production. *Vet Rec* 126:305-306.

63. Keener, K. M., M. P. Bashor, P. A. Curtis, B. W. Sheldon, and S. Kathariou. 2004. Comprehensive Review of *Campylobacter* and Poultry Processing. *Comp Rev Food Sci and Food Saf* 3:105-116.

64. Khakhria, R., and H. Lior. 1992. Extended phage-typing scheme for *Campylobacter jejuni* and *Campylobacter coli*. *Epidemiol Infect*. 108:403-414.

65. Laconcha, I., N. Lopez-Molina, A. Rementeria, A. Audicana, I. Perales, and J. Garaizar. 1998. Phage typing combined with pulsed-field gel electrophoresis and random amplified polymorphic DNA increases discrimination in the epidemiological analysis of *Salmonella enteritidis* strains. *Int J Food Microbiol* 40:27-34.

66. Lara-Tejero, M., and J. Galan. 2001. CdtA, cdtB, and cdtC form a triparte complex that is required for cytolethal distending toxin activity. *Infect and Immun* 69:4358-4365.

67. Lawson, G., J. Leaver, G. Pettigrew, and A. Rowland. 1981. Some features of *Campylobacter sputorum* subsp. *mucosalis* subsp. nov., nom. rev., and their taxonomic significance. *Int J Sys Bact* 42:27-36.

68. Lindmark, H., C. Diedrich, L. Andersson, R .Lindqvist, and E. Olsson Engvall. 2006. Distribution of *Campylobacter g*enotypes on broilers during slaughter. *J Food Prot* 69:2902-2907.

69. Line, J. E., J. S. Bailey, N. A. Cox, N. J. Stern, and T. Thompkins. 1998. Effect of yeast-supplemented feed on *Salmonella* and *Campylobacter* populations in broilers. *Poult Sci* 77:405-410.

70. Line, J. E. and K. L. Hiett. 2007. Effect of coprophagia on colonization of broiler chicks with *Campylobacter jejuni*. Campylobacter Helicobacter and Related Organisms International Workshop.

71. Lior, H., D. L. Woodward, J. A. Edgar, L. J. Laroche, and P. Gill. 1982. Serotyping of C*ampylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. *J Clin Microbiol* 15:761-768.

72. Madden, R., A. Moran, and P. Scates. 1998. Frequency of occurrence of *Campylobacter* spp. in red meats and poultry in Northern Ireland and their subsequent subtyping using polymerase chain reaction-restriction fragment length polymorphism and the random amplified polymorphic DNA method. *J Appl Microbiol* 84:703-708.

73. Marshall, B. J., and C. S. Goodwin. 1987. Revised nomenclature of *Campylobacter pyloridis*. *Int J Sys Bacteriol* 37:68.

74. McClung, C., D. Patriquin, and R. Davis. 1983. *Campylobacter nitofigilis* sp. nov.; a nitorgen fixing bacterium associated with roots of *Spartina alterniflora* Loisel. *Int J Sys Bacteriol* 33:605-612.

75. Meinersmann, R. J., L.O. Helsel, P.I. Fields, and K. L. Hiett. 1997. Discrimination of *Campylobacter jejuni* isolates by *fla* gene sequencing. *J Clin Microbiol* 35:2810-2814.

76. Meinersmann, R. J., and K. L. Hiett. 2000. Concerted evolution of duplicate fla genes in *Campylobacter*. *Microbiologyl* 146:2283-2290.

77. Meinersmann, R. J., R. Phillips, K. L. Hiett, and P. Fedorka-Cray. 2005. Differentiation of *Campylobacter* populations as demonstrated by flagellin short variable region sequences. *Appl Environ Microbiol* 71:6368-6374.

78. Mellman, A., J. Mosters, E. Bartlet, P. Roggenten, A. Ammon, A. Friedrich, H. Karch, and D. Harmsen. 2004. Sequence-based typing of *flaB* is a more stable screening tool than typing of *flaA* for monitoring of *Campylobacter* populations. *J Clin Microbiol* 42:4840-4842.

79. Miller, W. G., and R. E. Mandrell. 2005. Prevalence of *Campylobacter* in the food and water supply. p. 101-164. *In* J.M.Ketley, and M.Konkel (eds.) *Campylobacter* Molecular and Cellular Biology. Horizon Bioscience, Norfolk, UK.

80. Miwa, N., T. Takegahara, K. Katsuya, and T. Hedeo. 2003. *Camplobacter jejuni* contamination of *C. jejuni*-negative flocks during processing in a Japanese slaughterhouse. *Int J Food Microbiol* 84:105-109.

81. Mooney, A., M. Clyne, D. Doherty, B. Kilmartin, and B. Bourke. 2001. *Campylobacter upsaliensis* exerts a cytolethal distending toxin effect on HeLa cells and T lymphocytes. *Path Med Microbiol* 147:735-743.

82. Nachamkin, I., K. Bohachick, and C. M. Patton. 1993. Flagellin gene typing of *Campylobacter* by restriction fragment length polymorphism analysis. *J Clin Microbiol* 31:1531-1536.

83. Nachamkin, I., H. Ung, and C. Patton. 1996. Analysis of HL and O serotypes of *Campylobacter* strains by the flagelin gene typing system. *J Clin Microbiol* 34:277-281.

84. Nachamkin, I., B. Allos, and T. Ho. 2000. *Campylobacter* and Gillian-Barrè Syndrome. p. 155-175. *In* I. Nachamkin, and M. B. Blaser (eds.) *Campylobacter*. ASM Press, Washington, DC.

85. Neill, S. D., J. L. Campbell, J. J. O'Brien, S. T. C. Weatherup, and W. Ellis. 1985. Taxonomic position of *Campylobacter cryaerophila* sp. nov. *Int J Sys Bacteriol* 35:342-356.

86. Newell, D. G. 2002. The ecology of *Campylobacter jejuni* in avian and human hosts and in the environment. *Int J Infect Dis* 6:3S16-3S21.

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

88. Newell, D. G., J. A. Frost, B. Duim, J. A. Wagenaar, R. H. Madden, J. van der Plas, and S. On. 2000. New developments in the subtyping of *Campylobacter* species. p. 27-44. *In* Nachamkin I, and M.Blaser (eds.) *Campylobacter*. ASM Press, Washington, D.C.

89. Newell, D. G. , J. E. Shreeve, M. Toszeghy, G. Domingue, S. A. Bull, T. J. Humphrey, and G. Mead. 2001. Changes in the carriage of *Campylobacter* strains by poultry carcasses during processing in abattoirs. *Appl Environ Microbiol* 67:2636-2640.

90. Newell, D. G., and J. A. Wagenaar. 2000. Poultry infections and their control at the farm level. p. 497-509. *In* I. Nachamkin and M. B. Blaser (eds.) *Campylobacter*. ASM press, Washington, D.C.

91. Nielsen, E., J. Engberg, and V. Fussing. 2001. Genotypic and Serotypic stability of *Campylobacter jejuni* strains during in vitro and in vivo passage. *Int J Med Microbiol* 291:379-385.

92. Nielsen, E., J. Engberg, V. Fussing, L. Petersen, C. Brogren, and S. On. 2000. Evaluation of phenotypic and genotypic methods for subtyping *Campylobacter jejuni* isolates from humans, poultry, and cattle. *J Clin Microbiol* 38:3800-3810.

93. Okrend, A. J., R. W. Johnston, and A. B. Moran. 1986. Effect of acid on the death rates at 52˚C of *Salmonella newport*, *Salmonella typhimurium*, and *Campylobacter jejuni* in poultry scald water. J Food Prot 49:500.

94. Owen, R., J. Hernandez, and A. Lastovica. 1993. PCR-based restriction fragment length polymorphism analysis of DNA sequence diversity of flagellin genes of *Campylobaccter jejuni* and allied species. *Mol Cell Probes* 7:471-480.

95. Park, S. 2005. *Campylobacter*: stress response and resistance. p. 279-299. *In* M.Griffiths (ed.) Understanding Pathogen Behavior, Virulence, Stress Response and Resistance. Woodhead Publishing, Cambridge.

96. Patton, C. M., I. K. Wachsmuth, G. M. Evins, J. A. Kiehlbach, B. D. Plikaytis, N. Troup, L. Tomkins, and H. Lior. 1991. Evaluation of 10 methods to distinguish epidemic-associated *Campylobacter* strains. *J Clin Microbiol* 29:680-688.

97. Pavlovskis, O.R. , D. M. Rollins, and R. L. Haberberger. 1991. Significance of flagella in colonization resistance of rabbits immunized with *Campylobacter* spp. *Infect Immun* 59:2259-2264.

98. Payne, R. E. M., M. D. Lee, D. W. Dreesen, and H. M. Bernhart. 1999. Molecular epidemiology of *Campylobacter jejuni* in broiler flocks using randomly amplified polymorphic DNA-PCR and 23S rRNA-PCR and role of litter in its transmission. *Appl Environ Microbiol* 65:260-263.

99. Pearson, A. D., M. H. Greenwood, T. D. Healing, R. K. Feltham, J. Donalson, 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.

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

101. Penner, J. L., and J. N. Hennessy. 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. *J Clin Microbiol* 12:732-737.

102. Pickett, C., E. C. Pesci, D. L. Cottle, G. Russell, A. N. Erdem, and H. Zeytin. 1996. Prevalence of cytolethal distending toxin production in *Campylobacter jejuni* and relatedness of *Campylobacter* spp. cdtB gene. *Infect Immun* 64:2070-2078.

103. Plastridge, W. N., L. F. Williams, and D. G. Trowbridge. 1964. Antibiotic sensitivity of physiologic groups of microaerophilic vibrios. *Am J Vet Res* 25:1295-1299.

104. Rhodes, K. M., and A. E. Tattersfield. 1982. Guillian-Barrè Syndrome associated with *Campylobacter* infection. *Br Med J* 285:173-174.

105. Rice, B., D. M. Rollins, E. T. Mallinson, L. Carr, and S. W. Joseph. 1997. *Campylobacter jejuni* in broiler chickens: colonization and humoral immunity following oral vaccination and experimental infection. *Vaccine* 15:1922-1932.

106. Ring, M., M.A. Zychowska, and R. Stephan. 2005. Dynamics of *Campylobacter* spp. spread investigated in 14 broiler flocks in Switzerland. *Avian Dis* 49:390-396.

107. Rosenquist, H., H. M. Sommer, N. L. Neilsen, and B. B. Christensen. 2006. The effect of slaughter on the contamination of chicken carcasses with thermotolerant *Campylobacter*. *Int J Food Microbiol* 108:226-232.

108. Russell, S.M. 2003. Intervention strategies for reducing *Salmonella* prevalence on Ready-to-Cook Chicken. Part One Processing. *Poult Int* 42:20-29

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

110. Salama, S., F. J. Bolton, and D. N. Hutchinson. 1989. Improved method for the isolation of Campylobacter jejuni and Campylobacter coli bacteriophages. Lett. Appl. Microbiol. 8:5-7.

111. Sanchez, M. X., W. M. Fluckey, M. M. Brashears, and S. R. McKee. 2002. Microbial profile and antibiotic susceptibility of *Campylobacter* spp. and *Salmonella* spp. in broilers processed in air-chilled and immersion chilled environments. *J Food Prot* 65:948-956.

112. Scarcelli, E., R.M. Piatti, R. Harakava, S. Miyashiro. 2005. Molecular subtyping of *Campylobacter jejuni* subsp. *jejuni* strains isolated from different animal species in the state of Sao Paulo, Brazil. *Braz J Microbiol* 36:378-382.

113. Schneitz, C. 2005. Competitive exclusion in poultry--30 years of research. *Food Control* 16:657-667.

114. Schouls ,L. M., S. Reulen, B. Duim, J. A. Wagenaar, R. J. L. Willems, K. Dingle, F. Colles, and J. Van Embden. 2003. Comparative genotyping of *Campylobacter* by amplified fragment length polymorphism, multilocus sequence typing, and short repeat sequencing: strain diversity, host range, and recombination. *J Clin Microbiol* 41:15-26.

115. Scientific Committee on Veterinary Measures Relating to Public Health. 1998. Benefits and limitations of antimicrobial treatments for poultry carcasses. Available from: http://europa.eu.int/comm/food/fs/sc/scv/out14_en.html. Brussels, Belgium: European Commission. Accessed August 29, 2007 .

116. Scientific Panel on Biological Hazards. 2005. Evaluation of the efficacy of peroxyacids for the use as an antimicrobial substance applied on poultry carcasses. *EFSA Journal* 306:1-10.

117. Shreeve, J. E., M. Toszeghy, A. Ridley, and D. G. Newell. 2000. Sequential spread of *Campylobacter* infection in multi-pen broiler house. *Avian Dis* 44:983-988.

118. Shreeve, J. E, M. Toszeghy, A. Ridley, and D. G. Newell. 2002. The carry-over of *Campylobacter* isolates between sequential poultry flocks. *Avian Dis* 46:378-285.

119. Sinhamhapatra, M., S. Biswas, A. K. Das, and D. Bhattacharyya. 2004. Comparative study of different surface decontaminants on chicken quality. *Br Poult Sci* 45:624-630.

120. Sjogren, E., Ruiz-Palacios, and B. Kaijser. 1989. Campylobacter jejuni isolations from Mexican and Swedish patients with repeated symptomatic and/or asymptomatic diarrhoea episodes. *Epidemiol Infect* 102:47-57.

121. Skirrow, M. B. 1982. *Campylobacte*r enteritis--the first five years. *J Hyg* 89:175-184.

122. Skirrow, M. B. 1994. Diseases due to *Campylobacter*, *Helicobacter,* and related organisms. *J Comp Pathol* 111:113-149.

123. Skirrow, M. B., and J. Benjamin. 1980. Differentiation of enteropathogenic *Campylobacte*r. *J Clin Pathol* 33:1122.

124. Skirrow, M. B., and M. B. Blaser. 2000. Clinical aspects of *Campylobacter* infection. p. 69-88. *In* I. Nachamkin and M. Blaser (eds.) Campylobacter. ASM Press, Washington, D.C.

125. Skirrow, M. B., and J.-P. Butzler. 2000. Foreword. p. xvii-xxii. *In* I. Nachamkin, and M. Blaser (eds.) Campylobacter. ASM Press, Washington, D.C.

126. Smith, T., and M. Taylor. 1919. Some morphological and biological characters of the spirilla (*Vibrio fetus*, n. sp.) associated with disease of the fetal membranes in cattle. *J Exp Med* 30:299-311.

127. Somers, E. B., Schoeni, J. L., and Wong, A. 1994. Effects of trisodium phosphate on biofilm and plaktonic cells of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium*. *Int J Food Microbiol* 22:269-276.

128. Stern, N. J., J. S. Bailey, L. C. Blankenship, N. A. Cox, and F. McHan. 1988. Colonization characteristics of *Campylobacter jejuni* in chick ceca. *Avian Dis* 32:330-334.

129. Stern, N. J., P. J. Fedorka-Cray, J. S. Bailey, N. A. Cox, S. E. Craven, K. L. Hiett, M. T. Musgrove, S. Ladely, D. Cosby, and G. C. Mead. 2001. Distribution of *Campylobacter* spp. In selected U.S. poultry production and processing operations. *J Food Prot* 64:1705-1710.

130. Swaminathan, B., T. Barrett, and the CDC PulseNet Task Force. 2000. A national molecular subtyping network for food-borne bacterial disease surveillance in the United States. p. 529-535. *In* Nachamkin I, and M.Blaser (eds.) *Campylobacter*. ASM Press, Washington, D.C.

131. Tanner, A., M. Listgarten, and J. Ebersole. 1981. *Wolinella* gen. nov. *Wolinella succinogenes* (*Vibrio succinogenes* Wolin et al.) comb. nov., *Wolinella recta* sp. nov., *Campylobacter concious* sp. nov. and *Eikenella corrodens* from humand with peridontal disease. *Int J Sys Bacteriol* 31:432-445.

132. Tauxe, R. V. , M. S. Deming, and P. A. Blake. 1985. *C. jejuni* infections on college campuses: A national survey. *Am J Public Health* 75:659-660.

133. Tenover, F.C., R. Arbeit, G. Archer, J. Biddle, and S. Byrne. 1994. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. *J Clin Microbio*l 32:407-415.

134. Thompson III, L. M., R. M. Smibert, J. L. Johnson, and N. R. Krieg. 1988. Phylogenetic study of the genus *Campylobacter*. *Int J Sys Bacteriol* 38:190-200.

135. Thompson, T.W., J.R. Blanton, J.E. Mann, M.M. Brashears, and C.Z. Alvarado. 2006. Reduction of *Campylobacter* spp. on poultry carcasses using various interventions under simulated industry conditions. *Poult Science Abstracts* 85:98.

136. Totten, P. A., C. L. Fennell, F. C. Tenover, J. M. Wezenberg, P. L. Perine, W. E. Stamm, and K. K. Holmes. 1985. *Campylobacter cinaedi* (sp. nov.) and *Campylobacter fenneliae* (sp. nov.): two new *Campylobacter* species associated with enteric disease in homosexual men. *J Infect Dis* 151:131-139.

137. Udayamputhoor, R. S., H. Hariharan, T. A. van Lunen, P. J. Lewis, S. Heaney, L. Price, and D. Woodward. 2003. Effects of diet formulations containing proteins from different sources on intestinal colonization by Campylobacter jejuni in broiler chickens. *Can J Vet Res* 67:204-212.

138. van den Bogaard, A. E. and E. E. Stobberingh. 1999. Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs 58:589-607.

139. Vandamme, P. 2000. Taxonomy of the family Campylobacteraceae. p. 3-26. *In* I. Nachamkin, and M.Blaser (eds.) *Campylobacter*. ASM Pres, Washington, D.C.

140. Veròn, M., and R. Chatelain. 1973. Taxonomic study of the genus *Campylobacter* Sebald and Veròn and designation of the neotype strain for the type species *Campylobacter fetus* (Smith and Taylor) Sebald and Veròn. *Int J Sys Bacteriol* 42:122-134.

141. Wagenaar, J. A., M. van Bergen, M. Mueller, T. A. Wassenaar, and R. Carlton. 2005. Phage therapy reduces *Campylobacter jejuni* colonization in broilers. *Vet Microbiol* 109:275-283.

142. 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* 82:244-230.

143. Wassenaar, T. A., N. M. C. Bleumink-Plyum, D. G. Newell, P. J. Nuijten, and B. A. van der Zeijst. 1994. Differential flagellin expression in a *flaAflaB*+ mutant of *Campylobacter jejuni*. *Infect Immun* 62:3901-3906.

144. Wassenaar, T. A., and D.G. Newell. 2000. Genotyping of *Campylobacter* spp. *Appl and Environ Microbiol* 66:1-9.

145. Wassenaar, T. A, S. On, and R. J. Meinersmann. 2000. Genotyping and the consequences of genetic instability. p. 369-380. *In* Nachamkin I, and M.Blaser (eds.) *Campylobacter*. ASM Press, Washington, D.C.

146. Wempe. J, C. Genigeorgis, T. Farver, and H. Yusufu. 1983. Prevalence of *Campylobacter jejuni* in two California chicken processing plants. *Appl Environ Microbiol* 45:355-359.

147. Widders, P., R. Perry, W. Muir, A. Husband, and K. Long. 1996. Immunizations of chickens to reduce intestinal colonization with *Campylobacter jejuni*. *Bri Poult Sci* 37:765-768.

148. Widders, P. R., L. M. Thomas, K. L. Long, M. A. Tokhi, M. Pannaccio, and E. Apos. 1998. The specificity of antibody in chickens immunized to reduce

intestinal colonization with *Campylobacter jejuni*. *Vet Microbiol* 64:39-50.

149. Woodward, D., and F. Rodgers. 2002. Identification of *Campylobacter* heat-stable and heat-labile antigens by combining the Penner and Lior serotyping schemes. *J Clin Microbiol* 40:741-745.

150. Wyszynska, A., A. Raczko, M. Lis, and E. K. Jagusztyn-Krynicka. 2004. Oral immunization of chickens with a virulent *Salmonella* vaccine strain carrying *C. jejuni 7*2Dz/92 *cjaA* gene elicits specific humoral immune response associated with protection against challenge with wild-type C*ampylobacter*. *Vaccine* 22:1379-1389.

151. Yao, R., D. H. Burr, and P. Guerry. 1997. CheY-mediated modulation of *Campylobacter jejuni* virulence.. *Mol Microbiol* 23:1021-1031.

152. Zhao, T and M. P. Doyle. 2006. Reduction of *Campylobacter jejuni* on chicken wings by chemical treatments. *J Food Prot* 69:762-767.

CHAPTER 3

GENETIC DIVERSITY OF *CAMPYLOBACTER* ON BROILER CARCASSES COLLECTED AT RE-HANG AND POST-CHILL IN 17 U.S. POULTRY PROCESSING PLANTS[*]

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Abstract

Campylobacter jejuni and *C. coli* are the most important human enteropathogens among the campylobacters. The objective of this study was to determine how diversity in Campylobacter found on chicken carcasses collected from re-hang and post-chill sites at 17 poultry processing plants in the United States is impacted during processing by sequencing the Short Variable Region of the *flaA* locus. Seventy percent of carcasses had one *flaA*-SVR type detected. *Campylobacter* genetic diversity decreased as carcasses proceeded through processing; carcasses sampled at re-hang had significantly more genetic diversity in *Campylobacter* populations than carcasses sampled at post-chill. There was more diversity in C*ampylobacter* on carcasses collected during winter than spring, summer, or fall. There were certain types that were present at re-hang that were not present at post-chill, and vice versa, suggesting that some subtypes may be prone to perish during processing while others may survive or persist despite stressors encountered with the processing environments.

Introduction

Campylobacter jejuni and *C. coli* are the most important human enteropathogens among the campylobacters, affecting an estimated 2.4 million cases each year in the U.S. alone (*4, 12*). Risks for contracting campylobacteriosis are consumption and handling of raw and undercooked poultry, most commonly chicken and turkey products, cross contamination with other foods, and consumption to contaminated milk and water (*2, 6, 19, 28, 33*).

Both *C. coli* and *C. jejuni* colonize the intestines of food-producing animals and humans (*29*). The favored environment appears to be the intestines of avians, which may be due the body temperature of 42˚ C, which is the optimal growth temperature for *Campylobacter*. There have been many investigations into the possible sources of contamination of poultry flocks with *Campylobacter*, but no definitive factor has been determined to explain its incidence (*6, 10*). Since *Campylobacter* is ubiquitous, it is easy for the flock to be exposed to many potential sources of contamination; current sampling and culture methods may not be adequate to recover all bacterial cells present. In some birds, particularly poultry, *Campylobacter* is a commensal. After one member of a flock has become contaminated, *Campylobacter* is transmitted from that animal to other animals.

C. jejuni and *C. coli* can be found on 90% of poultry in the U.S. (*32*) and from 18% to >90% of poultry in Europe, varying from country to country (*22*). Carryover contamination from a positive flock to a negative one is also a source for contamination in processing (*5, 22*). Cross-contamination in the slaughter plant due to contaminated equipment and processing water, is very difficult to control. Finished products that are

heavily contaminated with high numbers of microbes are considered undesirable from a food safety and quality point of view. There are various steps in processing designed to eliminate or control these microbes, such as chilling and multiple washes. In some instances, processing aids are used to further reduce the microbial load on chicken carcasses by removing surface contamination.

Measures taken to reduce or eliminate the prevalence of *Campylobacter* are being researched and applied in the food industry. Prevalence in foodstuffs is likely to have a huge impact on the health care industry, as *Campylobacter*-related illness and hospitalization costs are $8 billion per year in the United States alone (*9*). The underlying principle for reduction of *Campylobacter* infection in humans is to prevent colonization of poultry, or to reduce the prevalence on carcasses during processing. Clarification of the diversity and nature of *Campylobacter* infections helps in the investigations of disease. Molecular subtyping is an important tool for epidemiological studies; it helps in tracing sources and routes of transmission for human infection, identifying and monitoring specific strains over time and in different regions. Molecular subtyping also lends itself to the development of strategies to control transmission, sources and possible contamination of the food chain (*23, 25, 36*).

In this study, the Short Variable Region (SVR) of the flagellin locus was analyzed to determine the genetic diversity of populations of *Campylobacter* collected from rehang and post-chill sites at 17 broiler processing plants around the United States. We hypothesized that the diversity in *Campylobacter* populations would decline as carcasses move towards the end of processing, and the population diversity would not affected by the choice of chemical processing aid.

Materials and Methods

Origin of Isolates

Campylobacter isolates were from a previous USDA-FSIS study conducted in 2005 (*7*).

Isolation of Colony for DNA extraction

Isolates were removed from freezer and streaked for isolation on tryptic soy agar (TSA) (Remel, Inc; Lenexa, KS; USA) with 5% sheep blood, incubated at 42˚C for 24 to 36 hours. An isolated colony chosen for subculture was streaked for a lawn on TSA and grown for 24 hours to be used in DNA isolation.

DNA Isolation

DNA extraction was performed using an isolation kit from (Puregene; Gentra Systems; Minneapolis, MN) following the manufacturer's instructions.

PCR for speciation

An automated BAX PCR (DuPont Qualicon, Wilmington, DE) system was used to identify the species of the isolates as *C. jejuni* or *C. coli*. Reference strains for campylobacter included *C. coli* ATCC 33559 and *C. jejuni* ATCC 33560, as described (*12).* For the DNA samples that did not produce identifiable product during electrophoresis was then subject to a multiplex PCR as described by Wang (*36*).

PCR for flaA gene

FlaA-SVR PCR was performed as described by Meinersmann (*19*) with the following primers: FLA4F: 5'GGATTTCGTATTAACACAAATGGTGC 3' and FLA625RU: 5' CAAGWCCTGTTCCWACTGAAG 3', and the PCR cycles included: 94 ˚C for 2 min and then were cycled 30 times at 94 ˚C for 15 s, 50 ˚C for 30s, 72 ˚C for 1 min. Samples were incubated at 72 ˚C for 2 min and held at 4 ˚C until processed.

Amplification products were analyzed by electrophoresis at 130V for 60 mins using 1X TBE (0.89 Tris borate, 0.02M EDTA) running buffer on 2% agarose gels (Seakem LE agarose, Cambrex Bio Science Rockland, Inc., Rockland, ME). Gels were stained with 10mg/ml of an ethidium bromide solution (Sigma) and visualized on a UV gel documentation system. The PCR products were cleaned using the Qiagen BioRobot 3000 (Qiagen, Inc., Valencia, CA)

Sequencing

Cleaned PCR product was sent to the Integrated Biomolecular Resources laboratory within the Core Technologies at the Eastern Regional Research Center of the USDA ARS (600 East Mermaid Lane, Wyndmoor, PA) for dye-terminator DNA sequencing using Applied Biosystem 3730 DNA sequencer and the Applied Biosystem 3130 Genetic Analyzer (Applied Biosystems, Forest City, CA). Sequence data were assembled with Sequencher 4.7™ (Gene Codes Corporation, Inc; Ann Arbor, MI; USA). PAUP version 4.0b10 (Sinauer Associates, Inc., Sunderland, MA) was used to generate distance matrixes.

Statistical analysis

Statistical analysis was carried out using the Community Analysis Package 3.0, Species Diversity and Richness (2004, Pisces Conservation, Ltd. Lymington, UK) to find Simpson's Index and statistical differences through a randomization test by Solow (*30*).

Method for naming types

Nucleotide and peptide sequences for alleles of *Campylobacter* FlaA SVR have been collected on a curated database on the Internet (http://hercules.medawar.ox.ac.uk/flaA/) Each detected type was entered into the database to determine if its *flaA*-SVR sequence

was already published. Those types that were found in the database were identified by the published number. If a type was not found in the database, its closest relative was the basis of the name. For example, in this study there were 4 sequences most closely related to the published sequence of *flaA*-SVR type 54. These four were given the names 54.1, 54.2, 54.3, and 54.4. The current study made use of the *Campylobacter jejuni* Multi Locus Sequence Typing website (http://pubmlst.org/ campylobacter/) developed by Keith Jolley and Man-Suen Chan and sited at the University of Oxford.

Results and Discussion

Understanding how *Campylobacter* is transmitted and contaminates carcasses during processing will help in designing interventions to reduce or eliminate it in broiler production. Studying diversity of bacterial populations also may help scientists to identify bacteria that may have adapted for survival in the processing environment or may survive stresses and more likely to cause human sickness. In this study, we studied two distinct areas of a poultry processing line. We found that diversity declined as carcasses moved towards the end of processing,.

Out of 35 flocks, *C. jejuni* was the only species detected in 21 flocks, *C. jejuni* and *C. coli* were detected in 13 flocks (Table 1). There were three flocks primarily comprised of *C. coli,* and all samples from these flocks were taken during the spring sampling.

In total, 1249 isolates were sequenced; they were collected from 17 plants, representing 35 flocks. Seventy-three *flaA*-SVR types were identified (Figure 1). There was greater diversity in rehang samples than there was in post-chill samples. *Campylobacter* from plant 8 was the most diverse, with eighteen types present, while

plant 3 had the least diversity, with one type present (Data not shown). Plant 3 was used as the basis for comparison to find significant differences in all diversity indices. The five plants with the lowest diversity were significantly less diverse than the other 12 plants. Plant 3 had no diversity in rehang and post-chill samples, as seen in Table 2. Only *flaA*-SVR type 57 was found in this processing facility, which may suggest proliferation of one type on the farm and at arrival for slaughter, and possibly type 57 has flourished in this processing environment.

In this study, for the purposes of statistical analysis, diversity was analyzed in two ways: independent and dependent observations. Independent observations refer to type richness, or the number of types per sample. It does not account for the abundance of each type at each sampling occasion. A sampling occasion is defined as each instance a sample was taken. For example, there are four seasons and two sites (re-hang and post-chill) which makes eight possible sampling occasions for each detected type at each plant. By analyzing independent observations, equal weight was given to each type, minimizing the effect of abundance on diversity. For dependent observations, relative abundance of each independent observation was analyzed. It was important in this study to differentiate between the two types of observations to detect diversity changes because some variables, such as season, chemical treatment may affect dependent observations (abundance of a type) but not necessarily independent observations (number of types at each sampling occasion). When performing statistical analysis on independent observations, we did not detect a statistically significant difference in diversity for any variable (season, site, chemical). All tables and figures shown reflect the analysis of dependent observations, unless noted.

Campylobacter prevalence in poultry flocks varies with latitude; number of cecal organisms per bird but is generally higher in summer than in winter (*14, 34*). In the current data, fall was the least diverse season with a diversity index 0.71, as shown in Table 3. Winter was the most diverse season. When considering independent observations, summer was the most diverse season, with a diversity index of 0.98; however, there was no significant difference in diversities in independent observations. There is a peak in human *Campylobacter*-related sickness and prevalence in warmer months (*6, 16*). Migratory birds and insects are suggested sources (*6, 15*). Over all seasons, there was a significant difference in diversity among fall, winter, and spring and summer. There was no significant difference observed between spring and summer. Over the four samplings that roughly represented the four seasons, there was a significant difference in diversities between the rehang and post-chill sites in the fall and winter seasons and no significant difference in diversity between rehang and post-chill sites in the spring and summer, as shown in Table 4. When comparing the diversity of all rehang samplings over all seasons, there was no significant difference, the same was noted for as post-chill samples. When comparing the diversity for each site, not differentiating for plants, it was found that rehang is significantly more diverse than post-chill, with diversity indices of 0.9466 and 0.9243, respectively.

Chicken carcasses produced in the United States and in the United Kingdom are frequently contaminated with multiple subtypes of *Campylobacter* (*11, 18, 25*). Two carcasses, (0.5%) had four genotypes detected, as seen in Table 5. By far, the majority of carcasses, 70%, had one *flaA*-SVR type detected n them.

In accordance with the results presented by Lindmark (2006) there may be a correlation between high flock prevalence and presence of several genotypes on carcasses. In the current study, results from plant three were in agreement with Lindmark's observation. Plant 3 had the lowest numbers, 0.78 ± 0.23 log CFU/ml at re-hang, 0.00 ± 0.00 log CFU/ml post-chill and low prevalence (Table 7), and the lowest diversity indices of 0.00. Plant 8 had the highest diversity, 0.83 and one of the highest prevalence and numbers among the studied plants, 4.33 ± 0.10 log CFU/ml and 1.06 ± 0.11 log CFU/ml for re-hang and post-chill respectively. However, counter to Lindmarks' assertion, in Plant 4 had the highest numbers post-chill and almost the highest in re-hang samples, 1.19 ± 0.11 log CFU/ml and 4.38 ± 0.10 log CFU/ml, respectively, with a diversity index of 0.54.

Chemical processing aids are used in poultry processing facilities to reduce the bacterial loads on carcasses; they may change the microtopography of chicken skin kill, damage or remove bacteria (*13*). Some chemical processing aids have an effect on the pH of the rinse water in the processing facility. Trisodium phosphate (TSP) for example, raises the pH and reduces the efficiency of chlorine as an antimicrobial. It may be expected to see a higher prevalence and genetic diversity in facilities that used TSP as a chemical processing aid. This was observed for the TSP utilizing plants, 4, 8, 10, 17, and 20. Plants that utilize TSP had the highest genetic diversity in post-chill sites as well as the highest prevalence of *Campylobacter*, as shown in Table 6 and Table 8 while plants utilizing hypochlorous acid system (HAS) had the lowest genetic diversity in the post-chill sample, with one *flaA*-SVR type surviving chemical treatment detected. Plants

using a blend of food grade acids (FGA) and chlorine dioxide (CD) as processing aids did not have significantly more diversity in the rehang site than at the post-chill site.

Alter (*3*) found that AFLP type distribution changes during processing, beginning with a broad diversity at the beginning of slaughter, with only a few highly genetically related *Campylobacter* subtypes present after a 24 h chilling period. In the current study there was less diversity later in processing, but the types found may not be very closely related. A lower diversity was observed in the post-chill site when compared to the rehang site. the overall index of diversity for rehang samples was 0.9466 and for post-chill samples the overall index of diversity was 0.9243. A high diversity in *C. jejuni* genotypes was isolated at the beginning of the slaughter line, with a significantly lower diversity found at post-chill sites. In many plants, prevalence was very low in post-chill sites, which will affect diversity .

In *Campylobacter*, genetic recombination is known to occur. In some studies, isolates with similar PFGE profiles were concluded to be of clonal origin, a product of genomic rearrangements (*36*). In addition, Rivoal (*27*) suggests that genotypes with similar but not identical *fla* and *rib* rRNA gene profiles are derivatives of a more commonly isolated subtype. In the current study, there were some *flaA*-SVR types that were closely related, but more study is needed to prove if there is a single origin for these types.

It has been reported that carcasses sampled towards the end stage of processing have been contaminated with campylobacters even when the bacteria was not isolated from the chickens at the arrival at the abattoir (*1, 8, 21, 24*). In addition, it has been shown that the subtypes of campylobacters found on carcasses from colonized chicks are

not always those that were most prevalent in the guts of birds (*1, 24*). In the current study, there were several subtypes detected post-chill that were not detected at re-hang, which may be indicative of contamination occurring during processing (Figures 1 and 2). The scalding process and the chilling period put selective pressure on the *C. jejuni* population. Certain stress-resistant subpopulations may survive and ultimately remain in the food chain leading to the conclusion that different genotypes not only exhibit a different potential for colonization but also a different potential to survive environmental stressors (*3, 26, 31*). There were many types found at the re-hang site that were not found at post-chill (Figures 1 and 2). This may show that some types are prone to perish during processing while others survive to retail or some types may flourish in stressful environments.

A change in diversity for independent samples may be an indication of whether some types of *Campylobacter* are more resistant to treatment. However, because of the propensity of *C. jejuni* to engage in lateral gene transfer, the flaA-SVR cannot be expected to be linked to the trait that confers the resistance; we can only conclude that there are resistance traits.

For this study, diversity of *Campylobacter* on broiler carcasses was examined. It was found that diversity decreased as a carcass proceeded through processing, and the season during which the carcass was sampled may affect on the diversity of types detected. We also found that the chemicals used in reprocessing or added to chill tanks may impact the diversity of *Campylobacter*. It must be kept in mind that in this study, we considered independent observations, yet, the sample size was inadequate to determine statistical significance. For future research, it may be possible to include a

larger sample size to determine the effects of processing on independent observations. It may also be promising to study those types that are able to survive processing or proliferate in the processing environment to determine any special characteristics possessed, and their possible impact on human health.

References

1. Allen, V. M., S. A. Bull, J. E. L. Corry, G. Domingue, F. Jørgensen, J. A. Frost, R. Whyte, A. Gonzalez, N. Elviss, and T. J. Humphrey. 2007. *Campylobacter* spp. Contamination of chicken carcasses during processing in relation to flock colonization. *Int J Food Microbiol* 113:54-61.

2. 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.

3. Alter, T., F. Gaull, A. Froeb, K. Fehlhaber. 2005. Distribution of *Campylobacter jejuni* at different stages of a turkey slaughter line. *Food Microbiol* 22:245-51

4. Anonymous. 2007. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food--10 States, 2006. *Morb Mortal Wkly Rep*, United States Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

5. Atterbury, R. J., P. L. Connterton, C. E. R. Dodd, C. E. D. Rees and I. F. Connerton. Isolation and Characterization of Campylobacter Bacteriophages from Retail Poultry. *Appl Environ Microbiol*. 69: 4511-4518.

6. Barrios, P.R., J. Reirsen, R. Lowman, J.R. Bisaillon, P. Michael, V. Fridriksdóttir, E. Gunnarsson, N. Stern, O. Berke, S. McEwena, and W. Martin. 2006. Risk factors for *Campylobacter* spp. Colonization in Iceland. *Prev Vet Med*. 74:264-278.

7. Berrang, M. E., J. S. Bailey, S. F. Altekruse, B. Patel, W.K. Shaw Jr., Meinersmann, R. J., and P. J. Feorka-Cray. 2007. Prevalence and numbers

of *Campylobacter* on broiler carcasses collected at rehang and post-chill in 20 U.S. processing plants. *J Food Prot* 70:1556-1560.

8. Berrang, M. E., and J. A. Dickens. 2000. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant *J Appl Poult Res*. 9:43-47.

9. Buzby, J. C., B. M. Allos, and T. Roberts. 1997. The economic burden of *Campylobacter* associated Gillian-Barrè Syndrome. *J Infect Dis* 176 (Suppl 2):S192-S197.

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

11. Dickins, M. A., S. Franklin, R. Stefanova, G. E. Schutze, K. D. Eisenach, I. Wesley, and M. D. Cave. 2002. Diversity of Campylobacter isolates from retail poultry carcasses and from humans as demonstrated by pulsed-field gel electrophoresis. *J Food Prot* 65:957-962.

12. Englen, M. D. and P. J. Fedorka-Cray. 2002. Evaluation of a commercial diagnostic PCR for the identification of *Campylobacter jejuni* and *Campylobacter coli*. *Lett Appl Microbiol*. 35:353-356.

13. Friedman,C. J., J. Neiman, H. C. Wegener, and R. V. Tauxe. 2000. Epidemiology of *Campylobacter* infections in the United States and other industrialized nations. p. 121-138. *In* Nachamkin I, and M.Blaser (eds.) *Campylobacter*. ASM Press, Washingotn, D.C.

14. Hinton Jr, A., J. A. Cason, and K. Ingram. 2004. Tracking spoilage bacteria in commercial poultry processiing and refrigerated storage of poultry carcasses. *Int J Food Microbiol* 91:155-165.

15. Hudson, J. A., C. Nicol, J. Wright, R. Whyte, and S. K. Hasell. 1999. Seasonal variation of C*ampylobacter* types from human cases, raw chicken, milk, and water. *J Appl Microbiol* 87:115-124.

16. Jacobs-Reitsma, W. F. 1997. Aspects of epidemiology of *Campylobacter* in poultry. *Vet Q* 19:113-117.

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

18. Jolley, K. A., M. Chan, and M. Maiden. 2004. mlstdbNet - distributed multi-locus sequence typing (MLST) databases. *BMC Bioinformatics* 5:86.

19. Lindmark, H., C. Diedrich, L. Andersson, R .Lindqvist, and E. Olsson Engvall. 2006. Distribution of *Campylobacter g*enotypes on broilers during slaughter. *J Food Prot* 69:2902-2907.

20. Meinersmann, R. J., L.O. Helsel, P.I. Fields, and K. L. Hiett. 1997. Discrimination of *Campylobacter jejuni* isolates by *fla* gene sequencing. *J Clin Microbiol* 35:2810-2814.

21. Miller, W. G., and R. E. Mandrell. 2005. Prevalence of *Campylobacter* in the food and water supply. p. 101-164. *In* J.M.Ketley, and M.Konkel (eds.) *Campylobacter* Molecular and Cellular Biology. Horizon Bioscience, Norfolk, UK.

22. Miwa, N., T. Takegahara, K. Katsuya, and T. Hedeo. 2003. *Camplobacter jejuni* contamination of *C. jejuni*-negative flocks during processing in a Japanese slaughterhouse. *Int J Food Microbiol* 84:105-109.

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

24. Newell, D. G., J. A. Frost, B. Duim, J. A. Wagenaar, R. H. Madden, J. van der Plas, and S. On. 2000. New developments in the subtyping of *Campylobacter* species. p. 27-44. *In* Nachamkin I, and M.Blaser (eds.) *Campylobacter*. ASM Press, Washington, D.C.

25. Newell, D. G. , J. E. Shreeve, M. Toszeghy, G. Domingue, S. A. Bull, T. J. Humphrey, and G. Mead. 2001. Changes in the carriage of *Campylobacter* strains by poultry carcasses during processing in abattoirs. *Appl Environ Microbiol* 67:2636-2640.

26. Nielsen, E., J. Engberg, and V. Fussing. 2001. Genotypic and Serotypic stability of *Campylobacter jejuni* strains during in vitro and in vivo passage. *Int J Med Microbiol* 291:379-385.

27. Payne, R. E. M., M. D. Lee, D. W. Dreesen, and H. M. Bernhart. 1999. Molecular epidemiology of *Campylobacter jejuni* in broiler flocks using randomly amplified polymorphic DNA-PCR and 23S rRNA-PCR and role of litter in its transmission. *Appl Environ Microbiol* 65:260-263.

28. Rivoal K., C. Ragimbeau, G. Salvat, P. Colin, G. Ermel. 2005. Genomic diversity of *Campylobacter coli* and *Campylobacter jejuni* isolates recovered from

free-range broiler farms and comparison with isolates of various origins. *Appl Environ Microbiol* 71:6216-27.

29. Skirrow, M. B. 1982. *Campylobacte*r enteritis--the first five years. *J Hyg* 89:175-184.

30. Skirrow, M. B. 1994. Diseases due to *Campylobacter*, *Helicobacter,* and related organisms. *J Comp Pathol* 111:113-149.

31. Solow, A. R. 1993. A simple test for change in community structure. *J Anim Ecol* 62:191-193

32. Stern, N. J., J. S. Bailey, L. C. Blankenship, N. A. Cox, and F. McHan. 1988. Colonization characteristics of *Campylobacter jejuni* in chick ceca. *Avian Dis* 32:330-334.

33. Stern, N. J., P. J. Fedorka-Cray, J. S. Bailey, N. A. Cox, S. E. Craven, K. L. Hiett, M. T. Musgrove, S. Ladely, D. Cosby, and G. C. Mead. 2001. Distribution of *Campylobacter* spp. In selected U.S. poultry production and processing operations. *J Food Prot* 64:1705-1710.

34. Tauxe, R. V. , M. S. Deming, and P. A. Blake. 1985. *C. jejuni* infections on college campuses: A national survey. *Am J Public Health* 75:659-660.

35. 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* 82:244-230.

36. Wang, G., C. G. Clark, T. M. Taylor, C. Pucknell, C. Barton, L. Price, D. L. Woodward, and F. G. Rodgers. 2002. Colony multiplex PCR assay for

identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. *J Clin Microbiol* 40:4744-4747.

37. Wassenaar, T. A., and D.G. Newell. 2000. Genotyping of *Campylobacter* spp. *Appl and Environ Microbiol* 66:1-9.

38. Wassenaar, T. A, S. On, and R. J. Meinersmann. 2000. Genotyping and the consequences of genetic instability. p. 369-380. *In* Nachamkin I, and M.Blaser (eds.) *Campylobacter*. ASM Press, Washington, D.C.

Table 1: Percentage of *C. jejuni* and *C. coli* detected on carcasses from each of 17 commercial processing plants sampled across seasons in 2005.

Plant	Season	% *C. jejuni*	% *C. coli*	n
1	Spring	100	0	10
	Summer	100	0	16
2	Spring	100	0	34
3	Fall	100	0	43
4	Fall	100	0	42
	Spring	100	0	67
	Summer	96.55	3.45	58
5	Fall	84.38	15.62	32
	Winter	100	0	44
	Spring	2.78	97.22	72
6	Summer	100	0	80
7	Winter	100	0	41
	Spring	100	0	16
	Summer	100	0	48
8	Winter	78.38	21.62	37
	Spring	100	0	52
	Summer	83.33	16.67	48
9	Winter	100	0	20
10	Winter	100	0	38
	Spring	100	0	53

	Summer	100	0	39
11	Spring	3.28	96.72	61
14	Winter	100	0	32
	Spring	0	100	59
	Summer	100	0	55
15	Winter	89.74	10.26	39
	Spring	46.3	53.7	54
	Summer	90.32	9.68	31
16	Winter	100	0	11
	Summer	26.67	73.33	30
17	Winter	100	0	40
	Spring	100	0	35
	Summer	22.73	77.27	66
18	Winter	94.74	5.26	38
20	Summer	89.19	10.81	37

Table 2: Simpson's Index of Diversity for *Campylobacter* detected on broiler carcasses from 17 commercial processing plants at re-hang, post chill or combined, over all seasons

Plant	Simpson's Index of Diversity
3	0.000[a]
6	0.0282[a]
18	0.0715[a]
11	0.2361[a]
2	0.2390[a]
7	0.4518[b]
1	0.4629[b]
16	0.5229[b]
4	0.5442[b]
9	0.5538[b]
14	0.6796[b]
17	0.7672[b]
15	0.7746[b]
20	0.7748[b]
5	0.7882[b]
10	0.7904[b]
8	0.8313[b]

[a] Different superscripts represent significant difference in diversity in randomization test ($p \leq 0.05$)

Table 3: Simpson's Index of Diversity for *Campylobacter* detected on broiler carcasses during each season, all processing plants combined

Season	Simpson's Index of Diversity
FA	0.7170[a]
W	0.9195[b]
SP	0.8802[c]
SU	0.8960[c]

[a] Different superscripts represent significant difference by randomization test ($p \leq 0.05$).

Table 4: Simpson's Index of Diversity for *Campylobacter* detected on broiler carcasses during each season at re-hang and post-chill sites, all processing plants combined

Season	Site	Simpson's Index of Diversity
Fall	Re-hang	0.7684^{aZ}
	Post-chill	0.4805^{bY}
Winter	Re-hang	0.9136^{aZ}
	Post-chill	0.8667^{bY}
Spring	Re-hang	0.8772^{cZ}
	Post-chill	0.8196^{cY}
Summer	Re-hang	0.9127^{cZ}
	Post-chill	0.8065^{cY}

[a] Diversity indices within the same plant at different sites with different superscripts are significantly different by randomization test ($p \leq 0.05$).

[Z] Diversity index at the same Site in different plants with different superscripts are significantly different by randomization test ($p \leq 0.05$).

Table 5: Number of unique *flaA*-SVR types detected per broiler carcass [a]

No Types/ Carcass	Site	Total No Carcasses	% Total Carcasses	% Site
1	Re-hang	212	50.84%	68.17%
	Post-chill	81	19.42%	76.42%
2	Re-hang	86	20.62%	27.65%
	Post-chill	23	5.52%	21.70%
3	Re-hang	11	2.64%	3.54%
	Post-chill	2	0.48%	1.89%
4	Re-hang	2	0.48%	0.64%
	Post-chill	0	0.00%	0.00%

[a] ≤ four isloates tested per carcass n = 417

Table 6: Simpson's Index of Diversity for *Campylobacter* detected on broiler carcasses for carcass treatments at re-hang and post-chill, all processing plants combined

Carcass Treatment	Site	Simpson's Index of Diversity
ASC[1]	Re-hang	0.8455[a]
	Post-chill	0.5410[b]
CD[2]	Re-hang	0.5933[a]
	Post-chill	0.3857[a]
FGA[3]	Re-hang	0.4882[a]
	Post-chill	0.5779[a]
HAS[4]	Re-hang	0.5631[a]
	Post-chill	0.0000[b]
NO TREATMENT	Re-hang	0.6222[a]
	Post-chill	0.4962[b]
PA[5]	Re-hang	0.8563[a]
	Post-chill	0.6445[b]
TSP[6]	Re-hang	0.9306[a]
	Post-chill	0.8281[b]

[a,b] Values within carcass treatments with different superscripts are significantly different by randomization test ($p \leq 0.05$)

[1] ASC, Acidified sodium chlorite (Sanova), Ecolab Inc. St. Paul, MN

[2] CD, Chlorine dioxide, Ashland Specialty Chemical, Boonton, NJ

[3] FGA, Blend of food grade acids (FreshFX), SteriFX Inc., Shreveport, LA

[4] HAS, Hypochlorous acid system ($Tomco_2$), Tomco Equipment Co., Loganville, GA

[6] PA, Peroxyacetic acid based antimicrobial (Inspexx 100), Ecolab Inc. St. Paul, MN

[7] TSP, Trisodium phosphate, Danisco USA Inc., New Century, KS

Table 7: *Campylobacter* prevalence and numbers (mean log CFU/ml carcass rinse ± standard error) detected on broiler carcasses at re-hang and post-chill for 17 different commercial processing plants sampled four times, 2005 (*7*)

	Re-hang		Post-chill	
Plant	No.+[1]	log CFU/ml	No.+	log CFU/ml
1	22^{Y}	1.62^{A} ± .26	5^{Z}	0.15^{B} ± 0.07
2	30^{Y}	2.21^{A} ± 0.24	3^{Z}	0.28^{B} ± 0.16
3	10^{Y}	0.78^{A} ± 0.23	0^{Z}	0.00^{B} ± 0.00
4	40^{Y}	4.38^{A} ± 0.10	36^{Y}	1.19^{B} ± 0.11
5	39^{Y}	3.35^{A} ± 0.17	18^{Z}	0.45^{B} ± 0.11
6	20^{Y}	2.35^{A} ± 0.38	20^{Y}	0.93^{B} ± 0.16
7	35^{Y}	2.52^{A} ± 0.18	14^{Z}	0.38^{B} ± 0.08
8	40^{Y}	4.33^{A} ± 0.10	34^{Z}	1.06^{B} ± 0.11
9	16^{Y}	1.36^{A} ± 0.30	1^{Z}	0.05^{B} ± 0.05
10	40^{Y}	3.55^{A} ± 0.16	20^{Z}	0.47^{B} ± 0.11
11	29^{Y}	1.64^{A} ± 0.20	8^{Z}	0.27^{B} ± 0.10
14	40^{Y}	3.50^{A} ± 0.16	31^{Z}	0.89^{B} ± 0.10
15	39^{Y}	4.49^{A} ± 0.19	22^{Z}	0.41^{B} ± 0.07
16	39^{Y}	3.05^{A} ± 0.18	3^{Z}	0.08^{B} ± 0.06
17	40^{Y}	4.19^{A} ± 0.14	31^{Z}	0.96^{B} ± 0.11
18	28^{Y}	2.67^{A} ± 0.32	11^{Z}	0.34^{B} ± 0.10
20	30^{Y}	2.98^{A} ± 0.29	13^{Z}	0.34^{B} ± 0.09
mean	30^{Y}	2.66^{A} ± 0.06	14^{Z}	0.43^{B} ± 0.02

[1] number positive out of 40 tested, 10 per replication

[A,B] Mean *Campylobacter* numbers from different sites within the same plant with different superscripts are significantly different ($P<0.01$) by students T test.

[Y, Z] *Campylobacter* prevalence values from different sites within the same plant with different superscripts are significantly different ($P<0.01$) by Chi Square test for independence

Table 8. *Campylobacter* counts (mean log CFU/ml carcass rinse ± standard error) from whole broiler carcass rinse samples collected at re-hang and post-chill as affected by reprocessing chemical treatment (*7)*

		Mean log CFU *Campylobacter* per ml rinse		
Chemical	n	Re-hang	Post-chill	Reduction
HAS[1]	40	$1.62^{cd} \pm 0.26$	$0.15^{cd} \pm 0.07$	$1.47^{cd} \pm 0.26$
PA	100	$1.94^{cd} \pm 0.16$	$0.15^{cd} \pm 0.06$	$1.79^{bc} \pm 0.17$
ASC	140	$2.48^{bc} \pm 0.14$	$0.04^{d} \pm 0.02$	$2.33^{b} \pm 0.14$
FGA	120	$1.63^{d} \pm 0.17$	$0.31^{c} \pm 0.06$	$1.32^{cd} \pm 0.15$
TSP	250	$3.73^{a} \pm 0.08$	$0.76^{a} \pm 0.04$	$2.97^{a} \pm 0.07$
CD	70	$2.82^{b} \pm 0.26$	$0.41^{bc} \pm 0.07$	$2.41^{ab} \pm 0.24$
none	80	$2.43^{bc} \pm 0.21$	$0.65^{ab} \pm 0.10$	$1.78^{bd} \pm 0.15$
mean	800	2.66 ± 0.06	0.43 ± 0.02	2.23 ± 0.06

[a,b,c,d] Values within columns with no like superscripts are significantly different ($p < 0.05$) by Tukey's honest significant difference test.

Figure 1: Dendrogram showing relatedness of *Campylobacter flaA*-SVR types detected on broiler carcasses from for 17 different commercial processing plants sampled four times, 2005

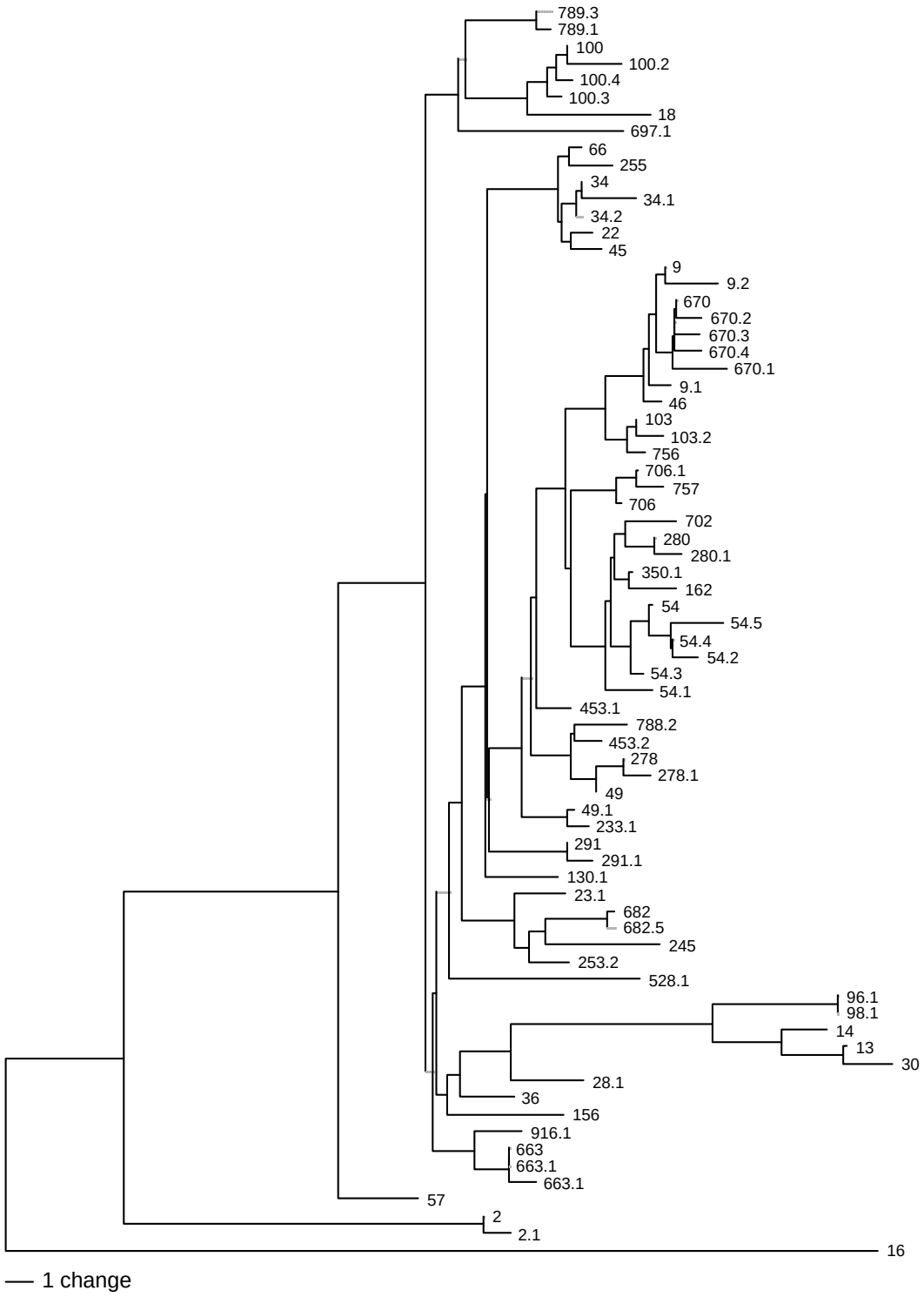

789.3
789.1
100
100.2
100.4
100.3
18
697.1
66
255
34
34.1
34.2
22
45
9
9.2
670
670.2
670.3
670.4
670.1
9.1
46
103
103.2
756
706.1
757
706
702
280
280.1
350.1
162
54
54.5
54.4
54.2
54.3
54.1
453.1
788.2
453.2
278
278.1
49
49.1
233.1
291
291.1
130.1
23.1
682
682.5
245
253.2
528.1
96.1
98.1
14
13
30
28.1
36
156
916.1
663
663.1
663.1
57
2
2.1
16
1 change

Figure 2: Frequency of *Campylobacter flaA*-SVR types detected across all plants and seasons, for re-hang and post-chill sites (Dependent observations)

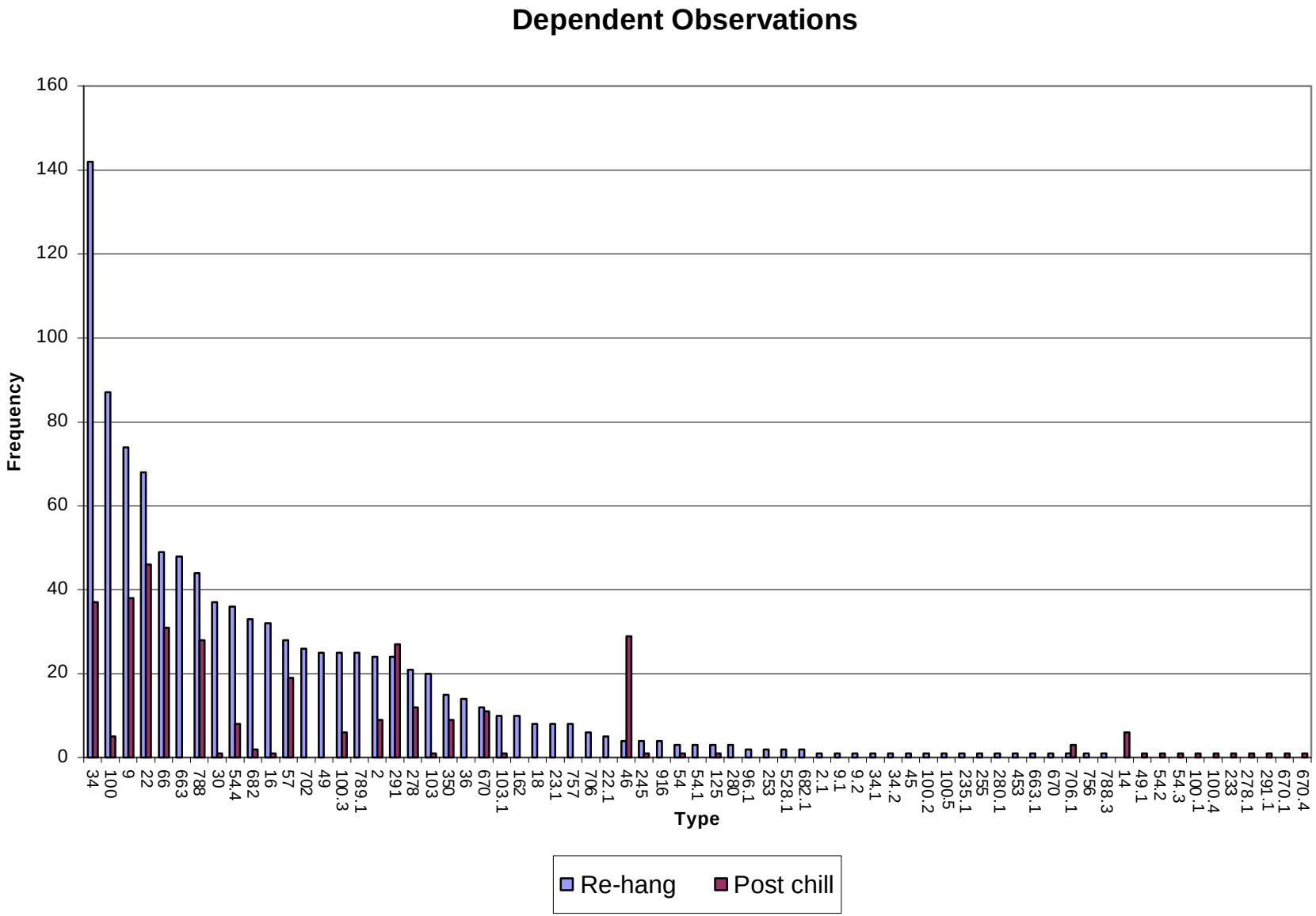
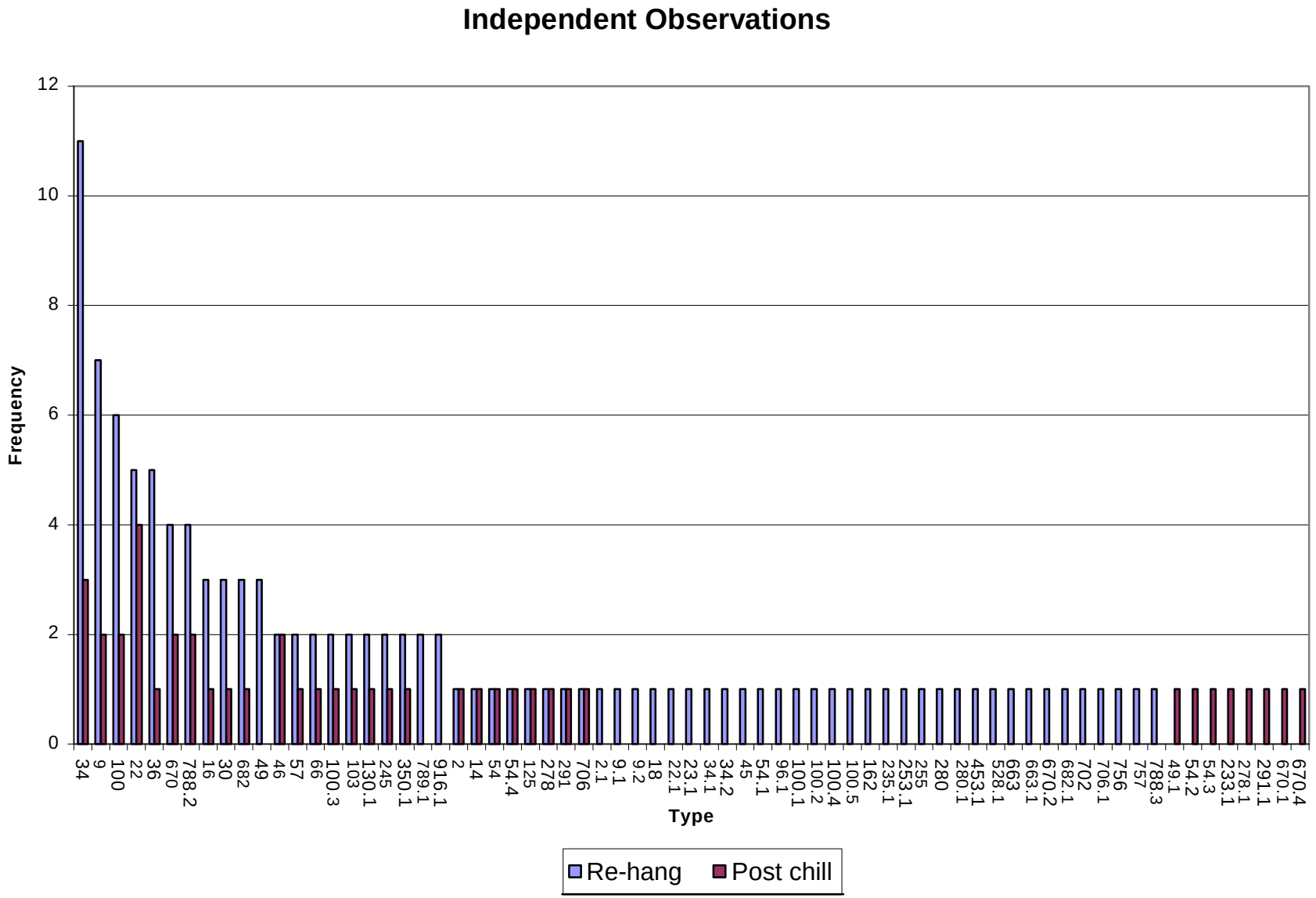

Dependent Observations
Frequency
160
140
120
100
80
60
40
20
0
34
100
9
22
66
663
788
30
54.4
682
16
57
702
49
100.3
789.1
2
291
278
103
350
36
670
103.1
162
18
23.1
757
706
22.1
46
245
916
54
54.1
125
280
96.1
253
528.1
682.1
2.1
9.1
9.2
34.1
34.2
45
100.2
100.5
235.1
255
280.1
453
663.1
670
706.1
756
788.3
14
49.1
54.2
54.3
100.1
100.4
233
278.1
291.1
670.1
670.4
Type
Re-hang
Post chill

Figure 3: Unique *Campylobacter flaA*-SVR types detected across all plants and seasons, for re-hang and post-chill sites (Independent observations)


Independent Observations
Frequency
0
2
4
6
8
10
12
Type
34
9
100
22
36
670
788.2
16
30
682
49
46
57
66
100.3
103
130.1
245
350.1
789.1
916.1
2
14
54
54.4
125
278
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49.1
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278.1
291.1
670.1
670.4
Re-hang
Post chill

CHAPTER 4

CONCLUSION

Campylobacter jejuni and *C. coli* are the most important human enteropathogens among the campylobacters, affecting an estimated 2.4 million cases each year in the U.S. alone. Risks for contracting campylobacteriosis are consumption and handling of raw and undercooked poultry, most commonly chicken and turkey products, cross contamination with other foods, and contaminated milk and water. *C. jejuni* and *C. coli* can be found on 90% of poultry in the U.S., and from 18% to >90% of poultry in Europe, varying from country to country. Carry over contamination from a positive flock to a negative one is a source for contamination in processing. In some instances, processing aids are used to further reduce the microbial load on chicken carcasses by removing surface contamination. However, use of these processing aids may have an additional effect on the microbial loads in addition to lowering counts. Clarification of the diversity and nature of *Campylobacter* helps in the investigations of disease.

In this study, the Short Variable Region (SVR) of the flagellin locus was analyzed to determine the genetic diversity of populations of *Campylobacter* collected from rehang (post-defeather) and post chill sites at 17 plants around the United States. Each population was analyzed for changes in diversity due to different variables. Diversity was affected by season, chemical processing aid, and site from which the sample was taken. It is also noted that several types may have been able to resist certain environmental stressors and adapt to the processing environment. Future study is needed

to determine any traits or characteristics that are prevalent in these types and enable survival and possible impacts on human health.