CAMPYLOBACTER, CHICKEN, AND THE REGULATORY PERFORMANCE
STANDARD

by

JANET SMITH

B.S., Piedmont College, 1986

A REPORT

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2013

Approved by:
Major Professor
Randall K. Phebus

Abstract

Campylobacter is recognized as a leading cause of bacterial gastroenteritis. In the United States, *Campylobacter* causes an estimated 600,000 illnesses and 55 deaths each year at a cost of over $1.3 billion. It is estimated that 80 percent of *Campylobacter* infections are foodborne with almost 50 percent of these cases attributed to poultry. Based on these statistics, *Campylobacter* and poultry is considered by some to be the riskiest pathogen-food combination. *Campylobacter* illness is usually self-limiting but serious illness and complications can occur. Serious illness requires treatment with antibiotics, but with emerging antibiotic resistance observed in *Campylobacter* isolates, treatment options might be limited. Therefore, it is of importance to reduce significantly the consumer's exposure to *Campylobacter* through poultry consumption. In July 2011, USDA FSIS's new performance standard for *Campylobacter* in chicken and turkey slaughter establishments went into effect. For chicken, the standard allows no more than eight *Campylobacter*-positive samples out of a fifty-one sample set. Methods for *Campylobacter* detection and enumeration include direct plating using a medium such as Campy-Cefex, MPN techniques, ELISA, and PCR. To meet the new performance standard the industry will need to consider improvements in poultry production. Improvements likely will not be limited to processing interventions such as scalding, picking, evisceration, and chilling. Improvements may include on-farm interventions such as enhanced biosecurity, use of competitive exclusion or vaccinations, good hygiene practices, and improved staging at introduction to processing. Post-processing interventions that might be considered include freezing or further processing (i.e. cooking) of poultry products from *Campylobacter*-positive flocks. Significant improvements in establishments' food safety programs are expected to occur to meet the standard and are predicted to result in an estimated reduction of 5,000 *Campylobacter* illnesses per year.

Table of Contents

List of Tables

Introduction

In July 2011, the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) implemented new performance standards for *Salmonella* and *Campylobacter* in chicken and turkey slaughter establishments. This implementation came during a time when consumer demand for safer food was increasing in response to the seemingly endless reports of food recalls due to associated human illnesses and even deaths because of the presence of pathogenic bacteria. These new requirements are just a small part of efforts focused toward improving food safety with the ultimate goal of reducing the public's exposure to pathogenic microorganisms in food and consequently reducing the incidence of foodborne illness. The regulation introduces a more stringent process control performance standard for *Salmonella* and introduces for the first time a performance standard for *Campylobacter.*

The promulgation of the *Campylobacter* performance standard comes almost simultaneously with the release of a report from the University of Florida's Emerging Pathogens Institute naming *Campylobacter* and poultry as the riskiest pathogen-food combination. This distinction is based on the high number of annual foodborne illnesses and costs of these illnesses attributed to *Campylobacter* acquired through the consumption of poultry. This same report also ranks *Campylobacter* number three in overall public health impact, outranked only by *Salmonella* and *Toxoplasma gondii* (Batz, Hoffmann, & Morris, 2011).

This report will describe the general characteristics of *Campylobacter*, its association with foodborne illness, public health significance, and role in poultry, particularly chicken, and the environment. Included in this report will be a summary of the features of the performance standard for *Campylobacter* in young chicken including the history behind the regulation. A concise review of specific methods for detection and cultivation found in literature will be

presented. Finally, this report will discuss strategies for the control of *Campylobacter,* issues to

be resolved, and future implications of the performance standard.

Campylobacter

Campylobacter is not a new organism but one described, although not given the official name, as a causative agent of diarrheal disease in animals and humans as early as 1886 (Post, n.d.). During the early 1900s, it was widely recognized in the veterinary community as a cause of spontaneous abortions in cattle and sheep and a cause of periodic diarrheal disease in these animals (Post, n.d.). These organisms were first classified as vibrios, but as distinct differences were recognized, a new genus *Campylobacter* was proposed in 1963 separating these organisms from the vibrios (Hoffman & Blankenship, 1985).

The *Campylobacter* genus is composed of fastidious organisms with distinct growth requirements. Due to this, recognition of the clinical significance of this organism was hampered early on until isolation techniques were developed. It was not until the 1970s that *Campylobacter* was first isolated from human samples and recognized in the medical community as an important cause of human enteritis (Hoffman & Blankenship, 1985). Skirrow described this new disease in the British Medical Journal in 1977 and further suggested its association with poultry (Post, n.d.). Today the *Campylobacter* genus consists of an estimated eighteen species with approximately half of these associated with human disease (Stern, Line, & Hui-Cheng, 2001). The genus will likely continue to evolve as molecular characterization techniques are further developed and additional knowledge is gained about the genus.

Campylobacters are gram-negative organisms exhibiting spiral rod morphology and corkscrew-type motility. They grow between 25°C and 45°C with the clinically significant species, described as thermotolerant, having an optimum growth temperature of 42°C. One distinguishing characteristic of the *Campylobacter* genus is the ability to grow only in a microaerophilic or reduced oxygen atmosphere. An atmosphere consisting of 5% oxygen, 10%

carbon dioxide, and 85% nitrogen is optimal for growth (as cited in Stern et al., 2001). *Campylobacters* are biochemically inert, not fermenting or oxidizing carbohydrates. They are noncompetitive with other flora; therefore, competing bacteria in a sample can easily overgrow and mask their presence.

Campylobacters* are very sensitive to environmental conditions such as drying, freezing, and oxygen (as cited in Chang, Mills, & Cutter, 2003). Considering *Campylobacter's* special growth requirements and sensitivity to environmental conditions, it seems this organism could be easily destroyed; however under adverse conditions, *Campylobacter* can change from the characteristic spiral shape to a coccoid form that is still essentially viable but nonculturable (Tangwatcharin, Chantahchum, Khopaibool, & Griffiths, 2006). This protection mechanism could explain why interventions that should reduce or eliminate *Campylobacter* are not always effective.

Campylobacter and Foodborne Illness

In 1942, Jensen reported four bacterial causes of foodborne illness: *Staphylococcus aureus, Salmonella, Clostridium botulinum,* and Streptococci (Miller, Smith, & Buchanan, 1998). Since that time, other pathogens including *Campylobacter* have emerged as significant illness causing foodborne contaminants. *Campylobacter,* although recognized as a causative agent of human disease since the 1970s, is still considered an emerging pathogen. According to the CDC definition, to have the distinction of being classified as an emerging pathogen, the incidence of an infectious agent in humans has to have dramatically increased in the past 20 years or be probable to increase in the future (as cited in Miller et al., 1998).

Campylobacter* is one of the leading causes of foodborne illness in industrialized countries. A 2011 report on foodborne illness stated *Campylobacter* and *Salmonella* were the

major contributors of foodborne illnesses in England, Wales, Australia, and the US (Scallan et

al., 2011). Friedman et al. attributed *Campylobacter* as being the leading cause of human

bacterial gastroenteritis worldwide (as cited in Engberg, Neimann, Nielsen, Aarestrup, &

Fussing, 2004). It has been estimated that up to 80 percent of *Campylobacter* infections are

foodborne (as cited in Smith, 2002). Table 1 is a compilation of the incidence of *Campylobacter*

in several countries in 2009. These data serve to underscore just how frequently *Campylobacter*

illnesses are reported.

Table 1: **Estimated rate of *Campylobacter* incidence per 100,000 population in 2009.**

Country	Incidence per 100,000
Australia	108.1
Canada	22.8
Europe (all combined)	45.57
Iceland	23.17
New Zealand	88.9
Norway	59.34
Switzerland	105.90
United Kingdom	106.32
United States	12.93

(C-EnterNet, 2012; EFSA, 2011; ESR, 2010; OzFoodNet, 2010)

While there is a strong case supporting the majority of *Campylobacter* illnesses as foodborne,

there are sporadic cases associated with recreational water use and handling of animals (Scallan

et al., 2011). Scallan et al. (2011) breaks down the distribution of *Campylobacter* illnesses in the

United States by probable source as follows: 6%, contact with animal feces; 5%, contact with pet puppy; 6%, contact with farm animals; 3%, consumption of untreated water, 80%, foodborne.

In the United States, foodborne illness data are collected through a cooperative effort of several agencies: CDC, select state health departments, USDA-FSIS, and FDA. These agencies form the Foodborne Diseases Active Surveillance Network (FoodNet). FoodNet covers roughly 15 percent of the US population and actively collects data of foodborne illnesses associated with several major pathogenic organisms including *Campylobacter* (CDC, FoodNet, 2011). Of 19,089 reported cases of foodborne illness in the United States in 2010, FoodNet data reveal 6,365 laboratory-confirmed cases of *Campylobacter* infection. This is an incidence of 13.58 per 100,000 people (CDC, FoodNet Figures, 2011). Table 2 shows the incidence of *Campylobacter* in the United States from 1996-2010. Unfortunately, these data do not correlate illnesses with specific food source.

Table 2: Number of laboratory-confirmed cases of *Campylobacter* infection and incidence per 100,000 population, Foodborne Disease Active Surveillance Network (FoodNet), United States

Year	Number of laboratory-confirmed cases	Incidence per 100,000 population	Surveillance population (millions)
1996	3367	23.59	14.27
1997	3960	24.55	16.13
1998	4022	19.42	20.71
1999	3832	14.82	25.86
2000	4708	15.36	30.65
2001	4751	13.61	34.90
2002	5064	13.34	37.95
2003	5272	12.60	41.85
2004	5686	12.79	44.45
2005	5692	12.68	44.87
2006	5770	12.70	45.42
2007	5871	12.78	45.94
2008	5854	12.61	46.41
2009	6058	12.93	46.86
2010	6365	13.58	46.86

(CDC, FoodNet Figures, 2011)

Of the clinically significant *Campylobacter* species, there are three responsible for the majority of human *Campylobacter* illnesses: *Campylobacter jejuni, Campylobacter coli,* and *Campylobacter lari* (Stern et al., 2001). *Campylobacter jejuni* is estimated to be the source in over 99 percent of cases (as cited in Smith, 2002). Robinson et al. estimated the percent illnesses caused by these species as follows: *Campylobacter jejuni* 90%*, Campylobacter coli* 7 to 8%*,* and *Campylobacter lari* 2-3% (as cited in Soncini et al., 2006).

Campylobacter gastroenteritis is usually self-limiting and resolves in 1-5 days (Smith, 2002). Since many cases of *Campylobacter* illness can be mild, it is likely the incidence of *Campylobacter* gastroenteritis is much higher than numbers reveal with many cases undiagnosed because medical attention is not sought. These infections would not be included in FoodNet data, which include only laboratory-confirmed cases. Tauxe estimated that "1 out of 100 individuals experiences a bout of campylobacteriosis each year" (as cited in Smith, 2002). A report from the Emerging Pathogens Institute estimates that *Campylobacter* causes more than 600,000 illnesses and 55 deaths each year at a cost of over $1.3 billion (Batz et al., 2011).

The typical characteristic of infection with *Campylobacter* is profuse diarrhea; however, symptoms can range from mild vomiting, cramping, and diarrhea to a more serious illness requiring hospitalization and antibiotic treatment (Lindblad, Hansson, Vagsholm, & Lindqvist, 2006). *Campylobacter* illness has been associated with serious complications such as Guillain-Barré and Miller-Fisher syndromes that result in acute neuromuscular paralysis, and Reiter's syndrome, which is a reactive arthritis (Stern et al., 2001). Hospitalization is required in approximately 10 percent of cases (as cited in Smith, 2002). According to 2010 FoodNet data, hospitalization was required in 928 cases (14.6%) (CDC. MMWR, 2011). Of these illnesses, eight deaths were attributed to *Campylobacter.* (CDC. MMWR, 2011).

The virulence of *Campylobacter* is associated with at least three factors: its ability to invade and damage intestinal mucosal cells, to produce cytotoxin, and to cross the intestinal mucosa and invade other sites (Zheng, Meng, Zhao, Singh, & Song, 2006). Not all strains are equally virulent. Besides differing host immune defenses, these differences in virulence may account to some degree for the varying severity of illnesses associated with *Campylobacter.* In cases associated with high fever, septicemia, or prolonged illness, antibiotic treatment is

necessary. Treatment with antibiotics can shorten the duration of *Campylobacter* illness and decrease the incidence of serious systemic infection (as cited in Gupta et al., 2004).

Erythromycin and fluoroquinolones such as ciprofloxacin are commonly used to treat *Campylobacter* infections (as cited in Larkin et al., 2006). Emerging antibiotic resistance among *Campylobacter* isolates and the subsequent decrease in effective antibiotic treatments has become a significant public health concern. Antibiotic resistance lessens therapeutic options for treating serious infections. Persons infected with antimicrobial-resistant strains of *Campylobacter* endure longer illness and are more frequently hospitalized (as cited in Nelson, Chiller, Powers, & Angulo, 2007).

According to the National Antimicrobial Resistance Monitoring System (NARMS), which began surveillance of *Campylobacter* antimicrobial resistance in 1997, resistance to ciprofloxacin significantly increased from 13 percent in 1997 to 19 percent in 2001 while resistance to macrolides, azithromycin, and erythromycin remained low at 1-3 percent (Gupta et al., 2004). The CDC reported the percent of *Campylobacter* isolates resistant to ciprofloxacin as 21 percent in 2002 and 26 percent in 2007 (as cited in Zhao et al., 2010). Roasto et al. (2007) attributed this emerging resistance primarily to the use of antibiotics in animal production and offered evidence from previously documented studies. In light of these findings, the FDA developed a new approach to animal drug approvals by examining "the probability of emerging antimicrobial resistance in animals as a result of drug use, the probability that these resistant bacteria will be transferred to humans, and the probability that these resistant bacteria will adversely affect human health" (as cited in Nelson et al., 2007). Harrison et al. stated, "In countries such as Denmark, where the use of enrofloxacin in poultry has been prohibited since

2000, a decrease in the percentage of fluoroquinolone-resistant *Campylobacter jejuni* and *Campylobacter coli* isolates has been detected" (as cited in Roasto et al., 2007).

Even though the development of resistance by the use of antibiotics in veterinary medicine is still debated, the FDA withdrew the approval for the use of enrofloxacin in poultry in 2005 and sarafloxacin use in poultry was voluntarily withdrawn in 2001 (Zhao et al., 2010). One study conducted in 1990, reported no findings of fluoroqinolone-resistant *Campylobacter* in 297 human isolates (Nelson, et al., 2007). This was prior to the approval of the use of fluoroquinolones in poultry production in 1995 and seems to corroborate the belief that the use of antibiotics in veterinary medicine can be a major contributor to emerging antimicrobial resistance. It has become a major objective worldwide to monitor developing antimicrobial resistance of enteric pathogens in food animals as a means of minimizing or eliminating the effect of antimicrobial use in animal production and subsequent development of resistance (Larkin et al., 2006).

According to a study by Zhao et al. (2010), despite these withdrawals, ciprofloxacin-resistant *Campylobacter* isolated from poultry still showed a slight increase. The 2010 NARMS report, National Antimicrobial Resistance Monitoring System: Enteric Bacteria Human Isolates Final Report, showed 22.4 percent of *Campylobacter* isolates were resistant to ciprofloxacin. When 2010 prevalence of resistant *Campylobacter* was compared to average prevalence of resistance in 2003-2007, there was no significant difference (NARMS, 2010). An interesting point that may need further study is whether the selective antimicrobials utilized in *Campylobacter* media are selecting for strains that are more resistant.

Campylobacter and Poultry

Campylobacter illness is most frequently associated with raw milk, poultry, beef, pork, and shellfish (Scallan et al., 2011). *Campylobacter* is part of the normal flora or at least a commensal organism of many warm-blooded animals including cattle, sheep, swine, turkey, chicken, wild birds and household pets. *Campylobacter* species are not host specific and can colonize a variety of animals and humans under ideal conditions (McCrea, Tonooka, VanWorth, Atwill, & Schrader, 2006). Wong et al. (2007) reported the following prevalence of *Campylobacter* in raw retail meat samples: 89.1% of chicken, 9.1% pork, 10% unweaned veal, 6.9% lamb and mutton, and 3.5% in beef. Additional studies found the prevalence of *Campylobacter* contamination of meats as follows: 90% poultry, 60% red meat, and 9-66.2% pork (as cited in Fosse et. al, 2006).

According to the USDA FSIS, there is "overwhelming" evidence that raw meat and poultry carry pathogenic organisms such as *Campylobacter* and consumption of these products presents risk to consumers (USDA FSIS, 1996). Wong et al. (2007) concluded that the prevalence in poultry is significantly higher than other meats and that chicken meat was more heavily contaminated than other raw meats. It seems chicken, and other fowl, may be ideal hosts due to their high body temperature, which is near the optimal growth temperature of 42°C for many thermotolerant *Campylobacter* species (McCrea et al., 2006). According to Harris and coworkers almost 50 percent of *Campylobacter* infections can be attributed to poultry (as cited in Stern & Pretanik, 2006).

Foodborne *Campylobacter* illness is usually sporadic and not associated with large outbreaks; however, of the reported outbreaks of *Campylobacter* infections, dairy foods were more often implicated with poultry following a close second (Department of Health & Human

Services, memorandum, January 18, 2011). Poultry was most commonly associated with

sporadic cases, with the Department of Health & Human Services stating that a higher risk of

Campylobacter illness from poultry was associated with eating out (memorandum, January 18,

2011). The Emerging Pathogens Institute reported that poultry is responsible for more foodborne

illnesses than any other food primarily due to the prevalence of *Salmonella* and *Campylobacter*

and estimated illnesses caused by contaminated poultry to be $2.4 billion annually (Batz et al.,

2011).

  Campylobacter jejuni is the predominant *Campylobacter* species isolated from broiler

chickens with *Campylobacter coli* the second most common (Lindblad et al., 2006). In a study

from Ireland, the species were distributed in raw chicken (fresh and frozen) as follows:

Campylobacter jejuni 69%, *Campylobacter coli* 30%, and *Campylobacter lari* 1% (Moore,

Wilson, Wareing, Humphrey, & Murphy, 2002). When colonized, chickens typically carry

Campylobacter in the intestinal tract, making fecal contents the primary source of

Campylobacter contamination (USDA FSIS, 1996). Several studies have shown that between

11-80 percent of the poultry examined showed the presence of *Campylobacter* in the intestines

(as cited in Soncini et al., 2006). Berrang et al. reported cecal contents could contain more than

7.0 log CFU/g of *Campylobacter* in infected birds (as cited in Berrang, Smith, & Hinton, 2006).

Processing of poultry, which exposes the intestinal contents, often results in spreading of

Campylobacter throughout the processing environment and to other carcasses (Fluckey et al.,

2003). After processing, USDA-FSIS data showed an average of 5,300 CFU of *Campylobacter*

per carcass and an 88 percent prevalence rate post chill (as cited in De Cesare, Sheldon, Smith,

& Jaykus, 2003).

As shown from the preceding data, poultry is a significant source of *Campylobacter.* According to the American Meat Institute, poultry consumption in the United States has risen steadily since the 1970s (AMI, 2009).   With the exception of a slight decline between 2007 and 2009, due in large part to economic factors, poultry consumption is again on the rise according to the National Chicken Council (Wattagnet, Apr 05, 2011).   With increasing consumption of poultry, chicken will continue to be a significant contributor to *Campylobacter*-associated foodborne illness. It is of primary importance that levels of *Campylobacter* in retail chicken be reduced or eliminated to minimize this risk to consumers.

The *Campylobacter* Performance Standard for Young Chickens

According to an internet survey, the top five food safety concerns are foodborne illness, food contaminants, pesticide exposure, antibiotic resistance, and environmental effects (Planet Matters and More, 2011). The Thomson Reuters PULSE Healthcare Survey of 2010 stated that 61 percent of respondents were concerned about food safety and contamination with over half of these indicating that meat was their biggest concern. The survey found that overall respondents felt the best approach for the industry to use to reduce the risk of foodborne illness was better quality controls followed by more inspections (Thomson, 2010). It should be noted that no amount of inspection could detect unseen microbiological hazards present in food. More inspection would further tax the overworked, under-budgeted USDA FSIS without significantly decreasing foodborne illness from pathogens present in the meat products. There must be a better alternative.

In 1906, the Federal Meat Inspection Act was passed partially in response to the unsanitary conditions in meatpacking houses exposed in Upton Sinclair's book, The Jungle. This act established sanitary standards for slaughter and meat processing plants and authorized

the USDA to monitor and inspect these operations. Initially the act did not include poultry but with growing consumer demand for poultry and processing, the Poultry Products Inspection Act was passed in 1957. The Food Safety and Quality Service was established by USDA in 1977 and later renamed The Food Safety Inspection Service (FSIS) in 1981. FSIS's mission was to manage an inspection program that would ensure meat, poultry, and eggs were safe and properly labeled (USDA FSIS, 1996).

With the inception of the Federal Meat Inspection Act in 1906, organoleptic measures were the sole means of evaluating the sanitary condition of meat and henceforth safety to the consumer. During the ensuing years, monumental changes occurred in the meat industry particularly in the processing of poultry. Along with the incorporation of automation, production increased as did consumer demand (USDA FSIS, 1995). These changes increasingly burdened the current poultry inspection system. In order to fulfill FSIS's food safety responsibilities there was need for change. No longer could the safety of meat and poultry be ensured by sensory measures. The agency began to recognize the need to address unseen hazards in poultry and the fact that pathogenic microorganisms were an increasing problem on raw meat and poultry.

In 1983, FSIS asked the National Academy of Sciences (NAS) to recommend measures to modernize the poultry inspection system (USDA FSIS, 1995). NAS recommended FSIS begin evaluating pathogenic organisms present on raw meat and poultry with an increase in microbial assessment of meat and poultry. A determination of what was needed to ensure the safety of meat and poultry could not be performed without evaluating what pathogenic organisms were associated with meat and poultry products. With this evaluation, then HACCP (Hazard Analysis and Critical Control Point) principles could be adopted to prevent

contamination rather than detect contamination (USDA FSIS, 1995). This would be the beginning of an important shift in improving meat and poultry safety.

HACCP was to accomplish three goals: prevent or delay growth of pathogens, reduce or destroy pathogens, and reduce the initial load thereby reducing subsequent contamination (CAST, 1994). Also in this timeframe, the Council for Agricultural Science and Technology (CAST) put together a team to ascertain what was currently known about foodborne pathogens (CAST, 1994). The 1994 report found that raw meats were most often implicated in foodborne illness and the application of HACCP principles would most likely reduce the number of foodborne illnesses from these foods. It was also recognized that different measures of control are required for different pathogens. There was not a one size fits all solution (CAST, 1994). One important recommendation from this team was that food-pathogen combinations be identified in order to establish controls to minimize risk (CAST, 1994).

In the July 25, 1996 Federal Register: 9 CFR Part 304, et al. Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) System; Final Rule,   FSIS established performance standards for *Salmonella* as an effort to monitor the effectiveness of the newly adopted Hazard Analysis Critical Control Point (HACCP) system in reducing food safety hazards (USDA FSIS, 1996). FSIS knew that current policies needed revision in order to comply with HACCP principles and there was a need to rely more heavily on performance standards (USDA FSIS, 1996). With this new rule, USDA FSIS (1996) outlined the following food safety goals:

(1) provisions for systematic prevention of biological, chemical, and physical hazards through adoption by meat and poultry establishments of science-based process control systems; (2) targeted efforts to control and reduce harmful bacteria on raw meat and

poultry products; (3) adoption of food safety performance standards that provide

incentives for innovation to improve food safety and to provide a measure of

accountability for achieving acceptable food safety results; (4) removal of unnecessary

regulatory obstacles to innovation; and (5) efforts to address hazards that arise throughout

the food safety continuum from farm to table. (p. 38807)

No longer would the government assume total responsibility for the production of safe food

products with their "command and control regulations" (USDA FSIS, 1996). Performance

standards ensured that industry would now shoulder the responsibility of producing safe food

(USDA FSIS, 1996).

The first targeted organism for the performance standard under the new HACCP based

system was *Salmonella*. *Salmonella* was selected as a monitoring tool due to the following: its

frequent isolation from poultry; the availability of current methods for detection; its distinction

as the most common cause of foodborne illness at that time; and the belief that any interventions

successful at reducing *Salmonella* incidence would be effective against other pathogens (USDA

FSIS, 1996). FSIS stated in this 1996 rule they would continue to collect data, adjust the

Salmonella performance standard, and set standards for other pathogens (USDA FSIS, 1996). In

FSIS's own words, "a standard is necessary to encourage innovation and provide the impetus for

continuing improvement and increasing effectiveness" (USDA FSIS, 1996). While *Salmonella*

was chosen as the "interim" target organism in the 1996 performance standard, FSIS

acknowledged the presence of other pathogens of public concern in poultry and requested

comments on the feasibility of targeting other pathogens (USDA FSIS, 1996). The use of the

term "interim" in the 1996 final rule certainly allows one to infer that performance standards for

other pathogens would be established in the future.

Today as before, it still is the agency's intention to rely heavily on performance standards. These standards are the means in which a plant can assess the effectiveness of their HACCP program for providing safe food (USDA FSIS, 1996). This philosophy is most evident in the newly implemented performance standard for *Campylobacter*. This performance standard is a result of the commissioning of the Food Safety Working Group by President Obama in March 2009 (USDA FSIS, 2010). This group's primary goals were to recommend improvements to U.S. food safety practices and regulations and ensure these were being followed and enforced (USDA FSIS, 2010). FSIS was specifically tasked with reducing Salmonella risk in poultry and developing other food safety plans. In response, the *Salmonella* performance standard was revised and a performance standard for *Campylobacter* was finally published (USDA FSIS, 2010). After years of speculation in the industry, it is only natural that *Campylobacter,* with its distinction as a major cause of foodborne illness, be selected as the next targeted organism.

Up until this time, it was not known what a *Campylobacter* performance standard would encompass. Would *Campylobacter* quantitation be required or would it be a determination of incidence by presence or absence testing? What species would be targeted? Would establishments be required to assess pre and post processing levels of *Campylobacter*? Would establishments be responsible for conducting the testing and reporting the results themselves or would FSIS collect and test the samples? What target level of *Campylobacter* would be established?

The performance standard for chicken is based on data obtained from FSIS's July 2007-June 2008 Nationwide Microbiological Baseline Data Collection Program: Young Chicken Survey. One of the main survey objectives was to gather information on the prevalence of select

bacteria in young chicken in order to establish regulatory policy (USDA FSIS, 2008). In this survey, the methodology used by FSIS was designed to be selective for three particular *Campylobacter* strains: *Campylobacter jejuni, coli, and lari.* These three species are responsible for the majority of human *Campylobacter* illnesses. While the performance standard does not specifically address these three species, the methodology used in the data collection and that will be used by FSIS in establishment verification testing is not optimized for recovery of other *Campylobacter* species (USDA FSIS, 2011).

Data gathered from the baseline survey for young chickens were from two distinct sample portions of the carcass rinse: a 1mL aliquot that was direct-plated and a 30mL aliquot that was enriched before plating. The data showed two important *Campylobacter* trends. The overall positive incidence in broiler chickens post chill for *Campylobacter* was found to be 40.23 percent (USDA FSIS, 2008). Additionally during processing, there was a reduction in *Campylobacter* incidence in chicken carcasses from 71.36 percent at rehang to 10.66 percent post chill (USDA FSIS, 2008). The latter data were determined from the 1mL aliquot of the 400mL carcass rinse.

Prior to the publication of the new performance standard, it had been assumed by many in the poultry industry, that a performance standard developed for *Campylobacter* would be a quantitative measure. Stern and Pretanik (2006) surmised that it was necessary to determine counts of *Campylobacter* on poultry carcasses in order to establish a "risk-acceptable count" on which industry could base control strategies. In contrast, the performance standard is a qualitative measure of incidence instead. The standard was set as no more than eight positive samples out of a 51-sample set when presence or absence of *Campylobacter* is determined by plating a 1mL portion of a 400mL post-chill carcass rinse (USDA FSIS, 2011). USDA FSIS

(2011) predicted, "as many as 5000 fewer cases of human illness due to *Campylobacter* might occur each year" with the implementation of the new performance standard.

The 1mL portion represents a theoretical detection limit of 1 CFU/mL or 400 CFU/carcass. In the original proposed rule, an additional 30mL enriched sample was to be tested representing a theoretical detection limit of 0.03 CFU/mL or 12 CFU/carcass. There was concern that the 1mL sample would detect only high levels of contamination while the enriched sample would more effectively detect lower levels of contamination but Stern and Pretanik (2006) found that higher recovery of *Campylobacter* using enrichment was not always true. The infectious dose of *Campylobacter* has been estimated to be approximately 500 to 800 cells (Cheng & Griffiths, 2003; Stern & Pretanik, 2006). One could conclude that testing a 1mL non-enriched sample would effectively detect levels that are potentially harmful to humans while the necessity of detecting levels lower than 400 CFU/carcass is not warranted. FSIS stated that even though the performance standard is based on a 1mL portion, data would be collected for the 30mL enriched sample to evaluate progress of the industry. "If there is no improvement in these data over time, FSIS may consider implementing the performance standard using the larger portion sample results as well" (USDA FSIS, 2011),

While this presence/absence determination simplifies the testing requirement for *Campylobacter,* Stern and Robach (2003) stated, "the mere presence of absence of *Campylobacter* spp. on broiler carcasses is an inadequate measure by which to account for the reduction in human disease." Although the baseline data showed a significant reduction in *Campylobacter* incidence during processing, this reduction may not be effective in reducing the risk to consumers (USDA FSIS, 2008). Authors of a New Zealand study stated that reducing the

number of bacteria on the carcass is more effective in reducing risk than trying to reduce the prevalence of positive carcasses (McIntyre, Lee, & Biggs, 2010b).

The performance standard corresponds to FSIS's 80-percent rule meaning an establishment actually operating at the performance standard has an approximately 80 percent chance of passing the set and therefore an approximately 20 percent chance of failing (USDA FSIS, 2010). If an establishment fails three consecutive sample sets, it is deemed not to have managed control of the process adequately to reduce the public's risk of pathogen exposure (USDA FSIS, 2010). It is unclear at this time what implications this has for the industry. New information and data obtained from FSIS's recurring baseline studies may warrant new approaches to microbial testing (USDA FSIS, 1996).

Methods for Detection of *Campylobacter*

Several cultural and non-cultural methods are available or are in development for the detection of *Campylobacter*. Cultural methods typically involve direct plating, enrichment, or MPN techniques. Cultural methods are often time-consuming and labor-intensive but produce isolates that can be further characterized. Non-cultural methods include techniques such as direct counting, conductance, immunoassay, PCR, spectroscopy, and biosensors. These techniques, while often providing rapid results have the disadvantages that they do not always provide a quantitative result and do not produce an isolate for further testing. Some methods employ a combination of cultural and non-cultural techniques.

Due to the fastidious nature and specific growth requirements of *Campylobacter,* isolation and identification can be difficult for the inexperienced laboratory technician. Identification is typically achieved through observation of characteristic morphology, distinct growth requirements, and only a few biochemical tests (Stern et al., 2001). The biochemical

inertness of the genus makes definitive species identification difficult. Table 3 demonstrates

some biochemical characteristics of selected species of *Campylobacter.*

Table 3: Biochemical Characteristics of Select *Campylobacter* species

Characteristic	*C. jejuni*	*C. coli*	*C. lari*	*C. fetus subsp. fetus*
Catalase	+	+	+	+
Oxidase	+	+	+	+
Glucose Utilization	-	-	-	-
H$_2$S, Lead Acetate	+	+	+	+
H$_2$S, TSI	-	+	D	-
Hippurate Hydrolysi**s**	+	-	-	-
Nitrate Reduction	+	+	+	+
Growth at 25°C	-	-	-	+
Growth at 42°C	+	+	+	-
Growth in 1% Glycine	+	+	+	+
Growth in 3.5% NaCl	-	-	-	-
Growth in 0.1% TMAO	-	-	+	D

D = 11% to 89% of strains are positive

(Stern et al., 2001)

All culture methods involve plating of the sample onto a growth medium, whether

directly, after enrichment, or using MPN techniques.   Growth media typically incorporate

antibiotics in the formula as selective agents to suppress growth of other competing bacteria.

These selective agents have a disadvantage in that they can also suppress stressed or susceptible

Campylobacter cells resulting in isolation of lower numbers or selection of specific

Campylobacter strains (Valdivieso-Garcia et al., 2007). Consequently, all enumeration methods are only an estimate of the number of *Campylobacter* present (Line, 2005).

In food matrices, *Campylobacter* counts can be low and require enrichment to reach numbers high enough to detect by plating. Enrichment involves the incubation of the sample in a selective broth to repair injured cells and enable growth to detectable levels. Enrichment may offer higher rates of recovery but one disadvantage is that initial *Campylobacter* numbers in the sample cannot be determined.   The enrichment choice may unknowingly select for certain *Campylobacter* strains or allow overgrowth of competing bacteria (Gharst, Hanson, & Kathariou, 2006).

The traditional MPN technique involves set-up and enrichment of samples in serial dilutions using multiple tubes for each dilution before plating on a culture medium.   The MPN technique is advantageous in that it can detect very low levels of contamination, <0.3 MPN/mL as compared to direct plating, 1 CFU/mL but the method is tedious and time-consuming. Direct plating requires up to two days before plates can be read. The MPN method requires an additional 1-2 days of enrichment before plating. Stern and Pretanik (2006) stated that the increased expense and time lost when using the MPN technique outweighs any statistical improvement in reported counts.

The use of the passive filter technique is becoming increasingly popular as a means of suppressing growth of contaminating organisms. A 0.45 or 0.65 µm pore-size cellulose membrane filter is applied directly to the medium of choice.   The prepared sample is then applied directly to the filter and allowed to absorb for 30 minutes to 1 hour before the filter is removed (Valdivieso-Garcia et al., 2007). In theory, this allows the highly motile *Campylobacter* to cross the filter and reach the medium while other bacteria become trapped

within the filter. Oyarzabal (2011) stated this technique results in a pure culture of

Campylobacter producing plates that are easier to interpret. One study suggested use of a

hydrophobic grid membrane filter, a slight variation of the passive filter technique, in

combination with plating. The filter method could effectively eliminate the need for antibiotics

in the medium therefore eliminating the possibility of selection or suppression of growth

(Valdivieso-Garcia et al., 2007).

     There are many media formulations available for the enrichment and isolation of

Campylobacter. Table 4 lists a few media and the selective agents commonly used for the

isolation of *Campylobacter.*

Table 4: Common Selective Media for the Isolation of *Campylobacter*

Enrichment Media	Selective Agents
Bolton Broth	Cefoperazone, Cycloheximide, Vancomycin
Doyle & Roman Broth	Cycloheximide, Polymycin B, Vancomycin
Hunt & Radle Broth	Cefoperazone, Cycloheximide, Trimethoprim, Vancomycin
Park & Sanders Broth – Formula A	Trimethoprim, Vancomycin
Park & Sanders Broth – Formula B	Cefoperazone, Cycloheximide
Preston Broth	Cycloheximide, Polymyxin B, Rifampicin, Trimethoprim, Vancomycin
Plating Media	
Blaser-Wang Agar	Cephalothin, Novobiocin, Polymyxin B, Vancomycin
Campy-Cefex Agar	Cefoperazone, Cycloheximide
CCDA	Amphotericin B, Cefoperazone, Sodium deoxycholate
Karmali Agar	Cefoperazone, Cycloheximide, Vancomycin
Skirrow	Polymyxin B, Trimethoprim, Vancomycin

(Post, n.d.)

Several other formulations including chromogenic-selective agar plates are also available. These plates employ selective agents and an indicator dye to color colonies. The indicator dye makes the colonies easy to visualize and count against the light background of the agar. According to Corry et al. (as cited in Line, 2005), "The number of formulations proposed for the isolation of thermophilic *campylobacter*s probably exceeds that for any other group of bacteria…."

Oyarzabal, Macklin, Barbaree, and Miller (2005) evaluated six common *Campylobacter* media: Campy-Cefex, modified Campy-Cefex, modified charcoal cefoperazone deoxycholate agar (CCDA), Karmali, CAMPY, and Campy-Line agars. They found while Campy-Cefex, modified Campy-Cefex, modified CCDA, and Karmali Agars were not significantly different in performance, Campy-Cefex and its modification produced the best results. *Campylobacter* colonies were difficult to see on CCDA due to the opacity of the medium. When cost of these agars were compared, modified Campy-Cefex also proved to be less expensive due to the replacement of laked horse blood with lysed horse blood and replacement of cycloheximide with amphotericin B (Oyarzabal et al., 2005). Supplements required in *Campylobacter* media are the main contributors to cost.

For samples not requiring enumeration, immunoassay and polymerase chain reaction (PCR) provide alternatives to culture. These often require enrichment before running the assay and are more expensive than traditional culture techniques but can eliminate some of the difficulties in interpreting culture plates. Several PCR assays have been developed for the qualitative determination of *Campylobacter* in food samples. PCR is highly sensitive and rapid and can detect enriched counts as low as 10,000 CFU/mL (Cheng & Griffiths, 2003). PCR can also be performed without enrichment in order to lessen the amount of time to result; however, food matrices often contain PCR inhibitors that must be overcome. Techniques such as dilution,

centrifugation, immunomagnetic separation, and gel filtration can be used to decrease these inhibitors (Cheng & Griffiths, 2003). However, a point to consider is a technique such as dilution will decrease the sensitivity of PCR. Alternative means such as membrane filtration, surface adhesion PCR, or paramagnetic beads can be used to concentrate and separate *Campylobacter* from contaminating bacteria without decreasing sensitivity. A report stated that *Campylobacter* could be concentrated using these techniques by as much as 2 log and thus improve detection so that samples could be assayed without enrichment (Shaw, 2009). One report stated that although enrichment is effective in recovering low numbers of bacteria, *Campylobacter* is not always recoverable by enrichment. Some strains are recovered only through direct plating (Gharst et al., 2006).

Quantitative PCR techniques have been developed but some of these often underestimate the amount of bacteria present (Rijpens & Herman, 2002).   In a small survey of naturally contaminated poultry carcass rinses obtained pre-evisceration, *Campylobacter* counts obtained from direct plating on Campy-Cefex agar were compared to counts obtained from a quantitative real-time PCR assay without enrichment. No correlation in *Campylobacter* counts was noted between the methods (unpublished). This demonstrates the necessity for further experimentation. Many variables could have had an effect on the results of this small survey. Interpretation is especially difficult because there is no standard method for comparison.   Faster, more sensitive, PCR methods with an emphasis on elimination of the enrichment process are desired to improve food-testing protocols used to support industry process control programs.

Other promising alternatives to plating, immunoassay, and PCR are currently in development for the detection of pathogens including *Campylobacter.* Raman spectroscopy and biosensors show promise for future use in the food industry. Raman spectroscopy can be used to

detect specific pathogens because each pathogen has a unique molecular fingerprint. By using silver nanorod substrates in conjunction with this technology, it is possible to increase the spectral signal so a sample can be analyzed within minutes without extraction or amplification (Shaw, 2009). Biosensors also have the potential of being used within food production areas to provide immediate feedback on the presence of bacterial pathogens even with numbers as low as <100 CFU/mL (Rasooly & Herold, 2006). The ability to detect *Campylobacter* in a matter of minutes in a food matrix as compared to hours or days would greatly enable producers to minimize consumer risk and reduce foodborne illnesses.

While personal preference and experience play a big role in whether a cultural, non-cultural, or combination method is used, some factors to consider are as follows: the species desired to cultivate, the presence or absence of competing bacteria, whether quantitation is required, and the limit of sensitivity desired. Many testing laboratories rely solely on the isolation of clinically significant species by direct plating on a selective medium, incubating in a microaerophilic atmosphere at an increased temperature of 42°C, examining for typical colony morphology followed by microscopic examination for characteristic shape and motility. Further testing using latex agglutination may be performed but identification is generally only to the genus level. Stern et al. (2001) stated that differentiation of species within the thermotolerant clinically significant *Campylobacter* species is unnecessary in food microbiology.

The National Advisory Committee on Microbiological Criteria for Foods recommended direct plating on Campy-Cefex or modified Campy-Cefex as the best methodology for enumerating *Campylobacter* in poultry carcass rinses (NACMCF, 2007). Campy-Cefex was the medium of choice for direct plating in FSIS's July 2007- June 2008 Nationwide Microbiological Baseline Data Collection Program: Young Chicken Survey (USDA FSIS, 2008). For ease of

comparison of results from future baseline studies and for verification testing required under the

Campylobacter performance standard, FSIS will continue to utilize Campy-Cefex for direct

plating of poultry carcass rinses (USDA FSIS, 2010). The industry will likely employ FSIS's

methodology for their own in-plant *Campylobacter* testing in order to have an equivalent

measurement with which to evaluate their processing interventions. An informal poll of ten

poultry companies in the United States revealed that 90 percent would use the FSIS method for

their *Campylobacter* testing (unpublished). Of those surveyed, two companies will substitute a

differential/selective medium in the place of Campy-Cefex agar. Three companies will

implement enrichment for qualitative results in addition to the direct plating outlined in the FSIS

method, with one of these using PCR for detection. One company will perform *Campylobacter*

testing utilizing a modified MPN technique.

Strategies for *Campylobacter* Control in Chickens

Although *Campylobacter*-associated illness from poultry versus other sources has not

been definitively determined, reducing *Campylobacter* in poultry is one way of accomplishing

the goal of reducing total human illnesses from *Campylobacter* (Codex, 2002). While the

performance standard focuses on reduction of *Campylobacter* through processing, the goal of

reducing contamination of processed poultry may be better accomplished by a focus on

eliminating the pathogen in live animals and preventing fecal contamination during processing

(Hinton, Cason, Hume, & Ingram, 2004). A comprehensive approach to *Campylobacter*

reduction in poultry should focus on three areas: production, processing, and food preparation

(Miller et al., 1998). The Codex Committee on Food Hygiene, in a FAO/WHO joint study

(2002), presented risk management strategies that may be helpful in reducing *Campylobacter*-

associated illnesses. These are summarized as follows:

- Establishment of codes of practice and standards

- Development and implementation of farm interventions

- Improvement in processing hygiene and antimicrobial interventions

- Improvement in consumer education

FSIS expects to see an improving trend in *Campylobacter* prevalence with the implementation of the performance standard. One area of concern to the industry is that there is probably a limit to what reductions can be achieved in the processing plant. Stern and Pretanik (2006) stated that the limits of HACCP intervention for control and reduction of *Campylobacter* might have already been reached. Regardless of the current state of the industry, until new and improved processing technologies including faster, more reliable detection methods are developed, the lowest limit will be reached (Stern & Robach, 2003). Other areas outside of the processing environment where controls could be applied, such as the farm and post-processing, will need to be considered (Stern & Robach, 2003).

Campylobacter on the farm is the initial point of contamination for chickens; however, this is not always statistically correlated with carcass contamination (Stern & Robach, 2003). Reduction of *Campylobacter* should begin at the live production stage (Stern and Pretanik, 2006). This task is quite difficult because the initial source and mechanism of colonization of broilers is still unidentified (Rasschaert, Houf, Van Hende, & De Zutter, 2006). It is certainly difficult if not impossible to begin to reduce *Campylobacter* levels if the initial source cannot be identified because once introduced *Campylobacter* spreads quickly. Other processing interventions will be less effective if some level of control is not applied at the source or as close to the source as possible. To obtain significant *Campylobacter* reductions in poultry the stages where intervention can be most effective need to be identified (Codex, 2002).

Live production factors to consider are horizontal transmission, vertical transmission, vectors, climate, water, feed, litter, biosecurity, and hygiene. The contribution of these factors to *Campylobacter* prevalence is largely unstudied. A New Zealand study theorized that infection is primarily horizontal during rearing but there are conflicting views on the significance of vertical transmission from the egg to chick (McIntyre, Lee, & Biggs, 2010a). In a study of turkey, data suggested that vertical transmission occurs (Harvey et al., 2004). This data was further corroborated by a report that concluded that *Campylobacter* could be passed through the fertile egg (Cox, et al., 2002). Another study indicated that chicken become colonized by horizontal transmission although vertical transmission cannot be totally excluded (as cited in Rasschaert, Houf, & De Zutter, 2007). Nadeau, Messier, and Quessy (2002) found no evidence of horizontal transmission of *Campylobacter* but suggested there must be a common source of contamination. Regardless of the route of transmission, the primary goal should be prevention of initial colonization. Natural colonization of poultry with *Campylobacter* usually occurs after 3 weeks of age and by market age almost all flocks are positive (McCrea, Macklin, Norton, Hess, & Bilgili, 2006).

Feed, water, and litter should be evaluated as potential sources of contamination (Codex, 2002). Water cannot be eliminated as a possible source of *Campylobacter* since contaminated water has been implicated as an important risk factor in human *Campylobacter* illnesses (as cited in Fang, Yang, Shih, Chou, & Yu, 2006). On the farm, water often remains in water pipes and drinkers for extended periods leading to the formation of biofilms (Trachoo, Frank, & Stern, 2002). Biofilm formation may offer protection and enhance survival of *Campylobacter*. To eliminate this risk, the farm water source should be chlorinated. Periodic sanitizing of the water

pipes and drinkers with chlorine should also be considered an integral part of a *Campylobacter* control program (Trachoo & Frank, 2002).

Feed has been considered a vehicle for introduction of pathogenic bacteria into poultry. *Salmonella* has frequently been implicated in contaminated feed, however no correlation has been found between contaminated feed and *Campylobacter* (Whyte, McGill, & Collins, 2003). Whyte et al. (2003) found no *Campylobacter* after surveying various stages in the feed production process as well as the feed mill environment. Feed does not appear to be an important factor in the introduction of *Campylobacter* and subsequent flock contamination.

Litter may be an important factor in transmission of *Campylobacter*. Research has shown there is a correlation between litter contamination and flock contamination; however, research has not proven a definitive correlation between contaminated litter and rates of contamination at the plant level (Fluckey et al., 2003). In a study of litter contamination, Fluckey et al. (2003) found *Campylobacter* present in litter on 100 percent of sampling days.

Other factors that may influence the transmission of *Campylobacter* on the farm are rodents, insects, pets, livestock, nearby farms and human traffic (Fluckey et al., 2003; as cited in Gharst et al., 2006). Human traffic, particularly from other poultry flocks has been associated with transmission of bacteria in broilers (as cited in Smith et al., 2004). The importance of good farm management practices including biosecurity in minimizing the spread of *Campylobacter* cannot be ignored.

Transportation to the processing plant has been found to increase levels of *Campylobacter* on the chicken (Stern, Clavero, Bailey, Cox, & Robach, 1995). Transport is stressful for the bird and often results in a high incidence of defecation. For birds colonized with *Campylobacter,* this soiling contaminates the transport crate, the bird, as well as other birds in

transit. Chickens arriving at the processing plant often have high *Campylobacter* numbers on the

exterior surface. Crates that have not been thoroughly washed and disinfected can be a source of

Campylobacter contamination of chickens (Arsenault, Letellier, Quessy, Morin, & Boulianne,

2007; Rasschaert et al., 2007). Many studies however, reported that crate washing is mostly

ineffective in eliminating *Campylobacter* contamination (as cited in Rasschaert et al., 2007).

This information supports the statement by Stern et al. (1995) in emphasizing the importance of

reducing colonization of the birds at the farm level.

A FAO/WHO study identified competitive exclusion as one possible method of reducing

Campylobacter colonization in birds; henceforth preventing or reducing vertical and/or

horizontal transmission of the bacterium (Codex, 2002). Thus far, competitive exclusion has had

only limited and nonreproducible success in preventing colonization with *Campylobacter* (as

cited in Stern et al., 2005).   Bacteriocins, which are toxins produced by bacteria that inhibit

growth of similar bacterial strains, show promise in reducing levels of *Campylobacter* in

colonized chickens (Stern et al., 2005). Stern et al. (2005) demonstrated *Campylobacter*

reductions from 4.6 to 6.3 log CFU/g of feces using bacteriocins. Probiotics have also been

studied to determine their effect on colonization and transmission of pathogens. One author

suggests that while certain probiotics may not be totally effective in preventing colonization,

these may prevent the expression of virulence of *Campylobacter* (Ding, Wang, & Griffiths,

2005).

Stern et al. (1995) stated that levels of *Campylobacter* arriving at the processing plant

have a direct effect on the contamination of processing equipment and subsequent potential

cross-contamination.   Russell (2010) estimates that seventy-one percent of chickens entering

the processing plant are contaminated with *Campylobacter*. Table 5 shows the occurrence of *Campylobacter* in selected areas within the processing plant.

Table 5: Occurrence of *Campylobacter* in a poultry processing plant

Sample types	% of positive samples
Surfaces:	
Conveyor belt	3.3
Cages	20
Boxes	0
Drains	10
Water:	
Scalding	30
Chilling	6.7
Chicken carcasses:	
Before plucking	20
After plucking	30
Before evisceration	33.3
Before chilling	16.7
After chilling	20

(Reiter, Bueno, Lopez, & Jordano, 2005)

In this study by Reiter et al. (2005) thirty samples were obtained from each assessed area. Sixty-three percent of the chickens were found to be positive for *Campylobacter* in the intestines and may correlate to incoming contamination rates at the plant.

Upon arrival at the processing plant, the practice of separating and processing *Campylobacter* positive flocks after known *Campylobacter* negative flocks could reduce the

levels of *Campylobacter* during processing (Codex, 2002). To be fully effective, this measure would require fast and reliable testing methods. It is thought that this practice would not be suitable when *Campylobacter* prevalence is high (Codex, 2002). A New Zealand study found that good hygiene, appropriate equipment maintenance, and adequate intervention at key steps during processing are still the most effective at minimizing and reducing *Campylobacter* contamination in the final product (McIntyre et al., 2010b). Measures such as correct adjustment and operation of evisceration equipment, replacement of old equipment, correct operation of chilling with the addition of antimicrobial processing aids such as chlorine, acidified sodium chlorite, and trisodium phosphate (TSP) will aid in reducing *Campylobacter* (McIntyre et al., 2010b). Processing interventions to consider as potential *Campylobacter* control points could be scalding, plucking, evisceration, water quality, chilling, and packaging (Codex, 2002).

It should be recognized that there is no one intervention for raw poultry that will significantly reduce or eliminate *Campylobacter* with the exception of irradiation. At the current time, irradiation is not widely accepted by consumers. Kemp and Schneider stated "to be 99.9% effective in the reduction of *Campylobacter* spp. a single intervention step would require a consistent reduction capability of up to 3.7 log CFU/mL" (as cited in Oyarzabal, Hawk, Bilgill, Warf, & Kemp, 2004). Reduction is best achieved with a multi-hurdle approach of several interventions working together. Ollinger and Moore (2009) identified seven steps at which process controls can be applied that are effective in reducing pathogen levels when used in concert: scalder, rehang, evisceration, cropper, inside-outside bird washer, on-line reprocessing, and chilling.

In general, washing at key processing steps will reduce *Campylobacter* numbers. Experts suggest that reductions seen from washing are primarily from physical removal of the organisms

rather than the action of disinfecting treatments (FAO/WHO, 2009b). Several effective

antimicrobial treatments can be applied at key processing steps to enhance *Campylobacter*

reduction. These antimicrobial treatments work by preventing bacterial attachment in addition to

inactivating bacteria (Arritt, Eifert, Pierson, & Sumner, 2002). Oyarzabal (2005) listed the

following common commercial antimicrobials used in poultry processing during prechill, on-line

reprocessing, chilling and postchill operations:

- Acidified sodium chlorite (ASC)

- Cetylpyridinium chloride (CPC)

- Chlorine

- Chlorine dioxide

- Ozone

- Peroxyacetic acid (PAA)

- Trisodium phosphate (TSP)

Campylobacter numbers can be as high as 7.5 log CFU/g in feathers (as cited in

Oyarzabal, 2005). Scalding has been shown to lower numbers of *Campylobacter* associated with

the carcass primarily by washing dirt and feces from the exterior of the bird; however, if the

scalder is poorly maintained and dirt and feces allowed to accumulate, numbers could increase

(FAO/WHO, 2009b). A scalder temperature of 58°C or higher significantly reduces

Campylobacter on carcasses (as cited in Oyarzabal, 2005). Scalding can potentially remove

more microorganisms than any other process but this reduction is short-lived because it has been

shown that picking causes the numbers of *Campylobacter* to increase significantly (Berrang et

al., 2006). *Campylobacter* present in the feathers can be transferred to the skin during picking

(as cited in Berrang et al., 2006; Scherer et al., 2006). In addition, fecal material is expelled from the ceca and cloaca during picking further contaminating the carcass (Berrang et al., 2006).

Processes such as rehang, evisceration, and cropping control pathogens by limiting human contact (Ollinger & Moore, 2009). It is important to maintain equipment to minimize rupture of crop or viscera (FAO/WHO, 2009b). Inside-outside bird washers in conjunction with an antimicrobial spray have been shown to reduce levels of *Campylobacter* by an average of 0.5 to 1.3 log CFU/mL of whole carcass rinse (Codex, 2011). These reductions however, are not always consistent (as cited in Oyarzabal, 2005). On-line reprocessing sprays incorporating chemicals such as acidified sodium chlorite have the potential to reduce *Campylobacter* numbers by up to 2 log CFU/mL (Codex, 2011).

Chilling seems to be the intervention on which most establishments rely for significant reductions of bacterial pathogens. In the U.S., immersion chilling is the standard. Most studies demonstrate reduction of bacterial numbers after chilling but this does not always correspond to a reduction in prevalence (Oyarzabal et al., 2004). Northcutt, Berrang, Dickens, Fletcher, & Cox (2003) indicated that prevalence might actually increase due to bacteria washing off contaminated carcasses and depositing on uncontaminated carcasses during the immersion chilling process. There is concern over the potential cross-contamination that may occur in the immersion chilling process but is more likely to occur where there is poor operation and maintenance of the immersion chilling system. Smith, Cason, & Berrang (2005) found evidence that bacterial prevalence can decrease during immersion chilling operations when the optimum operating parameters are maintained.

Addition of chlorine in the immersion chiller results in lower numbers of *Campylobacter* but chlorine quickly loses effectiveness as organic material builds in the chill water (Berrang et

al., 2007). *Campylobacter* reductions could range from 0.5 to 3.3 log CFU/mL of chiller water

depending on the age of the water or the organic load present (Oyarzabal, 2005). Chlorine

dioxide in chiller water reduced *Campylobacter* by 90 percent but it efficacy is also affected by

organic material (as cited in Oyarzabal, 2005). Careful consideration and monitoring of loading,

water circulation and replacement, chlorination levels or other antimicrobial treatments, and pH

in the operation of immersion chillers can result in a minimum reduction of *Campylobacter* of

2.5 log cycles (McIntyre et al., 2010b).

In a study by Huezo, Northcutt, Smith, Fletcher, and Ingram (2007), air-chilled carcasses

showed a reduction in *Campylobacter* of up to 1.4 log CFU/mL of carcass rinse with an average

reduction of 0.8 log on poultry carcasses. Reduction of bacteria by air chilling systems may be from

the drying effect of the air on the surface and body cavity of the poultry carcasses. This drying

reduces bacteria recovery by decreasing water activity, retarding bacterial growth and injuring

bacteria (as cited in Huezo et al., 2007). Other studies however have shown little change in bacteria

levels for air-chilled poultry carcasses (as cited in Allen, Burton, Corry, Mead, & Tinker, 2000). In

the assessment of prevalence, several studies report that air or immersion chilling, had little reducing

effect upon *Campylobacter* populations. In a study by Huezo et al. (2007) on inoculated carcasses,

Campylobacter prevalence was 100 percent before chill and 100 percent after chill for both chilling

methods. *Campylobacter* counts prior to chilling were 3.4 log CFU/mL of carcass rinse for both

chilling methods. The level of reduction was similar for both air and immersion chill methods, 1.4

log CFU/mL and 1.0 log CFU/mL respectively (Huezo et al., 2007). These reductions were not

considered significantly different.

Decontamination during postchill steps may be more effective than prechill interventions

in reducing *Campylobacter* (Oyarzabal et al., 2004). Interventions involving sprays, dips, or

baths applied near the end of processing result in greater reductions in *Campylobacter*

(Oyarzabal, 2005). The use of postchill dips is becoming more popular. Application of acidified

sodium chlorite as a postchill dip reduced *Campylobacter* numbers to less than 0.2 log CFU/mL

(Oyarzabal, 2005).   TSP applied as a postchill dip reduced *Campylobacter* numbers by as much

as 1.5 log CFU/mL of carcass rinse (Oyarzabal, 2005).

       As the preceding information suggests, *Campylobacter* is susceptible to most

antimicrobial treatments (Stern & Pretanik, 2006). It is important to test each antimicrobial

intervention in your own processing operation. Efficacy, cost, and potential sensory changes

should be considered when choosing an antimicrobial treatment. Manufacturer's data should be

used only as a guide because real world effects may be different. Oyarzabal (2005) stated that

even with intervention at several processing steps using antimicrobial treatments, concentrations

of 0.5 to 1 log CFU/mL are still common. Regardless of initial *Campylobacter* numbers and

combinations of treatments applied, all plants reduced *Campylobacter* numbers to about the

same level by end of processing (Berrang et al., 2007).

       Campylobacter does not continue to grow on raw poultry products, as can other bacteria

such as spoilage organisms and other pathogens (Stern & Pretanik, 2006). Since it does not grow

at refrigerator or room temperatures, the numbers of organisms on the product at the end of

processing represent the highest counts associated with the finished product. At this stage,

almost all interventions have been exhausted. Adding to the problem of remaining counts,

studies have found that *Campylobacter* viability is maintained and survival enhanced at 4°C

(Tangwatcharin et al., 2006). It may be worthwhile to consider what intervention, if any, can be

implemented at the end of process to further reduce *Campylobacter* numbers. One study reported

that freezing at less than -18°C for as little as 24 hours significantly reduced levels of

Campylobacter (Birk et al., 2006). Regulators in Norway saw significant reductions in

Campylobacter prevalence in poultry after instituting a plan that required meat from *Campylobacter*-positive flocks to be frozen or heat-treated before marketing (Hofshagen & Kruse, 2005). It can be argued that these additional steps result in less exposure to consumers thereby reducing the risk of illness.

After the product leaves the processing plant and enters the retail market, little can be done to further reduce *Campylobacter* numbers and subsequent consumer exposure. *Campylobacter* illness occurs through the consumption of undercooked meat or through cross-contamination. Packaging may also play an important role in consumer exposure to *Campylobacter*. The use of modified atmosphere or vacuum packaging may enhance the survivability of *Campylobacter* (Hendricks, Boyle, Kastner, & Fung, 2000). One report in the United Kingdom indicated that up to 40 percent of packaging of fresh chicken in the marketplace is externally contaminated with *Campylobacter* (FoodQuality, 2011). This statistic does not necessarily hold true in all cases but another study found that, overall, 3.0 percent of poultry packaging was externally contaminated with *Campylobacter* (Burgess, Little, Allen, Williamson, & Mitchell, 2005). External contamination may be a result of leaking packages.

Although undercooked poultry is a risk factor, *Campylobacter* is easily destroyed by heating. One study concluded that the sporadic nature of illness indicates that illnesses are largely a result of cross-contamination that occurs in the home during food preparation (as cited in Moore, Sheldon, & Jaykus, 2003). As many as 3 to 4 log CFU could be transferred from a contaminated stainless steel work surface to ready to eat food during food preparation even 1-2 hours after contamination (Moore et al., 2003). Another study showed no significant reduction in counts from contaminated work surfaces after 1 hour and that *Campylobacter* could survive longer on wood and plastic surfaces (Wanyenya, Muyanja, & Nasinyama, 2004). Consumer

education on food preparation and safe handling remain important in minimizing consumer risk once the food has entered the home. One study showed that consumers were often unable to prevent cross-contamination (FAO/WHO, 2009b). A CAST report (1994) stated if pathogens enter the food supply they will cause illness so if their presence can be prevented, no amount of mishandling can cause foodborne illness.

Future Implications

The effect this U.S. regulatory performance standard for *Campylobacter* in raw poultry will have in reducing foodborne *Campylobacter* illness is only speculative at this time. In order to determine its effect on foodborne illness acquired from consuming poultry, a quantitative risk assessment is necessary. This task has proven difficult because little data exists on the direct relationship between pathogen contamination and foodborne illness. Even with FoodNet surveillance there is no correlation of reported foodborne illnesses to the implicated foods. FAO/WHO initiated work on risk assessment for poultry and *Campylobacter* in 2001 with the hopes of determining consumer risk as well as estimating the change in risk that interventions were likely to create (Codex, 2002). The study, published in 2009, found this determination very complex. Several key findings were presented by FAO/WHO (2009a) in the report on the risk assessment of *Campylobacter* in broiler chickens and are quoted below:

- Reduction in retail prevalence of test-positive chicken products has a roughly proportional effect in risk reduction.
- Reduction in the contamination level of test-positive chicken products has a somewhat more complex relationship with the estimate of risk. For highly contaminated products, moderate reductions in the contamination level have

relatively mild effects. As the contamination level is further reduced, further reductions have increasing relative impact, and eventually yield significant relative-risk reductions.

- Between-flock prevalence is roughly proportional to the risk of illness. The presence of cross-contamination between flocks complicates this slightly due to risk from test-negative flocks that become contaminated by test-positive flocks during transport and in the slaughter plant.

- Reduction in within-flock prevalence clearly reduces the overall estimate of risk, but with a less than proportional rate due to the presence of cross-contamination in the slaughter process, which increases the within-flock prevalence for carcasses during processing.

- A number of scenarios were compared wherein the contamination levels in the processing environment were reduced. The analysis indicates the greatest benefit from reduced total loading of the intestinal tract of birds (thereby reducing the total load on the system). In addition, the benefits of reductions in levels of contamination that take place early in the processing stages can be undermined by cross-contaminated processes later in the processing environment.

- Freezing of poultry will inactivate *Campylobacter* slowly over time. This has been suggested and implemented as a risk mitigation measure, particularly for test-positive flocks in some countries…. (p. 92)

This risk assessment supported the conclusion that it is difficult to develop a risk profile with "direct applicability at any specific location or under any circumstance" for *Campylobacter* (Codex, 2002).

Several countries other than the United States have active *Campylobacter* control

strategies. Many of these however, are not mandatory and do not involve any sanctions or

penalties. It seems in these instances that strategies have been less effective in reducing

Campylobacter. Australia implemented a mandatory HACCP program for poultry processors in

1997 requiring microbiological testing to verify process control; however, there were no specific

targets set for *Campylobacter* (FSANZ, 2010a). A baseline survey performed in 2008 revealed a

Campylobacter prevalence of 84.3 percent in processed poultry (FSANZ, 2010a). As of 2010,

there have been no new regulatory requirements for primary processing or on-farm regulatory

measures implemented. In 2005, the UK implemented a "Cleaner Farms, Better Flocks"

program designed to enhance biosecurity measures and improve hygiene at the farm level (as

cited in FSANZ, 2010a). This non-mandatory program was still far from achieving the goal of

reducing *Campylobacter*-positive chickens by 50 percent by 2010. The prevalence in fresh

chicken remained largely unchanged from the baseline of 70 percent in 2005 to 65 percent in

2008. (as cited in FSANZ, 2010a).

New Zealand implemented processing targets for *Campylobacter* in 2007 with the goal of

reducing human illnesses by 50 percent after five years (as cited in FSANZ, 2010a). Along with

the implementation of good hygiene practices, use of processing aids, and control measures on

the farm, processors are required to sample three carcasses per day for *Campylobacter* and meet

a regulatory limit (FSANZ, 2010a). As of 2011, this limit was a moving window target of $\leq$

6000 CFU/carcass (NZFSA, 2011). When this target is exceeded two or more times out of a

moving window of nine samples, corrective actions must be taken and are dependant on how

much the target has been exceeded (FSANZ, 2010a; NZFSA, 2011). In a press release, the New

Zealand Food Safety Authority stated that this strategy has been successful in reducing human

illnesses by 50 percent (as cited in FSANZ, 2010a). No information was provided on human illness statistics to support this statement; however, within the first year of implementation, the 57 percent positive carcass prevalence had been reduced to 30.6 percent (as cited in FSANZ, 2010a).

Iceland maintains a unique situation in which strict controls can be applied to poultry to minimize consumer exposure to *Campylobacter*. Iceland produces poultry in a closed system meaning essentially all poultry produced in the country is for it own population. They accept no imports. This enables Iceland to apply unique strategies for control of *Campylobacter*. In 2000, Iceland implemented preslaughter testing of broilers where flocks are tested for the presence of *Campylobacter* prior to processing (Callicott et al., 2008).   If a flock tests positive for *Campylobacter,* the flock is processed at the end of the day or work week then frozen or further processed (i.e. cooked) before distribution (Callicott et al., 2008). One study reported the *Campylobacter*-positive broiler flock prevalence in Iceland to be 15 percent in 2008 (FSANZ, 2010a).   Iceland's poultry production system is very similar to the United States except in size. Strategies used by Iceland, which work effectively for small operations, would be very cumbersome and cost prohibitive to implement in a large system such as the United States' poultry production system (Callicott et al., 2008).

Actual foodborne illness reductions in the United States attributable to the enactment of this performance standard will not be known for some time. USDA FSIS (2011) estimates a potential reduction in human illnesses caused by *Campylobacter* of 5,000 per year. This estimation is not based on any real risk assessment scenarios, but based on prior experience with *Salmonella* (USDA, 2010).   The USDA FSIS performance standard for *Campylobacter* was derived from data obtained from baseline studies performed during a period from July 2007 to

June 2008. It is not known whether the industry has achieved reductions in *Campylobacter* after

this data collection but prior to the implementation of the performance standard. Stern and

Pretanik (2006) reported that over the past 10 years the industry has reduced *Campylobacter*

contamination with a subsequent decrease in human illness during the same period. It remains to

be seen if the industry can further reduce *Campylobacter* contamination to a level to ensure

public safety.

What if the limit of *Campylobacter* reductions has already been reached in the processing

environment as theorized by Stern and Pretanik (2006)? For a HACCP program to be most

effective in reducing or eliminating consumer risk from *Campylobacter*, potential control points

must be considered not only during processing or at the end of the processing line, but before

entry into the processing plant. According to data obtained from 1995 to 2001, Stern and

Pretanik (2006) determined there have been no reductions in *Campylobacter* counts on the farm.

Campylobacter prevalence of chicken in the processing plant is directly related to the condition

of the flock on the farm (Berrang et al., 2007). Animal production and on-farm interventions

must be explored in order to further impact *Campylobacter* levels on finished products.

Based on the New Zealand study a significant reduction in prevalence and concentration

of *Campylobacter* present on the carcass must occur before any reduction in human illness is

achieved (McIntyre et al., 2010a). What defines a significant reduction is still unknown. USDA

FSIS intends to perform continued data collection to monitor the industry's response to the

performance standard. If USDA FSIS monitoring finds unacceptable or no progress in reducing

Campylobacter, actions - whatever those may be, will be taken to reduce consumer risk (USDA,

2011). Actions taken could be posting of establishment results publically, more intensive

sampling, establishment food safety audits (FSAs) leading to other enforcement actions, and/or withdrawal of inspection services.

Market forces often play an important role in the production of food products with reduced pathogen risk to consumers. One study found that while regulations accounted for approximately one-third of the reductions in pathogen-positive samples, two-thirds of the reductions were based on "management determined actions" or actions which are solely at the discretion of the producer (Ollinger & Moore, 2009). Ollinger and Moore (2009) stated that these actions are most often motivated by fear of loss of consumer confidence, ability to obtain higher price for the product, or other such incentives that can be garnered in exchange for greater attention to food safety process control such as minimum purchase agreements. A good example of market forces producing reductions in *Campylobacter* in poultry is Danish broiler growers are paid a premium to produce *Campylobacter*-free chicken, which in turn is sold by the processor at a premium price to consumers who are willing to pay the higher price for *Campylobacter*-free chicken (as cited in FSANZ, 2010b).

What if failure to meet the performance standard is influenced by the timing of an establishment's sample set and the seasonality of *Campylobacter?* Several reports have found increased prevalence of *Campylobacter* during warmer months (Hosfshagen & Kruse, 2005; Scherer et al., 2006; FAO/WHO, 2009b). Meldrum, Tucker, and Edwards (2004) stated "the observation of the distinct seasonal variation in *Campylobacter* contamination stresses the importance of carrying out long-term surveys on chickens, rather than snapshot surveys, which may give an artificially high or low result, depending on the season sampled."

What if industry reduction in the prevalence of *Campylobacter* in poultry does not result in significant reductions in human foodborne illnesses? Reevaluation of the performance

standard and assessment of what magnitude of reduction is required to significantly reduce foodborne illnesses would be required. More data collection and risk assessment is necessary. More serious consideration may be given to end product interventions such as freezing, heat treatment, or irradiation.

Summary

Campylobacter and poultry have proven to be the riskiest pathogen-food combination concerning annual human foodborne illnesses and costs of these illnesses. The performance standard for *Campylobacter* is part of USDA's long term effort to "ensure that appropriate microbial testing is conducted and appropriate criteria and standards exist to reduce the food safety hazards posed by harmful bacteria on raw poultry" (USDA, 1996).

Campylobacter, although considered a fragile organism due to its fastidious nature, has proven to be difficult to control in poultry production. Processing interventions that have proven successful for the reduction of other pathogens such as *Salmonella* have proven inadequate for *Campylobacter.* The limit of effectiveness of processing interventions may have been reached or may soon be reached unless new technologies are developed. It is apparent that the entire food chain needs to be considered in efforts to produce safer food (FAO/WHO, 2009b). Currently there are no recognized effective interventions at the farm level to reduce *Campylobacter* (McIntyre, 2010b). With increased consumer pressure to ensure food safety, establishments will be forced to develop new strategies for control. These strategies may include changes in plant layout, investment in equipment, hiring more workers devoted to safety, increased training, development of innovative sanitation and operating procedures, implementation of multilayer interventions working in concert to kill or reduce pathogens, and research into animal production technologies that can minimize or prevent pathogen colonization

(Ollinger & Moore, 2009). As stated so well by Solomon and Hoover in 1999, "it appears that any future attempts to control *Campylobacter* enteritis will depend on both improved poultry husbandry techniques as well as increased awareness on the part of the consumer, a true 'farm-to-fork' approach."

References

Allen, V. M., Burton, C. H., Corry, J. E. L., Mead, G. C., & Tinker, D. B. (2000), Investigation of hygiene aspects during air chilling of poultry carcasses using a model rig. *British Poultry Science,* 41: 575-583.

American Meat Institute. (April 2009). AMI fact sheet: US meat and poultry production & consumption: An overview. Available at www.meatami.com/ht/a/GetDocumentAction/i/48781. Accessed December 3, 2011.

Arritt, F. M., Eifert, J. D., Pierson, M. D., & Sumner, S. S. (2002). Efficacy of antimicrobials against *Campylobacter jejuni* on chicken breast skin. *The Journal of Applied Poultry Research, Vol. 11, No. 4,* 358-366. (Winter).

Arsenault, J., Letellier, A., Quessy, S., Morin, J., & Boulianne, M. (2007). Prevalence and risk factors for *Salmonella* and *Campylobacter* spp. carcass contamination in turkeys slaughtered in Quebec, Canada. *Journal of Food Protection, Vol. 70, No. 6,* 1350-1359.

Batz, M. B., Hoffmann, S., & Morris, J. G., Jr. (2011). Ranking the risks: The 10 pathogen-food combinations with the greatest burden on public health. Emerging Pathogens Institute, University of Florida.

Berrang, M. E., Smith, D. P., & Hinton, A. Jr. (2006). Application of distilled white vinegar in the cloaca to counter the increase in *Campylobacter* numbers on broiler skin during feather removal. *Journal of Food Protection, Vol. 69, No. 2,* 425-427.

Berrang, M. E., Bailey, J. S., Alterkruse, S. F., Patel Jr., W. K., Meinersmann, R. J., & Fedorka-Cray, P. J. (2007). Prevalence and numbers of *Campylobacter* on broiler carcasses collected at rehang and postchill in 20 U.S. processing plants. *Journal of Food Protection, Vol. 70, No. 7,* 1556-1560.

Birk, T., Rosenquist, H., Brondsted, L., Ingmer, H., Bysted, A., & Christensen, B. B. (2006). A comparative study of two food model systems to test the survival of *Campylobacter jejuni* at -18˚C. *Journal of Food Protection, Vol. 69, No. 11,* 2635-2639.

Burgess, F., Little, C. L., Allen, G., Williamson, K., & Mitchell, R. T. (2005). Prevalence of *Campylobacter, Salmonella,* and *Escherichia coli* on the external packaging of raw meat. *Journal of Food Protection, Vol. 68, No. 3,* 469-475.

Callicott, K. A., Harðardóttir, H., Georgsson, F., Reiersen, J., Friðriksdóttir, V., Gunnarsson, E., …Stern, N. J. (2008). Broiler *Campylobacter* contamination and human campylobacteriosis in Iceland. *Applied and Environmental Microbiology, Vol. 74, No. 21,* 6483-6494.

CDC. FoodNet. (2011). FoodNet – Foodborne Diseases Active Surveillance Network. Available at www.cdc.gov/foodnet. Accessed November 19, 2011.

CDC. FoodNet Figures. (2011). FoodNet facts and figures – number of infections and incidence per 100,000 persons, Tables 2a and 2b. Last modified July 8, 2011. Available at www.cdc.gov/foodnet/factsandfigures/2009/incidence.html. Accessed November 19, 2011.

CDC. MMWR. (2011). Vital signs: Incidence and trends of infection with pathogens transmitted commonly through food – foodborne diseases active surveillance network, 10 U.S. sites, 1996—2010. *Morbidity and Mortality Weekly Report (MMWR), June 10, 2011.* Available at www.cdc.gov/mmwr/preview/mmwrhtml/mm6022a5.htm?s_cid=mm6022a5_w Accessed November 19, 2011.

C-EnterNet. (2012). C-EnterNet 2009 Annual Report. National integrated enteric pathogen surveillance program. Public Health Agency of Canada. Last modified 2012-02-23. Available at www.phac-aspc.gc.ca/c-enternet/pubs/2009/index-eng.php. Accessed May 15, 2012,

Chang, V. P., Mills, E. W., & Cutter, C. N. (2003). Comparison of recovery methods for freeze-injured *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter coli* in cell suspensions and associated with pork surfaces. *Journal of Food Protection, Vol. 66, No. 5,* 798-803.

Cheng, Z., & Griffiths, M. W. (2003). Rapid detection of *Campylobacter jejuni* in chicken rinse water by melting-peak analysis of amplicons in real-time polymerase chain reaction. *Journal of Food Protection, Vol. 66, No. 8,* 1343-1352.

Codex Alimentarius Commission. (2002). Discussion paper on risk management strategies for *Campylobacter* spp. in poultry. Joint FAO/WHO Food Standards Programme, CX/FH 03/5-Add.2. (March).

Codex Alimentarius Commission. (2011). Guidelines for the control of *Campylobacter* and *Salmonella* in chicken meat. CAG/GL 78-2011. Available at www.codexalimentarius.net/download/standards/.../CXG_78e.pdf. Accessed January 29, 2012.

Council for Agricultural Science and Technology (CAST) Task Force. (1994). Foodborne pathogens: Risks and Consequences. Task Force Report No. 122 (September).

Cox, N. A., Stern, N. J., Musgrove, M. T., Bailey, J. S., Craven, S. E., Cray, P. F.,…Hiett, K. L. (2002). Prevalence and level of *Campylobacter* in commercial broiler breeders (parents) and broilers. *The Journal of Applied Poultry Research, Vol. II, No. 2,* 187-190.

De Cesare, A., Sheldon, B. W., Smith, K. S., & Jaykus, L. (2003). Survival and persistence of *Campylobacter* and *Salmonella* species under various organic loads on food contact surfaces. *Journal of Food Protection, Vol. 66, No. 9,* 1587-1594.

Ding, W., Wang, H., & Griffiths, M. W. (2005). Probiotics down-regulate *fla*A σ^{28} promoter in *Campylobacter jejuni*. *Journal of Food Protection, Vol. 68, No. 11,* 2295-2300.

EFSA. (2011). Scientific Report of EFSA and ECDC. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. *EFSA Journal 2011;9(3):2090.* Available at www.efsa.europa.eu/en/efsajournal/doc/2090.pdf. Accessed May 15, 2012.

Engberg, J., Neimann, J., Nielsen, E. M., Aarestrup, F. M., Fussing, V. (2004). Quinolone resistant *Campylobacter* infections in Denmark: Risk factors and clinical consequences. *Emerging Infectious Diseases, Vol. 10, No. 6.*

ESR. (2010). Annual report concerning foodborne disease in New Zealand 2009. Available at www.foodsafety.govt.nz/elibrary/industry/FW10040_FBI_report_May_2010.pdf. Accessed May 15, 2012.

Fang, S. W., Yang, C. J., Shih, D. Y., Chou, C. C., & Yu, R. C. (2006). Amplified fragment length polymorphism, serotyping, and quinolone resistance of *Campylobacter jejuni* and *Campylobacter coli* strains from chicken-releated samples and humans in Taiwan. *Journal of Food Protection, Vol. 69, No. 4,* 775-783.

FAO/WHO. (2009a). Microbiological risk assessment series 12, Risk assessment of *Campylobacter* spp. in broiler chickens: Technical report. Available at www.who.int/foodsafety/publications/micro/MRA12.pdf. Accessed November 5, 2011.

FAO/WHO. (2009b). Microbiological risk assessment series 19, *Salmonella* and *Campylobacter* in chicken meat, meeting report. Available at www.who.int/foodsafety/publications/micro/MRA19.pdf. Accessed November 5, 2011.

FoodQuality news.com. (2011). External poultry packaging harbours *Campylobacter* threat –

    report. January 25, 2011.   Available at

    www.foodqualitynews.com/content/view/print/354827. Accessed January 25, 2011).

Fluckey, W. M., Sanchez, M. X., McKee, S. R., Smith, D., Pendleton, E., & Brashears, M. M.

    (2003). Establishment of a microbiological profile for an air-chilling poultry operation in the

    United States. *Journal of Food Protection, Vol. 66, No. 2,* 272-279.

Fosse, J., Laroche, M., Rossero, A., Federighi, M., Seegers, H., & Magras, C. (2006). Recovery

    methods for detection and quantification of *Campylobacter* depend on meat matrices and

    bacteriological or PCR tools. *Journal of Food Protection, Vol. 69, No. 9,* 2100-2106.

FSANZ. (2010a). Baseline survey on the prevalence and concentration of *Salmonella* and

    Campylobacter in chicken meat on-farm and at primary processing. Available at

    www.foodstandards.gov.au/_srcfiles/Poultry%20survey%20rept%20March%202010.pdf.

    Accessed May 15, 2012.

FSANZ. (2010b). Final assessment report. Proposal P282, primary production & processing

    standard for poultry meat. Available at

    www.foodstandards.gov.au/_srcfiles/P282%20Poultry%20PPPS%20FAR%20FINAL.pdf.

    Accessed May 15, 2012.

Gharst, G., Hanson, D., & Kathariou, S. (2006). Effect of direct culture versus selective

    enrichment on the isolation of thermophilic *Campylobacter* from feces of mature cattle at

    harvest. *Journal of Food Protection, Vol. 69, No. 5,* 1024-1027.

Gupta, A., Nelson, J. M., Barrett, T. J., Tauxe, R. V., Rossiter, S. P., Friedman, C. R.,…Angulo,

    F. J. (2004). Antimicrobial resistance among *Campylobacter* strains, United States, 1997-

    2001. *Emerging Infectious Diseases, Vol. 10, No. 6,* 1102-1109.

Harvey, R. B., Droleskey, R. E., Sheffield, C. L., Edrington, T. S., Callaway, T. R., Anderson, R. C.,…Nisbet, D. J. (2004). *Campylobacter* prevalence in lactating dairy cows in the United States. *Journal of Food Protection, Vol. 67, No. 7,* 1476-1479.

Hendricks, R. A, Boyle, E. A. E., Kastner, C. L., & Fung, D. Y. C. (2000). Compilation of intervention methods and conditions, and ingredient limits, for controlling *Campylobacter jejuni* in meat and poultry products. *Journal of Rapid Methods and Automation in Microbiology, Vol. 8, No. 4,* 285-305.

Hinton Jr., A., Cason, J. A., Hume, M. E., & Ingram, K. D. (2004). Use of MIDI-fatty acid methyl ester analysis to monitor the transmission of *Campylobacter* during commercial poultry processing. *Journal of Food Protection, Vol. 67, No. 8,* 1610-1616.

Hoffman, P. S., & Blankenship, L. C. (1985). Significance of *Campylobacter* in foods. *Developments in Food Microbiology – 2,* 91-120.

Hofshagen, M. & Kruse, H. (2005). Reduction in flock prevalence of *Campylobacter* spp. in broilers in Norway after implementation of an action plan. *Journal of Food Protection, Vol. 68, No. 10,* 2220-2223.

Huezo, R., Northcutt, J. K., Smith, D. P., Fletcher, D. L., & Ingram, K. D. (2007). Effect of dry air or immersion chilling on recovery of bacteria from broiler carcasses. *Journal of Food Protection, Vol. 70, No. 8,* 1829-1834.

Larkin, C., Donkersgoed, C., Mahdi, A., Johnson, P., McNab, B., & Odumeru, J. (2006). Antibiotic resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from hog, beef, and chicken carcass samples from provincially inspected abattoirs in Ontario. *Journal of Food Protection, Vol. 69, No. 1,* 22-26.

Lindblad, M., Hansson, I., Vagsholm, I., & Lindqvist, R. (2006). Postchill *Campylobacter* prevalence on broiler carcasses in relation to slaughter group colonization level and chilling system. *Journal of Food Protection, Vol. 69, No. 3,* 495-499.

Line, J. E. (2005). *Campylobacter:* Laboratory detection (and enumeration) methods. 2005 *Campylobacter* Control Conference, Atlanta Georgia. February 23, 2005.

McCrea, B. A., Macklin, K. S., Norton, R. A., Hess, J. B., & Bilgili, S. F. (2006). A longitudinal study of *Salmonella* and *Campylobacter jejuni* isolates from day of hatch through processing by automated ribotyping. *Journal of Food Protection, Vol. 69, No. 12,* 2908-2914.

McCrea, B. A., Tonnoka, K. H., VanWorth, C., Atwill, E. R., & Schrader, J. S. (2006). Colonizing capability of *Campylobacter jejuni* genotypes from low-prevalence avian species in broiler chickens. *Journal of Food Protection, Vol. 69, No. 2,* 417-420.

McIntyre, L., Lee, J., & Biggs, R. (2010a). Campylobacteriosis in New Zealand part one – the early days. *International Food Hygiene, Vol. 21, No. 5,* 21-22.

McIntyre, L., Lee, J., & Biggs, R. (2010b). Campylobacteriosis in New Zealand part two – tackling the problem. *International Food Hygiene, Vol. 21, No. 6,* 21-22.

Meldrum, R. J., Tucker, D., & Edwards, C. (2004). Baseline rates of *Campylobacter* and *Salmonella* in raw chicken in Wales, United Kingdom, in 2002. *Journal of Food Protection, Vol. 67, No. 6,* 1226-1228.

Miller, A. J., Smith, J. L., & Buchanan, R. L. (1998). Factors affecting the emergence of new pathogens and research strategies leading to their control. *Journal of Food Safety, Vol. 18, No. 4,* 243-263.

Moore, C. M., Sheldon, B. W., & Jaykus, L. (2003). Transfer of *Salmonella* and *Campylobacter* from stainless steel to romaine lettuce. *Journal of Food Protection, Vol. 66, No. 12,* 2231-2236.

Nadeau, E., Messier, S., & Quessy, S. (2002). Prevalence and comparison of genetic profiles of *Campylobacter* strains isolated from poultry and sporadic cases of campylobacteriosis in humans. *Journal of Food Protection, Vol. 65, No. 1,* 73-78.

NACMCF. (2007). Analytical utility of *Campylobacter* methodologies. *Journal of Food Protection, Vol. 70, No. 1,* 241-250.

NARMS. (2010). National antimicrobial resistance monitoring system: Enteric bacteria, human isolates final report. Available at www.cdc.gov/narms/pdf/2010-annual-report-narms.pdf. Accessed March 13, 2012.

Nelson, J. M, Chiller, T. M., Powers, J. H., & Angulo, F. J. (2007). Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: A public health success story. *Food Safety, CID 2007:44 (1 April),* p. 977-980.

Northcutt, J. K., Berrang, M. E., Dickens, J. A., Fletcher, D. L., & Cox, N. A. (2003). Effect of broiler age, feed withdrawal, and transportation on levels of coliforms, *Campylobacter, Escherichia coli,* and *Salmonella* on carcasses before and after immersion chilling. *Poultry Science, Vol. 82, No. 1,* 169-173.

NZFSA. (2011). Schedule I, national microbiological database programme. Available at www.foodsafety.govt.nz/elibrary/industry/animal-products-national-nmd/schedule-2011.pdf. Accessed May 15, 2012.

Ollinger, M. & Moore, D. (2009). The interplay of regulation and market incentives in providing food safety. *USDA Economic Research Report, Number 75*, July.

Oyarzabal, O. A. (2005). Reduction of *Campylobacter* spp. by commercial antimicrobials applied during the processing of broiler chickens: A review from the United States perspective. *Journal of Food Protection, Vol. 68, No. 8,* 1752-1760.

Oyarzabal, O. A. (2011). Fifth Workshop on *Campylobacter* isolation and identification from foods. Alabama State University, Montgomery Alabama. May 24-26, 2011.

Oyarzabal, O. A., Hawk, C., Bilgill, S. F., Warf, C. C., & Kemp, G. K. (2004). Effects of postchill application of acidified sodium chlorite to control *Campylobacter* spp. and *Escherichia coli* on commercial broiler carcasses. *Journal of Food Protection, Vol. 67, No. 10,* 2288-2291.

Oyarzabal, O., Macklin, K., Barbaree, J. & Miller, R. (2005). Evaluation of agar plates for the direct enumeration of *Campylobacter* spp. from poultry carcass rinses. *Applied and Environmental Microbiology, June 2005,* 3351-3354.

OzFoodNet. (2010). Monitoring the incidence and causes of disease potentially transmitted by food in Australia: Annual report of the OzFoodNet network, 2009. *Communicable Diseases Intelligence, Vol. 34, No. 4.* Available at www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3404b.htm. Accessed May 15, 2012.

Planet Matters and More website (September 13, 2011). Top 5 food safety concerns. Available at www.planetmattersandmore.com/food-safety-issues/top-5-food-safety-concerns/. Accessed September 29, 2011.

Post, D. E. (n.d.). Food-borne pathogens monograph number 3 *Campylobacter*. Oxoid Folio 463.

Rasooly, A., & Herold, K. (2006). Biosensors for the analysis of food- and waterborne pathogens and their toxins. *Journal of AOAC International, Vol. 89, No. 3,* 873-883.

Rasschaert, G., Houf, K., Van Hende, J., & De Zutter, L. (2006). *Campylobacter* contamination during poultry slaughter in Belgium. *Journal of Food Protection, Vol. 69, No. 1,* 27-33.

Rasschaert, G., Houf, K., De Zutter, L. (2007). External contamination of *Campylobacter*-free flocks after transport in cleaned and disinfected containers. *Journal of Food Protection, Vol. 70, No. 1,* 40-46.

Reiter, M. G. R., Bueno, C. M. M., Lopez, C., & Jordano, R. (2005). Occurrence of *Campylobacter* and *Listeria monocytogenes* in a poultry processing plant. *Journal of Food Protection, Vol. 68, No. 9,* 1903-1906.

Rijpens, N. P., & Herman, M. F. (2002). Molecular methods for identification and detection of bacterial food pathogens. *Journal of AOAC International, Vol. 85, No. 4,* 984-995.

Roasto, M., Juhkam, K., Tamme, T., Horman, A., Hakkinen, L., Reinik, M.,…Hanninen, M. (2007). High level of antimicrobial resistance in *Campylobacter jejuni* isolated from broiler chickens in Estonia in 2005 and 2006. *Journal of Food Protection, Vol. 70, No. 8,* 1940-1944.

Russell, S. (2010). Control of *Salmonella, Campylobacter* and other bacteria in raw poultry. *Food Safety Magazine, April/May 2010,* 40-43.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M., Roy, S.,…Griffin, P. M. (2011). Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases, Vol. 17, No. 1*

Scherer, K., Bartelt, E., Sommerfeld, C., & Hildebrandt, G. (2006). Quantification of *Campylobacter* on the surface and in the muscle of chicken legs at retail. *Journal of Food Protection, Vol. 69, No. 4,* 757-761.

Shaw, D. P., Cason, J. A., & Berrang, M. E. (2005). Effect of fecal contamination and cross-contamination on numbers of coliform, *Escherichia coli, Campylobacter,* and *Salmonella* on immersion-chilled broiler carcasses. *Journal of Food Protection, Vol. 68, No. 7,* 1340-1345.

Smith, James L. (2002). *Campylobacter jejuni* infection during pregnancy: Long term consequences of associated bacteremia, Guillain-Barré syndrome, and reactive arthritis. *Journal of Food Protection, Vol. 65, No. 4,* 696-708.

Smith, K., Reimers, N., Barnes, H. J., Bong, C. L., Siletzky, R., & Kathariou, S. (2004). *Campylobacter* colonization of sibling turkey flocks reared under different management conditions. *Journal of Food Protection, Vol. 67, No. 7,* 1463-1468.

Solomon, E. B. & Hoover, D. G. (1999). *Campylobacter jejuni*: a bacterial paradox. *Journal of Food Safety, Vol. 19, No. 2,* 121-136.

Soncini, G., Valnegri, L., Vercellotti, L., Colombo, F., Valle, D., Franzoni, M., & Bersani, C. (2006). Investigation of *Campylobacter* in reared game birds. *Journal of Food Protection, Vol. 69, No. 12,* 3021-3024.

Stern, N. J., Clavero, M. R. S., Bailey, J. S., Cox, N. A., & Robach, M. C. (1995). *Campylobacter* spp. in broilers on the farm and after transport. *Poultry Science, 74:*937-941.

Stern, N. J., Line, J. E., & Hui-Cheng, C. (2001). *Campylobacter. Compendium of Methods for the Microbiological Examination of Foods,* 301-310.

Stern, N. J., & Pretanik, S. (2006). Counts of *Campylobacter* spp. on U.S. broiler carcasses. *Journal of Food Protection, Vol. 69, No. 5,* 1034-1039.

Stern, N. J., & Robach, M. C. (2003). Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. *Journal of Food Protection, Vol. 66, No. 9,* 1557-1563.

Stern, N. J., Svetoch, E. A., Eruslanov, B. V., Kovalev, Y. N., Volodina, L. I., Perelygin, V. V.,…Levchuk, V. P. (2005). *Paenibacillus polymyxa* purified bacteriocin to control *Campylobacter jejuni* in chickens. *Journal of Food Protection, Vol. 68, No. 7,* 1451-1453.

Tangwatcharin, P., Chantahchum, S., Khopaibool, P., & Griffiths, M. W. (2006). Morphological and physiological responses of *Campylobacter jejuni* to stress. *Journal of Food Protection, Vol. 69, No. 11,* 2747-2753.

Thomson Reuters (July 2010). National survey of healthcare consumers: Food safety. Available at www.factsforhealthcare.com/pressroom/NPR_report_FoodSafety0710.pdf. Accessed September 29, 2011.

Trachoo, N., & Frank, J. F. (2002). Effectiveness of chemical sanitizers against *Campylobacter jejuni*-containing biofilms. *Journal of Food Protection, Vol. 65, No. 7,* 1117-1121.

Trachoo, N., Frank, J. F., & Stern, N. J. (2002). Survival of *Campylobacter jejuni* in biofilms isolated from chicken houses. *Journal of Food Protection, Vol. 65, No. 7,* 1110-1116.

USDA FSIS. (1995). Pathogen reduction; Hazard analysis and critical control point (HACCP) system; Proposed rule. *Federal Register* 60:6774-6889. (February 3).

USDA FSIS. (1996). Pathogen reduction; Hazard analysis and critical control point (HACCP) system; Final rule. *Federal Register* 61:38806-38989. (July 25).

USDA FSIS. (2008). The nationwide microbiological baseline data collection program: Young

chicken survey. July 2007-June 2008. Available at

www.fsis.usda.gov/PDF/Baseline_Data_Young_Chicken_2007-2008.pdf. Accessed May 27,

2011.

USDA FSIS. (2010). New performance standards for *Salmonella* and *Campylobacter* in young

chicken and turkey slaughter establishments; New compliance guides. *Federal Register*

75:27288-27294. (May 14).

USDA FSIS. (2011). New performance standards for *Salmonella* and *Campylobacter* in young

chicken and turkey slaughter establishments: Response to comments and announcement of

implementation schedule. *Federal Register* 76:15282-15290. (March 21).

Valdivieso-Garcia, A., Harris, K., Riche, E., Campbell, S., Jarvie, A., Popa, M.,...Rahn, K.

(2007). Novel *Campylobacter* isolation method using hydrophobic grid membrane filter and

semisolid medium. *Journal of Food Protection, Vol. 70, No. 2,* 355-362.

Wanyenya, I., Muyanja, C., & Nasinyama, G. W. (2004). Kitchen practices used in handling

broiler chickens and survival of *Campylobacter* spp. on cutting surfaces in Kampala,

Uganda. *Journal of Food Protection, Vol. 67, No. 9,* 1957-1960.

Wattagnet.com (April 5, 2011). US chicken consumption increases in 2010 after three-year

decline. Available at www.wattagnet.com/PrintPage.aspx?id=21655. Accessed December 3,

2011.

Whyte, P., McGill, K., & Collins, J. D. (2003). A survey of the prevalence of *Salmonella* and

other enteric pathogens in a commercial poultry feed mill. *Journal of Food Safety, Vol. 23,*

No. 1, 13-24. (April).

Wilson, J. E., Wilson, T. S., Wareing, D. R., Humphrey, T. J., & Murphy, P. G. (2002).
Prevalence of thermophilic *Campylobacter* spp. in ready-to-eat foods and raw poultry in
Northern Ireland. *Journal of Food Protection, Vol. 65, No. 8,* 1326-1328.

Wong, T. L., Hollis, L., Cornelius, A., Nicol, C., Cook, R., & Hudson, J. A. (2007). Prevalence,
numbers, and subtypes of *Campylobacter jejuni* and *Campylobacter coli* in uncooked retail
meat samples. *Journal of Food Protection, Vol. 70, No. 3,* 566-573.

Zhao, S., Young, S. R., Tong, E., Abbott, J. W., Womack, N., Friedman, S. L., and McDermott,
P. F. (2010). Antimicrobial resistance of *Campylobacter* isolates from retail meat in the
United States between 2002 and 2007. *Applied and Environmental Microbiology, Dec. 2010,*
7949-7956.

Zheng, J., Meng, J., Zhao, S., Singh, R., & Song, W. (2006). Adherence to and invasion of
human epithelial cells by *Campylobacter jejuni* and *Campylobacter coli* isolates from retail
meat products. *Journal of Food Protection, Vol. 69, No. 4,* 768-774.