Perspectives on Food-Safety Issues of Animal-Derived Foods

Edited by Steven C. Ricke and Frank T. Jones

The University of Arkansas Press
Fayetteville
2010

Manufactured in the United States of America

ISBN-10: 1-55728-919-0
ISBN-13: 978-1-55728-919-3

14 13 12 11 10 5 4 3 2 1

Text design by Ellen Beeler

♾ The paper used in this publication meets the minimum requirements of the American National Standard for Permanence of Paper for Printed Library Materials Z39.48-1984.

Library of Congress Cataloging-in-Publication Data

Perspectives on food-safety issues of animal-derived foods / edited by Steven C. Ricke and Frank T. Jones.
p. cm.
Includes bibliographical references and index.
ISBN 978-1-55728-919-3 (hardback : alk. paper)
1. Food—Microbiology. 2. Food of animal origin—United States—Safety measures.
I. Ricke, Steven C., 1957– II. Jones, Frank T.
QR115.P47 2010
664.001'579--dc22

2009041590

This publication was made possible by the support of the Food Safety Consortium through the U.S. Department of Agriculture Cooperative State Research, Education, and Extension Service.

18

Fluoroquinolone-Resistant *Campylobacter jejuni* in Raw Poultry Products

Ramakrishna Nannapaneni, Omar A. Oyarzabal, Steven C. Ricke, and Michael G. Johnson

Introduction

Campylobacter is a leading cause of foodborne illnesses, with 21.7 cases every 100,000 persons in the United States (Anonymous 1999; Altekruse et. al. 2006) and 30.2 cases every 100,000 persons in Canada (Galanis 2007). Although outbreaks of campylobacteriosis are rare and usually linked to the consumption of raw milk (Evans et al. 1996; Korlath et al. 1985) or contaminated water (Jones and Roworth 1996; Koenraad, Ayling et al. 1995; Koenraad, Jacobs-Reitsma et al. 1995; Mentzing 1981), there are between two and eight million estimated cases of campylobacteriosis, most as sporadic cases with no association to single-source outbreaks, and 200 to 800 estimated deaths in the United States annually (Moore et al. 2006). Infections with foodborne *Campylobacter* can lead to Guillain-Barré syndrome, one of the most common causes of flaccid paralysis in the United States in the last 50 years (Tam et al. 2006; Mishu et al. 1993).

Surveys of commercial broiler farms have shown that broilers frequently carry large numbers of *Campylobacter* in their intestinal contents, and during processing, highly contaminated carcasses can cross-contaminate carcasses of *Campylobacter*-free birds, thereby increasing the incidence of contaminated retail products (Miwa et al. 2003; Potturi-Venkata et al. 2007). In a recent U.S. study, the largest (24%) population attributable fraction (PAF) was associated to the consumption of poultry prepared in restaurants (Friedman et al. 2004). Therefore, poultry meat contaminated with *C. jejuni* is considered a significant risk factor for enteritis (Anonymous

1999, 2007; Newell and Wagenaar 2000), and consumption of undercooked poultry meat is considered a significant risk factor for human campylobacteriosis (Kramer et al. 2000).

A two-year study conducted by the Minnesota Department of Health found that 88% of raw poultry sampled from local supermarkets tested positive for *Campylobacter* (Smith et al. 1999). Recent studies suggest that 65 to 85% retail raw chicken carcasses are *Campylobacter* positive with approximately 1 to 4.82 $\log_{10}$ CFU/carcass of *Campylobacter* load per carcass rinse (Nannapaneni et al. 2005a, 2006a; Oyarzabal et al. 2005, 2007; Potturi-Venkata et al. 2007).

With the introduction of the Hazard Analysis and Critical Control Points systems in the United States, poultry processors have been required to meet performance standards for *Salmonella* in their products (Anonymous 1996). In the last few years, the Food Safety and Inspection Services of the U.S. Department of Agriculture (FSIS-USDA) has been evaluating the need for the implementation of similar performance standards for *Campylobacter* spp. The FSIS-USDA has not set a processing standard for *Campylobacter* (incidence or numbers) on raw or further processed poultry products yet. However, the FSIS-USDA is performing a new annual nationwide young chicken microbiological baseline data-collection program that may provide the necessary information to establish such a performance (FSIS-USDA Notice 31–07).

Fluoroquinolone-Resistant *Campylobacter* Strains

Until the early 1990s, most *C. jejuni* and *C. coli* were susceptible to fluoroquinolones (Fliegelman et al. 1985). However, the incidence of fluoroquinolone resistance (*FR*), and macrolide resistance, has been increasing in recent years and in some countries fluoroquinolones may, unfortunately, be of limited use for the empiric treatment of campylobacteriosis (Engberg et al. 2001; Reina et al. 1994; Sánchez et al. 1994). In other countries, the incidence of antimicrobial resistance has remained low over the years (Unicomb et al. 2003). Yet, the risk of acquiring *FR-Campylobacter* strains varies from country to country, even among countries with high incidence of *FR* strains. For instance, the risk for a tourist visiting Thailand is much higher than the same tourist visiting Spain, although both countries have an incidence of around 80% *FR-Campylobacter* strains (Hakanen et al. 2003; Hoge 1998; Ruiz 1998). Erythromycin, a macrolide antimicrobial, remains the drug of choice for the treatment of confirmed or presumptive campylobacteriosis (Allos 2001; Engberg et al. 2001; Kist 2002), and resistance to this antimicrobial may pose a more serious threat to public health.

For some, one of the reasons for the increase in *FR* among *Campylobacter* strains relates to the use of fluoroquinolones in food production animals, an

event that has always been discussed in countries with high incidence of *FR-Campylobacter* strains (Unicomb et al. 2003). It has also been suggested that fluoroquinolone-resistant *C. jejuni* causes a more prolonged diarrheal disease than susceptible strains (Gupta et al. 2004; Smith et al. 1999). Nevertheless, a recent investigation summarizing the correlation between *FR-Campylobacter* infections and the severity of the diarrheal diseases produced by these *C. jejuni* strains has concluded that there is no data to support a prolonged, more severe disease hypothesis by these strains (Wassenaar et al. 2007).

Antimicrobial resistance has increased substantially in *Campylobacter* over the past two decades (FDA-CVM 2000). Molecular subtyping has revealed an association between *C. jejuni* strains isolated from chicken products and *C. jejuni* strains isolated from domestically acquired human cases of campylobacteriosis (Smith et al. 1999). Fluoroquinolone-resistant *C. jejuni* strains have been found to be ecologically competitive, rapidly replacing fluoroquinolone-susceptible strains in fluoroquinolone-treated chickens (Zhang et al. 2003), and persisting regardless of antimicrobial usage. In a five-year study conducted from 2002 to 2006, *Campylobacter* was detected on 57 to 96% of carcasses sampled with total *Campylobacter* load ranging from 0.90–4.82 $\log_{10}$ CFU/carcass. Ciprofloxacin-resistant *Campylobacter* CFU ($\log_{10}$ 0.90 or greater CFU/carcass) were found on 20 to 60% of sampled carcasses and total ciprofloxacin-resistant *Campylobacter* load ranging from 0.90–3.95 $\log_{10}$ CFU/carcass. While some reductions were seen for carcasses with higher loads of total *Campylobacter* or total ciprofloxacin-resistant *Campylobacter* during the five-year period from 2002 to 2006, random colony picks on CA and CCA confirmed the continued presence of high degree of ciprofloxacin-resistant *C. jejuni* (ciprofloxacin MIC's ranging from ≥16 to ≤32 μg/ml) in these retail raw chicken carcass rinses (Nannapaneni et al. 2005a, 2006a). A new method to enumerate fluoroquinolone-resistant *Campylobacter* has been developed by using a lethal dose of ciprofloxacin in *Campylobacter* selective agar plates to kill all ciprofloxacin-sensitive *Campylobacter* and selectively isolate naturally occurring ciprofloxacin-resistant *Campylobacter* (Nannapaneni et al. 2005a).

Virulence Mechanisms of *C. jejuni*

Several virulence factors have been characterized in *C. jejuni.* The most studied factor is the bipolar flagella, which comprises a system with an intricate regulatory network (Hendrixson and DiRita 2003; Wosten et al. 2004; Carrillo et al. 2004) and whose channels are apparently used to export adhesive proteins that are involved in cell invasion (Konkel et al. 2004). The presence of flagella is also important for the colonization of intestinal cells in chickens (Wassenaar et al. 1993). The adherence of *C. jejuni* to epithelial cells is mediated by several multiple factors, such as

CadF, PEB1, JlpA, Fibronectin, a 43 kDa major outer membrane protein and lipopolysaccharides (Fry et al. 2000; Jin et al. 2003; Konkel et al. 1997, 1999; Krause-Gruszczynska et al. 2007; Lara-Tejero et al. 2002; Lee et al. 2003; Moser et al. 1997; Pei et al. 1998). Three *cdt* genes of the cytolethal distending toxin (CDT) group are also important pathogenicity factors (Pickett et al. 1996; Pickett and Whitehouse 1999; Lara-Tejero and Galan 2001). In addition, it is possible that nonspecific binding to lipids can mediate adherence (Szymanski and Armstrong 1996).

An invasion-associated marker (*imv*), a chromosomal marker associated with adherence and invasion in 85% of invasive and 20% of noninvasive *C. jejuni* isolates, has also been described (Carvalho et al. 2001), although the function of *imv* is not well understood. More recently, a lipoprotein termed CapA, for *Campylobacter* adhesion protein A, has also been reported to reduce the capacity of *C. jejuni* to attach and invade Caco-2 cells. *Campylobacter jejuni* strains with a *capA* insertion mutant failed to colonize and persist in chickens (Ashgar et al. 2007). An isogenic mutation in the gene virB11 encoding a putative component of a type-IV secretion system resulted in reduction in adherence and invasion (Bacon et al. 2000). The colonization ability of *C. jejuni* isolates was also compromised when some genes encoding methyl-accepting chemotaxis proteins, such as *docB* and *docC,* were mutated (Hendrixson and DiRita 2004).

Some *C. jejuni* cells also exhibit microtubule-, microfilament-, and caveolin-dependent mechanisms (Hu and Kopecko 1999; Konkel and Jones 1989; Oelschlaeger 1993) that play a critical role in pathogenesis (Biswas et al. 2003; Byrne et al. 2007). Although the pathogenic mechanisms of *C. jejuni* are still not completely understood, molecular tools based on microarrays are starting to elucidate the presence of virulence factors at the DNA level that could be used to potentially screen isolates for pathogenicity. *In vitro* models based on animals or cell lines, such as cell monolayers (Caco-2, Hep-2, HeLa or L-cells) or detached individual cells (β-lymphocyte derived hybridoma cells), will still play an important role in confirming the results found from microarray data (Fauchere et al. 1986; Friis et al. 2006; Wassenaar and Blaser 1999; Lee et al. 2003; Deun 2007).

To study attachment, recognition, and invasive mechanisms of *C. jejuni,* selected monoclonal antibodies have been developed that could be used to block selected binding sites on target Caco-2 and INT-cells, and to visualize *C. jejuni* invasion (Friis et al. 2005; Nannapaneni et al. 2006b; Qian et al. 2007). Various inhibitors of cells' functions, such as cytochalasin B (inhibitor of actin polymerization), vincristine, colchicine, nocodazole (inhibitor of microtubule polymerization), and filipin (caveolin inhibitor), have been used to examine internalization of *C. jejuni* into human intestinal cells (Byrne et al. 2007; Konkel and Jones 1989). *C. jejuni* isolates possessing CadF and binding fibronectin have been detected by

fibronectin-coated coverslips with and without the addition of an anti-fibronectin antibody, and the increase in tyrosine phosphorylation by *C. jejuni* isolates have been detected by an immunoprecipitation assay with anti-paxillin conjugated to Protein A beads.

Hu et al. (2006) utilized the inhibitors fillipin III, pertussis toxin, and cholera toxin to demonstrate that *C. jejuni* 81–176 interacts with G proteins in caveolae of the host cell membrane. Fillipin III disrupts the formation of caveolae while cholera toxin and pertussis toxin are G protein inhibitors. As in the microfilament-dependent pathway for invasion, the microtubule-dependent pathway relies on the phosphorylation of host cell proteins. Various inhibitors can be utilized to block phosphorylation such as staurosporine, a broad spectrum kinase inhibitor, or genistein, an inhibitor of tyrosine kinase phosphorylation. Specific inhibitors can also be incorporated such as Wortmannin and LY294022 to inhibit PI 3-kinase, PD98059 to indirectly inhibit ERK MAP kinase, and SB203580 to inhibit P38 MAP kinase. The MAP kinase pathway is thought to trigger factors involved in cytoskeletal rearrangement of the host cell resulting in bacterial invasion (Friis et al. 2005; Hu et al. 2006).

Although *C. jejuni* with different degrees of resistance against ciprofloxacin can be frequently isolated from rinses from retail raw chicken carcasses, their virulence differences and the effects of environmental stresses on invasiveness of *C. jejuni* have not been uncharacterized. A diverse set of *C. jejuni* isolates collected from rinses of retail poultry products were tested for the presence of the *iam* gene, and their virulence against Caco-2 and INT-407 cells evaluated (Nannapaneni et al. 2005b). These results showed that *C. jejuni* expressed high levels of invasiveness, and most of the isolates infected the cells within 2 hours after the challenge. Only 12% of the isolates were found to be minimally invasive. Invasiveness in Caco-2 increased with infection time and infection dose for selected ciprofloxacin-sensitive and ciprofloxacin-resistant *C. jejuni* tested.

Effects of Temperature on *C. jejuni*

C. jejuni cells are commonly exposed to temperature changes and low temperatures (~4°C) during food processing and storage. The colonization of the intestinal tract of chickens by *C. jejuni* occurs at a temperature of 42°C, but the invasion of human cells occurs at 37°C. A two-component regulatory system termed RacR-RacS (reduced ability to colonize) appears to be involved in a temperature-dependent signaling pathway. *C. jejuni* with a mutation of the response regulator gene *racR* had a reduced ability to colonize the chicken intestinal tract and resulted in temperature-dependent changes in its protein profile and growth characteristics (Brás et al. 1999).

C. jejuni is able to sense, adapt, and respond to temperature fluctuations, and heat shock proteins have been demonstrated to help this organism colonize intestinal surfaces and survive at higher temperatures (Stintzi 2003). Different gene expression patterns were observed in *C. jejuni* in response to sudden shifts in temperature, but their relation to differences in virulence gene expression are presently unknown. There may be either an induction or repression of different genes in response to a temperature shift from 37 to 42°C, but little is known about *C. jejuni* responses to low-temperature shocks (Stintzi 2003), although actively growing cells transferred to saline remained competent to invasion of Caco-2 cells when exposed to 4°C for up to four weeks (Nannapaneni et al., unpublished data).

During poultry processing and storage, *C. jejuni* is exposed to cold stress and the adaptation to low-temperature shocks may play a critical role in its survival and virulence mechanisms. We still do not understand if any of the known pathogenicity factors is activated by temperature shocks or other environmental changes occurring during poultry processing. It is also unclear if adherence and invasiveness among FR-*C. jejuni* strains changes after exposure to temperature shifts. These studies would reveal whether or not a connection exists between pathogenicity and environmental conditions present during poultry processing in *C. jejuni.*

Conclusions and Future Perspectives

There are serious data gaps in the risk-assessment models for *C. jejuni* (FDA-CVM 2000). For quantitative risk-assessment purposes, a better understanding of the counts and virulence properties of poultry isolates of *C. jejuni* is needed. There appears to be considerable diversity and virulence determinants of floroquinolone-resistant and -sensitive *C. jejuni* occurring on raw poultry products. This issue becomes particularly critical since antibiotic–resistant strains of *C. jejuni* can enter the poultry product chain at any point.

Rapid and accurate detection of virulent strains of *C. jejuni* isolated from food products is essential in any food-safety monitoring program. The question remains whether the highly invasive phenotypes isolated from raw poultry products have the same virulence toward humans as those *C. jejuni* strains isolated from human infections. The binding, invasion, and translocation of *C. jejuni* in Caco-2 and INT-407 cell models may increase or decrease when *C. jejuni* cells are exposed to temperature shocks, and examining these conditions will provide a better understanding of the potential risks associated with different sources of *C. jejuni* from poultry during preharvesting and processing (temperature shock).

References

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

Altekruse, S. F., N. J. Stern, P. I. Fields, and D. L. Swerdlow. 2006. *Campylobacter jejuni*—An emerging foodborne pathogen. Emerg. Infect. Dis. 5:28–35.

Anonymous. 1999. Incidence of foodborne illnesses: Preliminary data from the Foodborne Diseases Active Surveillance Network (FoodNet)—United States, 1998. MMWR 48 (09):189–194.

Anonymous. 2007. Dirty birds, even premium chickens harbor dangerous bacteria. Consumer Reports, January 2007: 20–23.

Ashgar, S. S. A., N. J. Oldfield, K. G. Wooldridge, M. A. Jones, G. J. Irving, D. P. J. Turner, and D. A. A. Ala'Aldeen. 2007. CapA, an autotransporter protein of *Campylobacter jejuni,* mediates association with human epithelial cells and colonization of the chicken gut. J. Bacteriol. 189:1856–1865.

Bacon, D. J., R. A. Alm, D. H. Burr, L. Hu, D. J. Kopecko, C. P. Ewing, T. J. Trust, and P. Guerry. 2000. Involvement of a plasmid in virulence of *Campylobacter jejuni* 81–176. Infect. Immun. 68:4384–4390.

Biswas, D., K. Itoh, and C. Sasakawa. 2003. Role of microfilaments and microtubules in the invasion of INT-407 cells by *Campylobacter jejuni.* Microbiol. Immunol. 47:469–473.

Brás, A. M., S. Chatterjee, B. W. Wren, D. G. Newell, and J. M. Ketley. 1999. A novel *Campylobacter jejuni* two-component regulatory system important for temperature-dependent growth and colonization. J. Bacteriol. 181:3298–3302.

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

Carrillo, C. D., E. Taboada, J. H. Nash, P. Lanthier, J. Kelly, P. C. Lau, R. Verhulp, O. Mykytczuk, J. Sy, W. A. Findlay, K. Amoako, S. Gomis, P. Willson, J. W. Austin, A. Potter, L. Babiuk, B. Allan, and C. M. Szymanski. 2004. Genome-wide expression analyses of *Campylobacter jejuni* NCTC11168 reveals coordinate regulation of motility and virulence by *flhA*. J. Biol. Chem. 279:20327–20338.

Carvalho, A. C. T., G. M. Ruiz-Palacios, P. Ramos-Cervantes, L. Cervantes, X. Jiang, and L. K. Pickering. 2001. Molecular characterization of invasive and noninvasive *Campylobacter jejuni* and *Campylobacter coli* isolates. J. Clin. Microbiol. 39:1353–1359.

Deun, K. V., F. Haesebrouck, M. Heyndrickx, H. Favoreel, J. Dewulf, L. Ceelen, L. Dumez, W. Messens, S. Leleu, F. V. Immerseel, R. Ducatelle, and F. Pasmans. 2007. Virulence properties of *Campylobacter jejuni* isolates of poultry and human origin. Med. Microbiol. 56:1284–1289.

Engberg, J., F. M. Aarestrup, D. E. Taylor, P. Gerner-Smidt, and I. Nachamkin. 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli:* resistance mechanisms and trends in human isolates. Emerg. Infect. Dis. 7:24–34.

Evans, M., R. Roberts, C. D. Ribeiro, D. Gardner, and D. Kembrey. 1996. A milk-borne *Campylobacter* outbreak following an educational farm visit. Epidemiol. Infect. 117:457–462.

Fauchere, J. L., A. Rosenau, M. Veron, E. N. Moyen, S. Richard, and A. Pfister. 1986. Association with HeLa cells of *Campylobacter jejuni* and *C. coli* isolated from human feces. Infect. Immun. 54:283–287.

FDA-CVM. 2000. Human health impact of fluoroquinolone resistant *Campylobacter* attributed to the consumption of chicken. Online at http://www.fda.gov/cvm/Documents/Risk_asses.pdf. Accessed July 7, 2009.

Fliegelman, R. M., R. M. Petrak, L. J. Goodman, J. Segreti, G. M. Trenholme, and R. L. Kaplan. 1985. Comparative in vitro activities of twelve antimicrobial agents against *Campylobacter* species. Antimicrob. Agents Chemother. 27:429–430.

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

Friis, L. M., C. Pin, B. M. Pearson, and J. M. Wells. 2005. *In vitro* cell culture methods for investigating *Campylobacter* invasion mechanisms. J. Microbiol. Methods 61:145–160.

Fry, B. N., S. Feng, Y. Y. Chen, D. G. Newell, P. J. Coloe, and V. Korolik. 2000. The *galE* gene of *Campylobacter jejuni* is involved in lipopolysaccharide synthesis and virulence. Infect. Immun. 68:2594–2601.

Galanis, E. 2007. *Campylobacter* and bacterial gastroenteritis. CMAJ 177:570–571.

Gupta, A., J. M. Nelson, T. J. Barrett, R. V. Tauxe, S. P. Rossiter, C. R. Friedman, K. W. Joyce, K. E. Smith, T. F. Jones, M. A. Hawkins, B. Shiferaw, J. L. Beebe, D. J. Vugia, T. Rabatsky-Her, J. A. Benson, T. P. Root, and F. J. Angulo for the NARMS Working Group. 2004. Antimicrobial resistance among *Campylobacter* strains, United States, 1997–2001. Emerg. Infect. Dis. 10:1102–1109.

Hakanen, A., H. Jousimies-Somer, A. Siitonen, P. Huovinen, and P. Kotilainen. 2003. Fluoroquinolone resistance in *Campylobacter jejuni* isolates in travelers returning to Finland: association of ciprofloxacin resistance to travel destination. Emerg. Infect. Dis. 9:267–270.

Hendrixson, D. R., and V. J. DiRita. 2003. Transcription of sigma54-dependent but not sigma28-dependent flagellar genes in *Campylobacter jejuni* is associated with formation of the flagellar secretory apparatus. Mol. Microbiol. 50:687–702.

Hendrixson, D. R., and V. J. DiRita. 2004. Identification of *Campylobacter jejuni* genes involved in commensal colonization of the chick gastrointestinal tract. Mol. Microbiol. 52:471–484.

Hoge, C. W., J. M. Gambel, A. Srijan, C. Pitarangsi, and P. Echeverria. 1998. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. Clin. Infect. Dis. 26:341–345.

Hu, L., and D. J. Kopecko. 1999. *Campylobacter jejuni* 81–176 associates with microtubules and dynein during invasion of human intestinal cells. Infect. Immun. 67:4171–4182.

Hu, L., J. P. McDaniel, and D. J. Kopecko. 2006. Signal transduction events involved in human epithelial cell invasion by *Campylobacter jejuni* 81–176. Microbial Pathogenesis 40:91–100.

Jin, S., Y. C. Song, A. Emili, P. M. Sherman, and V. L. Chan. 2003. JlpA of *Campylobacter jejuni* interacts with surface-exposed heat shock protein 90α and triggers signaling

pathways leading to the activation of NFk B and p38 MAP kinase in epithelial cells. Cell Microbiol. 5:165–174.

Kist, M. 2002. Impact and management of *Campylobacter* in human medicine—European perspective. Int. J. Infect. Dis. 6:44–48.

Koenraad, P. M., R. Ayling, W. C. Hazeleger, F. M. Rombouts, and D. G. Newell. 1995. The speciation and subtyping of campylobacter isolates from sewage plants and waste water from a connected poultry abattoir using molecular techniques. Epidemiol. Infect. 115:485–494.

Koenraad, P. M., W. F. Jacobs-Reitsma, T. Van der Laan, R. R. Beumer, and F. M. Rombouts. 1995. Antibiotic susceptibility of campylobacter isolates from sewage and poultry abattoir drain water. Epidemiol. Infect. 115:475–483.

Konkel, M. E., S. G. Garvis, S. L. Tipton, D. E. Anderson Jr., and W. Cieplak Jr. 1997. Identification and molecular cloning of a gene encoding a fibronectin-binding protein (CadF) from *Campylobacter jejuni.* Mol. Microbiol. 24:953–963.

Konkel, M. E., S. A. Gray, B. J. Kim, S. G. Garvis, and J. Yoon. 1999. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the *cadF* virulence gene and its product. J. Clin. Microbiol. 37:510–517.

Konkel, M. E., and L. A. Jones. 1989. Adhesion to and invasion of HEp-2 cells by *Campylobacter* spp. Infect. Immun. 57:2984–2990.

Konkel, M. E., J. D. Klena, V. Rivera-Amill, M. R. Monteville, D. Biswas, B. Raphael, and J. Mickelson. 2004. Secretion of virulence proteins from *Campylobacter jejuni* is dependent on a functional flagellar export apparatus. J. Bacteriol. 186:3296–3303.

Korlath, J. A., M. T. Osterholm, L. A. Judy, J. C. Forfang, and R. A. Robinson. 1985. A point-source outbreak of campylobacteriosis associated with consumption of raw milk. J. Infect. Dis. 152:592–596.

Kramer, J. M., J. A. Frost, F. J. Bolton, and D. R. A. Wareing. 2000. *Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. J. Food Prot. 63:1654–1659.

Krause-Gruszczynska, M., L. B. Van Alphen, O. A. Oyarzabal, T. Alter, I. Hänel, A. Schliephake, W. König, J. P. M. Van Putten, M. E. Konkel, and S. Backert. 2007. Expression patterns and role of CadF protein in *Campylobacter jejuni* and *Campylobacter coli.* FEMS Microbiol. Lett. 274:9–16.

Lara-Tejero, M., and J. E. Galán. 2001. CdtA, CdtB, and CdtC form a tripartite complex that is required for cytolethal distending toxin activity. Infect. Immun. 69:4358–4365.

Lara-Tejero, M., and J. E. Galán. 2002. Cytolethal distending toxin: limited damage as a strategy to modulate cellular functions. Trends Microbiol. 10:147–152.

Lee, R. B., D. C. Hassane, D. L. Cottle, and C. L. Pickett. 2003. Interactions of *Campylobacter jejuni* cytolethal distending toxin subunits CdtA and CdtC with HeLa cells. Infect. Immun. 71:4883–4890.

Jones, I. G., and M. Roworth. 1996. An outbreak of *Escherichia coli* O157 and Campylobacteriosis associated with contamination of a drinking water supply. Public Health 110:277–282.

Mentzing, L. O. 1981. Waterborne outbreaks of campylobacter enteritis in central Sweden. Lancet. 2(8242):352–354.

Mishu, B., A. A. Ilyas, C. L. Koski, F. Vriesendorp, S. D. Cook, F. A. Mithen, and

M. J. Blaser. 1993. Serological evidence of previous *Campylobacter jejuni* infection in patients with the Guillain-Barré syndrome. Ann. Intern. Med. 118:947–953.

Miwa, N., Y. Takegahara, K. Terai, H. Kato, and Y. Takeuchi. 2003. *Campylobacter jejuni* contamination on broiler carcasses of *C. jejuni*-negative flocks during processing in a Japanese slaughterhouse. Int. J. Food Microbiol. 84:105–109.

Moore, J. E., M. D. Barton, I. S. Blair, D. Corcoran, J. S. Dooley, S. Fanning, I. Kempf, A. J. Lastovica, C. J. Lowery, M. Matsuda, D. A. McDowell, A. McMahon, B. C. Millar, J. R. Rao, P. J. Rooney, B. S. Seal, W. J. Snelling, and O. Tolba. 2006. The epidemiology of antibiotic resistance in *Campylobacter.* Microbes Infect. 8:1955–1966.

Moser, I., W. Schroeder, and J. Salnikow. 1997. *Campylobacter jejuni* major outer membrane protein and a 59-kDa protein are involved in binding to fibronectin and INT 407 cell membranes. FEMS Microbiol. Lett. 157:233–238.

Nannapaneni, R., R. Story, K. C. Wiggins, and M. G. Johnson. 2005a. Concurrent quantitation of total *Campylobacter* load and total ciprofloxacin-resistant *Campylobacter* loads in rinses from retail raw chicken carcasses from 2001 to 2003 by direct plating method at 42°C. Appl. Environ. Microbiol. 71:4510–4515.

Nannapaneni, R., R. Story, K. C. Wiggins, and M. G. Johnson. 2005b. Highly virulent *Campylobacter jejuni* in retail raw chicken carcass rinses. Abst. No. P5–35 in 2005 IAFP Ann Mtg, Aug. 14–17, Baltimore, MD.

Nannapaneni, R., R. Story, K. C. Wiggins, and M. G. Johnson. 2006a. Total *Campylobacter* and total ciprofloxacin-resistant *Campylobacter* loads in rinses from retail raw chicken carcasses in 2005, 5p, in Food Safety Consortium Annual Report, Oct. 1–3, 2006. University of Arkansas, Fayetteville.

Nannapaneni, R., R. Story, K. C. Wiggins, and M. G. Johnson. 2006b. New monoclonal antibody probes against *Campylobacter* and *Campylobacter jejuni,* 6p, in Food Safety Consortium Annual Report, Oct. 1–3, 2006. University of Arkansas, Fayetteville.

Newell, D. G., and J. A. Wagenaar. 2000. Poultry infection and their control at the farm level. Pages 497–509 in *Campylobacter*. I. Nachamkin and M. J. Blaser, eds. American Society for Microbiology, Washington, DC.

Oelschlaeger, T. A., P. Guerry, and D. J. Kopecko. 1993. Unusual microtubule-dependent endocytosis mechanisms triggered by *Campylobacter jejuni* and *Citrobacter freundii.* Proc. Natl. Acad. Sci. 90:6884–6888.

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. J. Food Prot. 68:1752–1760.

Oyarzabal, O. A., R. Rad, and S. Backert. 2007. Conjugative transfer of a chromosomally-encoded antibiotic resistance from *Helicobacter pylori* into *Campylobacter jejuni.* J. Clin. Microbiol. 45:402–408.

Pei, Z., C. Burucoa, B. Grignon, S. Baqar, X. Z. Huang, D. J. Kopecko, A. J. Bourgeois, J. L. Fauchere, and M. J. Blaser. 1998. Mutation in the *pel1A* locus of *Campylobacter jejuni* reduces interactions with epithelial cells and intestinal colonization of mice. Infect. Immun. 66:938–946.

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

Pickett, C. L., and C. A. Whitehouse. 1999. The cytolethal distending oxin family. Trends Microbiol. 7:92–297.

Potturi-Venkata, L-P., S. Backert, S. L. Vieira, and O. A. Oyarzabal. 2007. Evaluation of logistic processing to reduce cross-contamination of commercial broiler carcasses with *Campylobacter* spp. J. Food Prot. 70:2549–2554.

Qian, H., E. Pang, Q. Du, J. Chang, J. Dong, S. L. Toh, F. K. Ng, A. L. Tan, and J. Kwang. 2008. Production of a monoclonal antibody specific for the major outer membrane protein of *Campylobacter jejuni* and characterization of the epitope. Appl. Environ. Microbiol. 74:833–839.

Reina, J., M. J. Ros, and A. Serra. 1994. Susceptibilities to 10 antimicrobial agents of 1,220 *Campylobacter* strains isolated from 1987 to 1993 from feces of pediatric patients. Antimicrob. Agents Chemother. 38:2917–2920.

Ruiz, J., P. Goñi, F. Marco, F. Gallardo, B. Mirelis, T. Jimenez De Anta, and J. Vila. 1998. Increased resistance to quinolones in *Campylobacter jejuni:* a genetic analysis of *gyrA* gene mutations in quinolone-resistant clinical isolates. Microbiol. Immunol. 42:223–226.

Sánchez, R., V. Fernández-Baca, M. D. Díaz, P. Muñoz, M. Rodríguez-Créixems, and E. Bouza. 1994. Evolution of susceptibilities of *Campylobacter* spp. to quinolones and macrolides. Antimicrob. Agents Chemother. 38:1879–1882.

Smith, K. E., J. M. Besser, C. W. Hedberg, F. T. Leano, J. B. Bender, J. H. Wicklund, B. P. Johnson, K. A. Moore, and M. T. Osterholm. 1999. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota 1992–1998. NEJM 340:1525–1532.

Stintzi, A. 2003. Gene expression profile of *Campylobacter jejuni* in response to growth temperature variation. J. Bacteriol. 185:2009–2016.

Szymanski, C. M., and G. D. Armstrong. 1996. Interactions between *Campylobacter jejuni* and lipids. Infect. Immun. 64:3467–3474.

Tam, C. C., S. J. O'Brien, and L. C. Rodrigues. 2006. Influenza, campylobacter and mycoplasma infections, and hospital admissions for Guillain-Barré syndrome, England. Emerg. Infect. Dis. 12:1880–1887.

USDA-FSIS. 1996. Pathogen reduction; hazard analysis and critical control point (HACCP) systems; final rule. Federal Register. Online at http://www.fsis.usda.gov/OA/fr/haccp_rule.htm.

USDA-FSIS. 2007. May 22, 2007 Notice 31–07. Nationwide Young Chicken Microbiological Baseline Data Collection Program Update. U.S. Department of Agriculture. Washington, DC.

Unicomb, L., J. Ferguson, T. V. Riley, and P. Collignon. 2003. Fluoroquinolone resistance in *Campylobacter* absent from isolates, Australia. Emerg. Infect. Dis. 9:1482–1483.

Wassenaar, T. M., and M. J. Blaser. 1999. Pathophysiology of *Campylobacter jejuni* infections of humans. Microbes Infect. 1:1023–1033.

Wassenaar, T. M., M. Kist, and A. de Jong. 2007. Review. Re-analysis of the risks attributed to ciprofloxacin-resistant *Campylobacter jejuni* infection. Int. J. Antimicrobial Agents 30:195–201.

Wassenaar, T. M., B. A. M. van der Zeijst, R. Ayling, and D. G. Newell. 1993. Colonization of chicks by motility mutants of *Campylobacter jejuni* demonstrates the importance of flagellin A expression. J. Gen. Microbiol. 139:1171–1175.

Wosten, M. M., J. A.Wagenaar, and J. P. van Putten. 2004. The FlgS/FlgR two-component signal transduction system regulates the fla regulon in *Campylobacter jejuni.* J. Biol. Chem. 279:16214–16222.

Zhang, Q., J. Lin, and S. Pereira. 2003. Fluoroquinolone-resistant *Campylobacter* in animal reservoirs: dynamics of development, resistance mechanisms and ecological fitness. Anim. Health Res. Rev. 4:63–71.