Published in final edited form as:
Food Microbiol. 2013 June ; 34(2): 425–430. doi:10.1016/j.fm.2013.01.007.

A "successful allele" at *Campylobacter jejuni* contingency locus *Cj0170* regulates motility; "successful alleles" at locus *Cj0045* are strongly associated with mouse colonization

Katherine Artymovich[7,**], **Joo-Sung Kim**[8,**], **John E. Linz**[1,2,4,*], **David F. Hall**[6], **Lauren E. Kelley**[1,4], **Harrison L. Kalbach**[4], **Sophia Kathariou**[5], **Jean Gaymer**[3], and **Brenda Paschke**[3]

[1]Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824

[2]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824

[3]Animal Care Program, Michigan State University, East Lansing, MI 48824

[4]National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824

[5]Department of Food, Bioprocessing, and Nutrition Sciences, North Carolina State University, Raleigh NC 27695

[6]Quality Assurance Analyst, Neogen Corporation, 620 Lesher Place, Lansing, MI. 48912

[7]Application Scientist, Essen Bioscience, 300 W. Morgan Road, Ann Arbor, MI. 48818

[8]Senior Researcher, Korea Food Research Institute, 516 Baekhyun-Dong, Bundang-Ku, Sungnam-Si, Gyeonggi-Do 463-746, Republic of Korea

Abstract

Campylobacter jejuni is an important foodborne pathogen of humans and its primary reservoir is the gastrointestinal (GI) tract of chickens. Our previous studies demonstrated that phase variation to specific "successful alleles" at *C. jejuni* contingency loci *Cj0045* (successful alleles carry 9G or 10G homopolymeric tracts) and *Cj0170* (successful allele carries a 10G homopolymeric tract) in *C. jejuni* populations is strongly associated with colonization and enteritis in C57BL/6 IL-10 deficient mice. In the current study, we strengthened the association between locus *Cj0170*, *Cj0045*, and mouse colonization. We generated 8 independent strains derived from *C. jejuni* 11168 strain KanR4 that carried a *Cj0170* gene disruption and these were all non motile. Two randomly chosen strains with the *Cj0170* gene disruption (DM0170-2 and DM0170-6) were gavaged into mice. DM0170-2 and DM0170-6 failed to colonize mice while the control strain that carried a "successful" *Cj0170* 10G allele was motile and did colonize mice. In parallel studies, when we inoculated *C. jejuni* strain 33292 into mice, the "unsuccessful" *Cj0045* 11G allele experienced phase variation to "successful" 9G and 10G alleles in 2 independent experiments prior to d4 post inoculation in mice while the "successful" 9G allele in the control strain remained

*Corresponding author: John E. Linz, 234B GM Trout Building, Michigan State University, East Lansing, MI 48824. jlinz@msu.edu 517-355-8474; 517-353-8963 (FAX).
**Artymovich and Kim were primary and equal contributors to this manuscript.

stable through d21 post inoculation or shifted to other successful alleles. These data confirm that locus *Cj0170* regulates motility in *C. jejuni* strain KanR4 and is a virulence factor in the mouse model. The data also support a possible role of locus *Cj0045* as a virulence factor in strain 33292 in infection of mice.

INTRODUCTION

Campylobacter jejuni is among the most important foodborne bacterial pathogens (Scallen et al., 2011) and chickens constitute the major reservoir for human disease (Zhao et al., 2010; reviewed in Wassenaar, 2011). More than 20 contingency genes were identified in *C. jejuni* human disease isolate NCTC11168 (Parkhill et al., 2000). *C. jejuni* contingency genes carry homopolymeric tracts of 8 or more bases in which insertions or deletions (indels) of 1 or more bases occur at high frequency. These indel mutations can result in frame shifts that alter protein activity and this process is called phase variation (Jerome et al., 2011; Kim et al., 2012). A number of studies have shown that contingency genes are involved in the synthesis and modification of surface antigens associated with lipo-oligosaccharide (LOS), flagella, and capsule (see Supplementary Table S1 for proposed gene functions), and that phase variation in homopolymeric tracts carried within these genes impacts gene expression, surface structure chemistry, and potential for colonization/enteritis in animal models and human volunteers (Ashgar et al., 2007; Ewing et al., 2009; Guerry et al., 2002, 2006; Hendrixson et al., 2006; Howard et al., 2009; Kakuda and DiRita., 2006; Kim et al., 2012; Linton et al., 2000; McNally et al., 2007; Prendergast et al., 2004; Wilson et al., 2010). Despite this body of work, little is known about the specific mechanisms by which contingency genes impact colonization and disease.

Our previous studies demonstrated that *C. jejuni* contingency genes are subject to phase variation in the chicken gastrointestinal tract and this process can promote colonization and disease in C57BL/6 IL-10 deficient mice, our model for human *C. jejuni* colonization and enteritis (Kim et al., 2012). We observed that 5 contingency genes (*Cj0045*, *Cj0170*, *Cj0685*, *Cj1305/06/10*, and *Cj1420*) were conserved among the 3 human disease isolates analyzed (*C. jejuni* NCTC11168, 33292, and 81-176) and phase variation to predominant (occur in >50% of the population) “successful alleles” of 2 of these contingency genes, *Cj0045* and *Cj0170*, was strongly associated with colonization and enteritis in mice (Kim et al., 2012). In contrast, phase variation was not detected at significant levels when *C. jejuni* was cultured under standard laboratory conditions to generate inocula for animal experiments. Predominant alleles associated with colonization are designated successful alleles; predominant alleles that are not associated with colonization and disease are designated “unsuccessful alleles”. These data prompted us to hypothesize that these contingency genes encode important virulence factors. To begin to test this hypothesis, in the current study, we assessed colonization potential of deletion mutants of *Cj0170* in the mouse model. Furthermore, we determined if “successful alleles” of *Cj0045* associated with enteritis in mice are observed in *C. jejuni* isolated from a limited number of wild and domesticated animals or after passage of these isolates through commercial broilers.

MATERIALS AND METHODS

C. jejuni strains, media and growth conditions

The *C. jejuni* strains used in this study are presented in Table 1 and include human clinical isolates 81-176 (Bacon et al., 2000), 33292 (ATCC, Manassas, VA), NCTC11168 (Parkhill et al., 2000; Kim et al, 2012) and 11168-23SKanR4 (kanamycin resistant [KanR4]) derived from NCTC11168 as described previously (Wilson et al., 2003, 2010). Single colony isolates ( ) of *C. jejuni* 33292 that carry successful or unsuccessful alleles of *Cj0045* as the

predominant allele (9G or 11G homopolymeric tract, respectively) and *Cj1420* (9G or 8G homopolymeric tract, respectively) as well as single colony isolates (strains) of *C. jejuni* KanR4 that carry a successful allele (10G homopolymeric tract) or unsuccessful allele (9G homopolymeric tract) of *Cj0170* were identified in previous work (Kim et al., 2012). Strains carrying gene disruptions in *Cj0170* (DM0170-2, DM0170-6) were constructed in current work as described below. Thirteen additional *C. jejuni* human disease isolates were chosen randomly from our strain collection for genotype analysis (see Table 1) and these strains were described in a previous study (Wilson et al., 2000).

C. jejuni strains were routinely grown on Trypticase Soy Agar supplemented with 5% sheep blood (TSBA) at 37°C under standard microaerobic conditions (10% CO_2, 10% H_2, 80% N_2). For selective growth of *C. jejuni*, the medium was supplemented with cefoperazone (20 μg/ml), vancomycin (10 μg/ml), and amphotericin B (2 μg/ml) (TSBA-CVA). To minimize background growth of other bacteria in mouse fecal and cecal samples, higher concentrations of cefoperazone (32 μg/ml) and amphotericin B (10 μg/ml) were used in TSBA-CVA. To select for *C. jejuni* KanR4, the growth medium was supplemented with kanamycin (30 μg/ml).

Gene disruption of *Cj0170* in *C. jejuni* strain KanR4

The 5′-end and flanking region of *Cj0170* were amplified from genomic DNA of strain KanR4 using primers PK1 (5′-GCGGTTCATGTCAAAGTATAG-3′) and PK2 (5′-CTTGGAAAGGAACACCGCCGAGCCT TTTT GTGTAGAATGTTGAC-3′) to yield a 481bp fragment designated PK1-PK2. The 3′-end and flanking region (*Cj0172*) of *Cj0170* were amplified from the same DNA sample using primers PK3 (5′-ACCCTTTAGGAACTAAAGGGCGTTGAAGGCAGTGTGGAGC-3′) and PK4 (5′-CAGAAGTAGGAGTGAAAGATAGC-3′) to yield a 629bp fragment designated PK3-PK4. A chloramphenicol acetyltransferase (CAT) gene was amplified from plasmid pRRC (Malik Tareen et al., 2010; Wang and Taylor, 1990) using primers C1 (5′-CTCGGCGGTGTTCC T TTCCAAG-3′) and C2 (5′-CGCCCTTTAGTTCCTAAAGGGT-3′) to yield a 798bp fragment designated C1–C2. The underlined bases in each primer identify overlapping regions. PCR conditions to amplify PK1-PK2, PK3-PK4, and CAT were as follows. An initial denaturation was conducted for 1 m 30 s at 94°C followed by 35 cycles consisting of denaturation for 30 s at 94°C, annealing for 30 s at 55°C, and extension for 30 s at 68°C. The reaction was completed by a final extension for 5 m at 68°C. PCR reactions contained 1x *Pfx*50 PCR buffer (Invitrogen, Carlsbad, CA), 0.2 mM of each dNTP, 0.5 pmol/μl of each primer, 0.1 unit/μl of high-fidelity *Pfx*50 DNA Polymerase (Invitrogen), and 2ng/μl of chromosomal DNA. PK1-PK2, PK3-PK4, and CAT were purified, concentrated, and pooled (approximately 300–400ng) to conduct overlap extension PCR to produce a 1864 bp *Cj0170* deletion cassette (Hansen et al., 2007). PCR conditions for overlap extension were as follows. We conducted an initial denaturation for 90 s at 94°C followed by 35 cycles consisting of denaturation for 30 s at 94°C, annealing for 1m at 54°C, and extension for 3 m at 68°C. The reaction was completed by a final extension for 5 m at 68°C. The 1864 bp PCR product was resolved on an agarose gel, purified with a QIAquick gel extraction kit, QIAGEN Inc., Valencia, CA), and concentrated (340 ng/μl of *Cj0170* deletion cassette). Correct construction of the deletion cassette was confirmed by PCR and DNA sequence analyses.

A previously published protocol was used to transform the *Cj0170* deletion construct into *C. jejuni* KanR4 (Wilson et al., 2003). Electroporation was conducted using a Gene Pulser II (Bio-Rad) at 2.5kV, 3003, and 25.0 μF. Time constants for the pulse ranged from 5.5 to 7.5 ms. After electroporation, the sample was transferred to 1.5 ml of Bolton broth in a 6-well plate, incubated for 6 h at 37°C, 5% CO_2 with shaking, spread on TSBA supplemented with kanamycin (30 μg/ml) and chloramphenicol (20 μg/ml), and incubated for 2 d under the

standard growth conditions. To confirm deletion of *Cj0170* in strain KanR4, the entire region carrying the deletion cassette was amplified from genomic DNA using primers PK1 and PK4 and the PCR product was confirmed to contain a target band of the expected size (1864 bp). The PCR product was purified and the CAT gene was amplified using primers C1 and C2.

DNA extraction and polymerase chain reaction (PCR)

DNA was extracted from *C. jejuni* populations and analyzed by PCR as described previously (Kim et al., 2012).

Fragment analysis

Fragment analysis amplifies and resolves populations of DNA fragments carrying mutations in the homopolymeric tract of *C. jejuni* contingency genes (see Wassenaar et al., 2002). This provides useful information regarding the frequency of contingency gene alleles in a bacterial population that differ by one or more nucleotide changes within the homopolymeric tract. For fragment analysis of *Cj0045*, *Cj0170*, *Cj0685*, and *Cj1420*, we used gene-specific primers (Supplementary Table S2) and methods described previously (Kim et al., 2012). Primer design for fragment analysis was based on the NCTC11168 genome sequence (Parkhill et al., 2000). Fragment analysis data for 19 contingency loci are found in Supplementary Table S3.

High throughput DNA sequence analysis

High throughput DNA sequence analysis was conducted on small regions of contingency genes spanning the homopolymeric tract to confirm fragment analysis data as described previously (Kim et al., 2012) (see Supplementary Table S2). Briefly, PCR products were cloned into pCR4–TOPO using the TOPO TA Cloning Kit for Sequencing (Invitrogen). Recombinant plasmids were transformed into One Shot TOP10 Competent *E. coli* cells following the manufacturer's instructions. Twenty or more colonies were picked from LB kanamycin (50 μg/ml) agar plates and cultured individually in LB broth containing kanamycin overnight at 37°C. Bacterial cell suspensions in glycerol (15%) were submitted to the Research Technology Support Facility at Michigan State University for sequence analysis using an ABI 3730 Genetic Analyzer (Applied Biosystems) with primers described in Supplementary Table S2.

Mouse colonization

To prepare inocula for mouse colonization studies, *C. jejuni* single-colony isolates and human disease isolates from frozen stock cultures (−80°C) were grown on TSBA for 2 d and the cells were suspended in TSB at an OD (600 nm) of 0.1–0.2. Cell suspensions (100 μl) were spread on TSBA plates which were then incubated under standard growth conditions (see above) for 12 to 14 h. Bacterial cells on agar plates were resuspended in TSB at an OD (600 nm) of 0.1–0.2 (Wilson et al., 2010).

C57BL/6 IL-10 – deficient mice (8–12 weeks old) were used as a model of human colonization and enteritis caused by *C. jejuni* as described previously (Mansfield et al., 2007; Kim et al., 2012). Mice (eight per group, one group for each strain) were gavaged orally with inocula carrying from 7.0×10^6 to 8.5×10^7 CFU *C. jejuni* in 200 μl using 5 French feeding tubes (Sovereign, Mansfield, MA). Mice had unlimited access to Teklad mouse breeder sterilizable diet (7904) (Harlan laboratories, Indianapolis, IN) and sterile water throughout the experiment. Mice showing severe disease symptoms (hunched back and diarrhea) were euthanized with CO_2 immediately. Fecal pellets were collected at d1, d4, d7 or d8, and d14 post inoculation and cecal tissue (20–60 mg) was removed during

necropsy at d21. Colonization by *C. jejuni* in these samples was analyzed by CFU determinations as described previously (Kim et al, 2012).

Motility

Motility of *C. jejuni* isolates was measured as described previously (Kim et al., 2012) with the following minor modifications. No antibiotics were included in TSA or TSB during inoculum preparation and motility halos were measured at 43 hours.

Statistical analysis

Fisher's exact test was used to compare the number of colonized mice between groups for statistical significance ($P<0.05$) using R free statistical software (http://cran.mtu.edu). A t-test was used to compare the level of mouse colonization between groups for statistical significance and to compare motility between single colony isolates (SC1, SC7, SC18, SC210) and gene disruption strains (DM0170-2 and DM0170-6. Differences were considered significant with probability values less than 0.05 ($P<0.05$).

RESULTS

Gene disruption of *Cj0170* reduces mouse colonization and motility in *C. jejuni* strain KanR4

We constructed strains DM0170-2 and DM0170-6 that carried a gene disruption in *Cj0170* and gavaged them into C57BL/6 IL-10 deficient mice (Table 2). Strain SCK7, the positive control which carries the 10G successful allele of *Cj0170* as the predominant allele, colonized 6 of 8 mice at d4 and 4 of 8 mice at d21. In contrast, strains DM0170-2 and DM0170-6 colonized 0 of 8 mice at d4 and 0 of 8 mice at d21 post inoculation; there was a statistically significant difference in ability to colonize mice by these 3 strains ($P < 0.05$). In the motility assay (see Table 3), the positive controls strains SCK1 (also carries the successful 10G allele of *Cj0170* as the predominant allele) and SCK7 were highly motile while negative control strains SCK18 and SCK21 (carry the 9G unsuccessful allele of *Cj0170* as the predominant allele) exhibited very low motility and the difference in motility between these positive and negative control strains was statistically significant ($p < 0.05$) as observed previously (Kim et al., 2012). DM0170-2 and DM0170-6 exhibited even lower levels of motility than SC18 and SC21 ($P< 0.05$) and this low level of motility was statistically different than the highly motile strains SC1 and SC7 ($P<0.05$).

Cj0045 phase variation from an unsuccessful 11G allele to successful 9 or 10G alleles occurs by d4 post inoculation and is strongly associated with successful mouse colonization

To help determine if phase variation in *Cj0045* contributed directly to mouse colonization or occurred as a result of colonization, we gavaged strains SC13 (carries the successful 9G allele as the predominant allele) as well as strains SC5 and SC9 (carry the unsuccessful 11 G allele as the predominant allele) into mice and conducted fragment analysis on the mouse inoculum and on *C. jejuni* isolated from cecal contents at d21as described previously (Kim et al., 2012). In addition, we also conducted fragment analysis on *C. jejuni* isolated from fecal pellets of colonized mice at d4 post inoculation; see Table 2 (colonization) and Table 4 (fragment analysis).

C. jejuni strains SC13, SC5, and SC9 each colonized mice at high frequency and the inoculum of each strain carried the expected predominant allele (Table 2 and Table 4); significant differences in the numbers of colonized mice were not observed. However, in mice colonized by strain SC13, the 9G successful allele remained the predominant allele throughout the 21 d experiment or it shifted to the successful 10G and/or 7G alleles (Table

4). Of particular importance, a shift from the 9G allele to the unsuccessful 11G or 8G alleles was not observed at significant levels so unsuccessful alleles were greatly under-represented in the colonized mice.

In contrast, the 11G predominant unsuccessful allele in strains SC5 and SC9 (59–64% initial allele frequency) nearly disappeared by d4 post inoculation. In 8 of 12 mice colonized by *C. jejuni* at d4 post inoculation, the 11G allele shifted to a successful 9G allele, 10G allele, or variants of these alleles (6G, 7G, or 12G) and this occurred prior to d4 post inoculation as the strains established stable colonization. In 4 of 12 mice colonized at d4, *C. jejuni* also carried the unsuccessful 8G allele (allele frequency ranging from 20–35%). In 3 of 4 of these mice, the successful 7G allele was present at higher frequency than the unsuccessful 8G allele. In only one mouse was the 8G allele present at greater frequency than the 7G allele. In strains SC5 and SC9, there was a strong selection bias against the unsuccessful 8G and 11G alleles by d4 post inoculation.

C. jejuni contingency loci *Cj0045* and *Cj0170* are highly conserved in human disease isolates

We used fragment analysis to detect the presence of *Cj0045* and *Cj0170* in 13 randomly chosen human disease isolates from our *C. jejuni* strain collection (Wilson et al., 2000) (Supplementary Table S4). The data demonstrated that *Cj0045* (detected in12 of 13 isolates) and *Cj0170* (12 of 13 isolates) were conserved in the vast majority of these human disease isolates. We identified the predominant allele for loci *Cj0170* and *Cj0045* in the 13 human disease isolates as a successful or unsuccessful allele (see Supplementary Table S4) based on criteria established in a previous study (Kim et al., 2012). Successful 9G and 10G alleles of *Cj0045* were carried in 9 of 13 isolates. Surprisingly, the unsuccessful 9G allele at locus Cj0170 was carried in 10 of 13 human disease isolates analyzed in this study.

DISCUSSION

The successful 10G allele of *Cj0170* is a positive regulator of motility

We previously demonstrated that *C. jejuni* strains SCK1 and SCK7 carry a successful 10G allele of *Cj0170*, are highly motile, and colonize mice at high frequency. In contrast, we observed that strains SCK18 and SCK21 carry an unsuccessful 9G allele of *Cj0170*, exhibit very low motility, and do not colonize mice (Kim et al., 2012). Analysis of the *Cj0170* nucleotide sequence and predicted protein sequence suggested that the 8G allele of *Cj0170* encodes a protein with a complete open reading frame while the 9G and 10G alleles encode truncated proteins (Kim et al., 2012); the 9G allele lacks 29 amino acids and the 10G allele lacks 33 amino acids. Disruption of *Cj0170* in 2 independent *C. jejuni* strains DM0170-2 and DM0170-6 in the current study blocked motility and ability to colonize mice.

We previously observed that the 8G allele of *Cj0170* is not present at high frequency in *C. jejuni* KanR4 populations isolated from the GI tract of broiler chickens or mice. Furthermore, we observed that the successful 10G allele is the predominat allele in successful KanR4 populations (populations that successfully colonized mice) and that the 9G allele is the predominant allele in unsuccessful populations (do not colonize mice) isolated from chickens (Kim et al., 2012). Based on these data, it is reasonable to propose that the 10G successful allele of *Cj0170* encodes a protein with modified function which positively regulates motility of *C. jejuni* and its ability to colonize in mice. In contrast, we propose that the 9G allele encodes either a nonfunctional protein or a protein with modified function, but this protein does not positively regulate motility in *C. jejuni* strain KanR4.

C. jejuni motility is required for colonization and disease in humans and animals (Dasti et al., 2010; Guerry, 2007) supporting the idea that loss of motility in strains DM0170-2 and

DM0170-6 directly results in loss of ability to colonize mice. Since colonization is an early and critical step in the disease process, we argue that the successful 10G allele of *Cj0170* is an important virulence factor in the mouse model. We do not know if the protein encoded by the 8G allele is functional and confers virulence. Future studies will focus on the differential roles of proteins encoded by these *Cj0170* alleles in motility and colonization.

Successful alleles of *Cj0045* are strongly associated with mouse colonization

We previously observed that phase variation from a predominant 11G unsuccessful allele of *Cj0045* to predominant 9G or 10G successful alleles occurred in strain 33292 after inoculation into mice but prior to necropsy at d21 and the occurrence of the 9G or 10G successful allele was strongly associated with mouse colonization (Kim et al., 2012). Since stable colonization is established by d4 post inoculation in most mice (Kim et al., 2012), we theorized that phase variation to a successful allele prior to d4 would support the idea that phase variation contributed directly to colonization. An important novel aspect of the current study is that shifts in allele frequency from predominant unsuccessful alleles to predominant successful alleles in *Cj0045* occurred prior to d4 post inoculation when *C. jejuni* establishes stable colonization of mice. We argue that this observation confirms a direct role for successful alleles of *Cj0045* in mouse colonization.

The unsuccessful 8G and 11 G alleles of *Cj0045* were greatly under-represented in *C. jejuni* isolated from fecal pellets at d4 and from cecal tissue d21 post inoculation suggesting a strong selection bias against these alleles. We argued previously that the 8G and 11G alleles are unsuccessful because they encode proteins in which the *Cj0045* open reading frame (ORF) is extended by a single amino acid. This results in an overlap between the translation stop codon of *Cj0045* and the translation initiation codon of *Cj0044* immediately downstream and this, in theory, could reduce the ability to reinitiate translation of the Cj0044 protein (Kim et al., 2012). The current study supports this argument. Future studies will determine whether *Cj0045*, *Cj0044* or both genes are associated with colonization and disease in mice.

Conclusions

A successful allele at contingency locus *Cj0170* regulates motility in *C. jejuni* strain KanR4. Successful alleles at locus Cj0045 in strain 33292 are strongly associated with ability to colonize mice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by a grant from the National Institutes of Health (R21 AI081714-02) and by Michigan AgBio Research (formerly the Michigan Agricultural Experiment Station). We thank Dr. Linda Mansfield for breeding C57BL/6 IL-10 deficient mice for these studies.

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Highlights

1. Locus *Cj0170* in *C. jejuni* strain KanR4 plays a direct role in mouse colonization.
2. Locus *Cj0170* encodes a positive regulator of motility.
3. Successful alleles at locus *Cj0045* are strongly associated with mouse colonization.

Highlights

We previously demonstrated that contingency loci *Cj0170* and *Cj0045* are closely associated with colonization and disease in C57BL/6 IL10-deficient mice, our model for colonization and disease in humans. We also demonstrated that passage through the chicken reservoir (Ross 308 broilers) promotes phase variation in these contingency loci and others to specific alleles that closely associate with ability to colonize and cause disease (Kim et al., 2012) – we call these successful alleles.

Novel contributions of the submitted manuscript include

1. We confirm that *Cj0170* in *C. jejuni* strain KanR4 plays a direct role in colonization in mice.
2. We demonstrate that *Cj0170* is a positive regulator of motility in *C. jejuni* strain KanR4 and this is the most likely mechanism by which it impacts colonization and disease in mice.
3. We strengthen the association between successful alleles of locus Cj0045 in strain 33292 and ability to colonize mice.
4. We demonstrate that 2 contingency loci (*Cj0045*, *Cj0170*) are highly conserved in 16 *C. jejuni* human disease isolates analyzed to date – these data prompt us to hypothesize that these 2 genes may be important virulence factors in humans.

Table 1

Bacterial strains used in this study.

Species	Strain	Source	Strain Donor/Reference
C. jejuni	NCTC11168	Human	ATCC; Parkhill et al., 2000
C. jejuni	KanR4 [a]	Human	Wilson et al., 2003, 2010
C. jejuni	CamR [a]	Human	Wilson et al., 2003, 2010
C. jejuni	33292	Human	ATCC
C. jejuni	81-176	Human	C. Pickett/Bacon et al., 2000
C. jejuni	4300	Human	M. Konkel (WSU)
C. jejuni	4239928	Human	M. Konkel (WSU)
C. jejuni	E961009	Human	M. Konkel (WSU)
C. jejuni	E972653	Human	M. Konkel (WSU)
C. jejuni	E972796	Human	M. Konkel (WSU)
C. jejuni	E9795412	Human	M. Konkel (WSU)
C. jejuni	3128	Human	I. Wesley (USDA
C. jejuni	3136	Human	I. Wesley (USDA)
C. jejuni	33291	Human	ATCC
C. jejuni	43429	Human	ATCC
C. jejuni	43433	Human	ATCC
C. jejuni	43470	Human	ATCC
C. jejuni	49349	Human	ATCC
C. jejuni	DM0170-2 [b]	Human	This study
C. jejuni	DM0170-6 [b]	Human	This study
C. *jejuni*	SCK1, SCK7[b]	Human	Kim et al., 2012
C. *jejuni*	SCK18, SCK21[b]	Human	Kim et al., 2012
C. *jejuni*	SC5, SC9[c]	Human	Kim et al., 2012
C. *jejuni*	SC13[c]	Human	Kim et al., 2012

[a] Derived from strain NCTC11168

[b] Derived from strain KanR4

[c] Derived from strain 33292

Table 2

Colonization of C57BL/6 IL-10 deficient mice by isolates of *C. jejuni* strains NCTC11168, KanR4, and 33292

Strain	Inoculum [a]	Day 4 (fecal sample) [b]	Day 21 (cecal sample) [c]
TSB	0	0	0
11168	7.5×10^6	1.6×10^4 (6/10 mice)	1.1×10^6 (6/10 mice)
SCK7	8.1×10^7	1.3×10^6 (6/8 mice)	2×10^5 (4/8 mice)
DM0170-2	7.5×10^7	0 (0/8 mice)	0 (0/8 mice)
DM0170-6	8.5×10^7	0 (0/8 mice)	0 (0/8 mice)
SC9	3.1×10^7	1.0×10^7 (6/8 mice)	1.0×10^7 (5/8 mice)
SC5	3.3×10^7	4.0 x10^6 (7/8 mice)	1.8×10^5 (6/8 mice)
SC13	1.6×10^7	8.9×10^7 (8/8 mice)	1.5×10^6 (8/8 mice)

[a] Inoculum cfu.

[b] Average cfu/g fecal pellet for 8 mice (SCK7, DM0170-2. DM0170-6, SC13, SC5, and SC9) or 10 mice (NCTC11168). The ratio in parentheses relates the number of mice positive for *C. jejuni* colonization versus the number of mice challenged.

[c] Average cfu/g cecal sample for 8 mice (SCK7, DM0170-2. DM0170-6, SC13, SC5, and SC9) or 10 mice (NCTC11168). The ratio in parentheses relates the number of mice positive for *C. jejuni* colonization versus the number of mice challenged

Table 3

Motility of isolates of *C. jejuni* strain KanR4

Isolate [a]	Colony No. [b]	Allele [c]	Motility [d]	Isolate	Colony No.	Allele	Motility
SCK1	1	10G	1.3	SCK21	1	9G	0.6
	2		1.1		2		0.6
	4		1.4		3		0.7
	5		1.2		5		0.5
	7		1.3		6		0.4
					7		0.5
SCK7	1	10G	1.9		8		0.5
	2		2.0				
	3		1.5	DM0170-2	1	KO	0.3
	4		2.1		3		0.5
	5		2.2		4		0.4
	6		2.1		5		0.3
	7		1.9		6		0.3
	8		1.9		7		0.4
					8		0.3
SCK18	1	9G	0.7				
	2		0.8	DM0170-6 [e]	1	KO	0.3
	3		0.7		2		0.2
	4		0.9		4		0.3
	5		0.6		5		0.3
	6		0.6		6		0.3
	7		0.5		7		0.2
	8		0.8		8		0.5

[a] Isolate designation

[b] 8 Mueller Hinton Motility Agar (MHA, 0.4% agar) plates were inoculated with the same population derived from each isolate. Plates 3, 6, 8 from isolate SCK1; plate 4 from isolate SC21; and plate 2 from isolate DM0170-2 were not analyzed due to moisture on the agar surface that artificially spread cells from the inoculation site.

[c] 10G allele of *Cj0170* (10G homopolymeric tract) is a successful allele; 9G allele is an unsuccessful allele; KO, represents a gene disruption in *Cj0170*.

[d] Numbers represent the size of the motility halo (cm) on MHA

[e] The motility halo on plate 3 was 3.0 cm. This value was treated as an outlier and was not used to calculate significance in differences in motility between isolates. However, the occurrence of this phenomenon is of interest because it suggests that a second site mutation in the genome can suppress the motility phenotype of the *Cj0170* disruption. Characterizing this phenomenon will be one focus of future work.

Table 4

Allele frequencies of *Cj0045* in inoculum (isolate), fecal pellets (d4), and/or cecal samples (d21).

Isolate	6G (−3)	7G (−2)	8G (−1)	9G (ORF)	10G (+1)	11G (+2)	12G (+3)	13G (+4)	14G (+5)
SC13 (S) [a]				90 [b]	10	0			
SC9 (U) [a]				2	34	59	5		
SC5 (U) [a]				0	36	64			
Mouse [e]									
78 d4 [c] SC9		38	31	31	0	0	0		
82 d4 SC9		41	16	43	0	0	0		
79 d21[d] SC9		1	6	26	64	3	0		
85 d21 SC9	15		0	52	17	0	6	10	
80 d4 SC9		37	36	27	0	0	0		
80 d21		13	4	80	3	0	0		
81 d4 SC9		42	0	58	0	0	0		
81 d21			0	0	1	6	38	50	5
87 d4 SC5			0	100	0	0			
88 d4 SC5		34	0	66	0	0			
89 d4 SC5		30	23	47	0	0			
90 d4 SC5		19	38	43	0	0			
86 d21 SC5			0	100	0	0			
92 d21 SC5		14	4	78	4	0			
96 d4 SC13		43	15	42	0	0			
93 d21 SC13		12	0	88	0	0			
94 d21 SC13		10	4	81	5	0			
95 d21 SC13	44		0	56	0	0			
98 d4 SC13		55	0	45	0	0			
98 d21		1	4	82	3	0			
100 d4 SC13		47	0	53	0	0			
100 d21	17		0	45	24	0		14	

[a] *Cj0045* allele frequencies in inocula used in oral gavage for isolates SC5, SC9, and SC13. S = successful allele; U = unsuccessful allele.

[b] Numbers represent allele frequencies as a percent of the total population.

[c] d4. Fecal pellet obtained from mouse at d4 post gavage.

[d] d21. Cecal tissue obtained from mouse upon necropsy at d21 post gavage.

[e] Mice colonized by *C. jejuni* isolates SC5, SC9, and SC13 designated with these isolate numbers.

Analysis for fecal pellet and cecal samples from mice 83, 84, 91, 97, and 99 were not completed. Unsuccessful 8G, 11G, and 14G alleles are highlighted in gray. All other alleles are successful alleles.