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# Outcome of infection of C57BL/6 IL-10$^{-/-}$ mice with *Campylobacter jejuni* strains is correlated with genome content of open reading frames up- and down-regulated *in vivo*

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ABSTRACT

Human *Campylobacter jejuni* infection can result in an asymptomatic carrier state, watery or bloody diarrhea, bacteremia, meningitis, or autoimmune neurological sequelae. Infection outcomes of C57BL/6 IL-10$^{-/-}$ mice orally infected with twenty-two phylogenetically diverse *C. jejuni* strains were evaluated to correlate colonization and disease phenotypes with genetic composition of the strains. Variation between strains was observed in colonization, timing of development of clinical signs, and occurrence of enteric lesions. Five pathotypes of *C. jejuni* in C57BL/6 IL-10$^{-/-}$ mice were delineated: little or no colonization, colonization without disease, colonization with enteritis, colonization with hemorrhagic enteritis, and colonization with neurological signs with or without enteritis. Virulence gene content of ten sequenced strains was compared *in silico*; virulence gene content of twelve additional strains was compared using a *C. jejuni* pan-genome microarray. Neither total nor virulence gene content predicted pathotype; nor was pathotype correlated with multilocus sequence type. Each strain was unique with regard to absences of known virulence-related loci and/or possession of point mutations and indels, including phase variation, in virulence-related genes. An experiment in *C. jejuni* 11168-infected germ-free mice showed that expression levels of ninety open reading frames (ORFs) were significantly up- or down-regulated in the mouse cecum at least two-fold compared to *in vitro* growth. Genomic content of these ninety *C. jejuni* 11168 ORFs was significantly correlated with the capacity to colonize and cause enteritis in C57BL/6 IL-10$^{-/-}$ mice. Differences in gene expression levels and patterns are thus an important determinant of pathotype in *C. jejuni* strains in this mouse model.



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## 1. Introduction

*Campylobacter jejuni* is a leading cause of sporadic food-borne enteritis throughout the world. Some individuals are refractory to primary *C. jejuni* infection or develop an asymptomatic carrier state [1]. In most instances, *C. jejuni* infection in humans causes acute enteritis with watery or bloody diarrhea that resolves within 7–10 days. Reactive inflammatory disorders can also occur after *C. jejuni* infection. In the gastrointestinal tract, transmural inflammatory changes in colon or small intestines may occur that resemble inflammatory bowel disease (IBD) [2,3], while joint disease can also result. In addition, peripheral neuropathies (Miller Fisher and Guillain Barré Syndromes) are known sequelae of *C. jejuni* infection where autoimmune mechanisms are triggered [4,5]. It is known that immunocompromised patients have both a higher incidence of infection with *Campylobacter* and more severe disease manifestations with profuse diarrhea and persistence of the organism [6,7]. Furthermore, those at the extremes of age or with underlying immune compromise are predisposed to bacteremia and subsequent spread to other organs [7,8]. In this study we focused on examining clinical phenotypes potentially arising from strain-specific attributes.

Attempts have been made to associate particular disease outcomes with particular pathogen genetic and/or phenotypic characteristics with mixed success [9,10]. The most compelling such association discovered to date has been that of strains expressing particular lipooligosaccharides with autoimmune neurological sequelae [11]. The most likely reason for this general lack of correspondence between pathogen genotype and clinical presentation is that *C. jejuni* is known to have extensive genetic variation driven by multiple mutagenic mechanisms [12] and lateral gene transfer within *C. jejuni* and between *C. jejuni* and its closest relative, *Campylobacter coli* [13,14].

C57BL/6 IL-10$^{-/-}$, NOD IL-10$^{-/-}$, and C3H/HeJ IL-10$^{-/-}$ murine models of *C. jejuni* 11168 infection [15,16] were recently developed and characterized in our laboratory; the C57BL/6 IL-10$^{-/-}$ model was used to explore the capacity of *C. jejuni* 11168 and six other *C. jejuni* strains to colonize and undergo genetic adaptation to a new host during serial passage [17]. Some data from the latter study are reproduced here under open license agreement with BioMed Central and BMC Microbiology; the relevant citation is given at each occurrence.

The initial stage of the serial passage experiment revealed that an array of *C. jejuni* strains from humans and animals produced a spectrum of clinical outcomes that reflected that seen in human campylobacteriosis. In addition, the results showed that some *C. jejuni* strains, but not others, were able to evolve increased pathogenicity during serial passage in mice. This ability—or lack of ability—to respond to selection indicates that the genomic content of each strain is crucial for its virulence phenotype. The term "pathotype" will be used below to denote the virulence phenotype of *C. jejuni* strains in C57BL/6 IL-10$^{-/-}$ mice, that is, the constellation of clinical, gross pathological, and histopathological outcomes of infection with a particular *C. jejuni* strain in C57BL/6 IL-10$^{-/-}$ mice.

The experiments reported here were undertaken to correlate disease phenotypes in the murine model with the genetic composition of *C. jejuni* isolates tested and to extend our understanding of the ability of the murine model to replicate the spectrum of *C. jejuni*-associated disease seen in humans. Similar approaches using combinations of *in vivo* and *in silico* methods have been used to define the "virulome," or the set of genes necessary for pathogenicity, *of Brucella suis* and *Burkholderia mallei* [18,19].

We therefore infected C57BL/6 IL-10$^{-/-}$ mice with fifteen additional *C. jejuni* strains from a variety of clinical and non-clinical sources. This strain set included six minimally passaged clinical isolates from the Centers for Disease Control and nine genome-sequenced strains. The genome-sequenced strains include nine *C. jejuni* subsp. *jejuni* strains: one chicken isolate, four isolates from human cases of enteritis, one isolate from a case of meningitis, two isolates from cases of Guillain Barré syndrome, one isolate from a case of Miller Fisher syndrome, and one *C. jejuni* subsp. *doylei* isolate from a case of bacteremia. We were able to delineate five pathotypes of *C. jejuni* in this murine model: no colonization, colonization with little or no disease, colonization with moderate or severe enteritis, colonization with hemorrhagic enteritis, and colonization with neurological sequelae with or without enteritis. A detailed description of the neurological sequelae will be published separately. In order to identify additional genes involved in host colonization and disease induction, we compared the gene expression profiles of a *C. jejuni* 11168 culture grown in broth to that of *C. jejuni* 11168 cells recovered from the ceca of germ-free mice. Ninety open reading frames (ORFs) were significantly ($P \leq 0.05$) up- or down-regulated more than two-fold in *C. jejuni* 11168 cells from mouse ceca, including thirty-nine up-regulated ORFs not previously shown to be involved in colonization or virulence.

Publically available sequence data and genomic analysis tools were used to compare the genome-sequenced strains. Among the genome-sequenced strains, neither total genomic content nor the composition of putative experimentally identified virulence factors correlated with pathotype. However, these results should be interpreted cautiously, since many of the genome-sequenced strains, especially strains 81-176 and 33560 (type strain), have been used in multiple laboratory studies and may have lost virulence during passage on laboratory media. The genomic content of each of the minimally passaged strains and of each of the previously studied strains [17] was compared to that of *C. jejuni* 11168 using a pan-genomic microarray developed for this study using data from the ten genome-sequenced *C. jejuni* strains tested in mice. Again, no correlation was found between pathotypes of *C. jejuni* strains in C57BL/6 IL-10$^{-/-}$ mice and either clonal complexes defined by multilocus sequence typing or total genome content. We therefore concluded that particular constellations of virulence genes, point mutations or indels (including phase variation in contingency genes) in individual loci, or differences in gene expression levels are likely to determine the pathotypes of individual strains.

A statistically significant correlation was found between colonization and disease phenotypes of both the set of genome-sequenced strains and the set of strains analyzed by microarray and genomic content of ninety ORFs that were up- and down-regulated at least two-fold in the ceca of germ-free C57BL/6 IL-10$^{-/-}$ mice. Finally, the results of strain comparisons based on documented virulence factors and the results of the gene expression study were used to establish a first approximation of the *C. jejuni* C57BL/6 IL-10$^{-/-}$ mouse virulome.

## 2. Materials and methods

### 2.1. C. jejuni strains, media, and growth conditions

*C. jejuni* strains used in these experiments are listed in Table 1. (Some data in Table 1 are reproduced from Bell *et al.* [17] under open license agreement with BioMed Central and BMC Microbiology). Strains D6844–D6849 were kindly supplied as blinded cultures by Drs. Collette Fitzgerald and Linda Demma of the Centers for Disease Control (CDC), Atlanta, GA, USA; strains CG8486 and CG8421 were gifts of Dr. Patricia Guerry; strain 84-25 was the gift of Drs. Martin Blazer and Nicole Iovine, and strain 81-176 was the gift of Dr. William Miller. Cultures to be inoculated