

Update on *Campylobacter* vaccine development

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ABSTRACT

Background: Acute diarrheal disease caused by viral, bacterial and parasitic infections are a major global health problem with substantial mortality and morbidity in children under five years of age in lower and middle income countries. However, a number of these infections also impact large segments of populations in upper income countries, as well as individuals who travel overseas for work, business or pleasure. *Campylobacter* has been and continues to be a leading cause of disease burden globally across all income countries.

Aims: The aim of this review is to describe recent understanding in burden of disease, consider the current landscape of *Campylobacter* vaccine development, and address the challenges that need to be overcome.

Sources: Relevant data from the literature as well as clinical trials described in European and US registries were used to conduct this review.

Content: Despite advances in population health, food security, improved sanitation, water quality and the reduction of poverty, *Campylobacter* infections continue to plague global populations. The emerging recognition of chronic health consequences attributed to this pathogen is changing the potential valuation of preventive interventions. Advancing development of new vaccines is a present opportunity and holds promise.

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Introduction

Acute enteric infections causing diarrhea and gastroenteritis are a global public health problem with high mortality and morbidity, particularly among children of the developing world. Each year, approximately 500,000 children under five years old die from severe, dehydrating diarrhoea and dysentery worldwide, and millions more are hospitalized, mostly in low-resource countries.¹ In addition, growing evidence finds that children are also susceptible to long-term physical and cognitive health consequences secondary to these frequent infections.²



While the overwhelming burden due to these infections is in the developing world, acute enteric infections are also a frequent cause for outpatient visits and hospitalization throughout the developed world with substantial societal and economic impact. For example, Scallan and colleagues published recently updated estimates for foodborne illness in the United States, which indicated that each year 31 major pathogens acquired in the United States caused 9.4 million episodes of diarrhoeal illness, 55,961 hospitalizations, and 1,351 deaths.³ While the acute consequences would appear to be of global significance and drive science and public health efforts to mitigate the problem, there is growing evidence linking such infections with a myriad of chronic health consequences including neurological, haematological and rheu-

matological systems.⁴ Furthermore, as with many other global infections, antibiotic resistance concerns among bacterial infections, particularly *Campylobacter* spp., have brought alarm to the potential worsening impact of these infections with decreasing effectiveness of commonly used antibacterials.

To fully address this problem, a multifaceted approach is needed including improvements in sanitation and hygiene, safe water, food security, breast-feeding, and adequate nutrition, and vaccination. While vaccines against some enteric pathogens are available, most are currently used for limited purposes, although efforts are underway to expand their utilization. Additionally, novel vaccines are in development, which promise hope to improve the health of at-risk populations from the developed and developing worlds. Herein, we review the current public health gap, understanding of pathogenesis, historical efforts, and current status of vaccine development for *Campylobacter*.

Burden of disease

It is estimated that *Campylobacter* infection is associated with 7.5 million disability adjusted life years (DALYs) globally, second only to rotavirus as a causative agent of diarrheal disease, with varying epidemiology across multiple populations.⁵ The 2015 global burden of disease estimates indicate over 25,000 deaths

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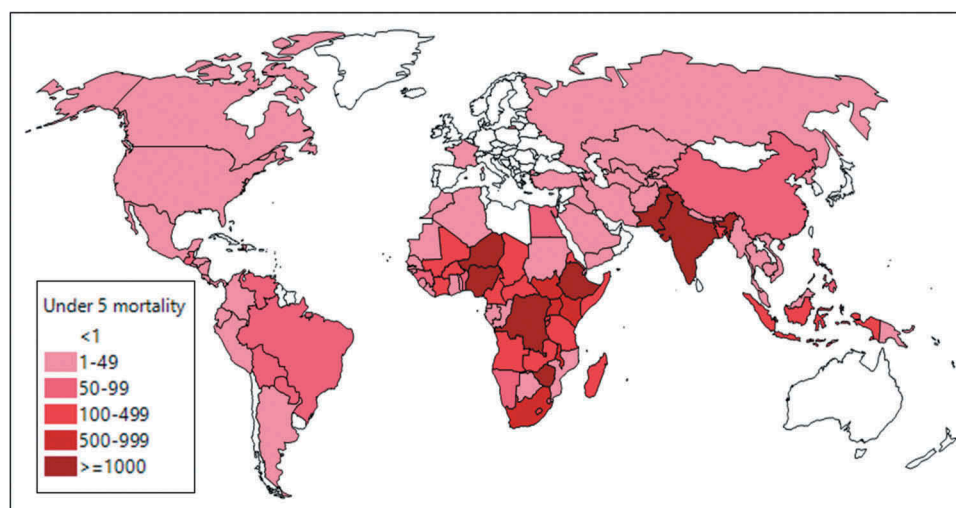


Figure 1. *Campylobacter*-attributable under 5 mortality (recreated from .

annually in children under 5 years of age with the highest rates in parts of Africa and Asia (Figure 1). Recent studies on diarrheal disease in several low and middle income countries (LMICs) have continued to highlight *Campylobacter* as a significant pathogen in children under five years of age.^{6,7} Despite the global burden of disease, the acute illness is often confounded by high infection rates in asymptomatic age-matched children.

Two landmark studies have recently described the pathogens associated with acute enteric infections in children under 5 living in multiple LMICs. In the Global Enteric Multicenter Study (GEMS), *C. jejuni* was identified as a leading cause of moderate to severe diarrhea in children aged 0–11 months as well as in children 2–5 years of age across all study sites; however, rates appeared to be highest at study sites in Bangladesh, Pakistan and India.⁶ These data are somewhat limited in their ability to determine the true burden of campylobacteriosis due to case-control design. In particular, using mathematical models, Havelaar and Swart demonstrated that for pathogens in which the force of infection is high, disease odds ratios may be biased towards the null and estimates of the population attributable fraction may underestimate the proportion of cases attributable to the organism.⁸ Additionally, microbiological methods have hampered all prior estimates of *Campylobacter* morbidity. Specifically, studies relying on culture-based methods of pathogen identification likely underestimate pathogen-specific disease burden. In fact, a re-analysis of GEMS data, highlighted a significant increase in the attributable incidence of *C. jejuni/coli* (as well as other enteric pathogens) to moderate to severe diarrhea when molecular based methods were used to identify disease etiology.⁹

In the Malnutrition and Enteric Disease (MAL-ED) study, designed as a birth cohort study, *Campylobacter* was the most common pathogen, identified in approximately 35% of diarrheal stools and 25% of non-diarrheal stools in children < 1 year of age.⁷ By 1–2 years of age, that proportion had increased to 40% in diarrheal stools and 35% in non-diarrheal stools. Estimates of adjusted attributable fraction highlight *Campylobacter* as the third leading cause of diarrhea in children < 1 year of age in the populations with the highest rates in two sites in South America (Fortaleza, Brazil and Loreto,

Peru) and in Venda, South Africa. By the second year of life, *Campylobacter* attributed to approximately 8% of all diarrheal cases, the highest of any diarrheal pathogen with the highest burden in Bhaktapur, Nepal and Loreto, Peru.

In addition to the morbidity associated with acute diarrheal illness in these populations, *Campylobacter* infection has been shown to be negatively associated with linear growth regardless of whether or not the infection is associated with acute illness.¹⁰ In children from the MAL-ED cohort study with *Campylobacter* infection, approximately 1 cm growth shortfall and an approximate 0.25 kg less weight gain was seen compared to children without *Campylobacter*; an effect that appeared to be most pronounced at 0–2 months of age.¹¹ Perhaps even more important than the association between *Campylobacter* infection and growth faltering is the potential link to environmental enteric dysfunction as recently described by Schnee and Petri.¹² If these associations are confirmed, current burden of disease estimates have likely grossly underestimated the morbidity associated with *Campylobacter* in children in LMICs.

In contrast to LMICs, the rate of asymptomatic infection is low; however, industrialized countries globally have documented a high rate of campylobacteriosis associated with consuming contaminated food and drink. In 2017 *Campylobacter* was the most commonly identified foodborne pathogen in the United States with approximately 10,000 cases confirmed in the 10 FoodNet sites performing active surveillance.¹³ In Europe, *Campylobacter* was also the leading cause of foodborne disease with over 200,000 cases in 2013, approximately 2.5 times the number of cases of *Salmonella*, another common foodborne pathogen.¹⁴ Similarly, data from OzFoodNet sites, Australia's foodborne disease surveillance system, have highlighted *Campylobacter* as the leading cause of foodborne disease with an estimated 116 notifications per 100,000 population.¹⁵ While these data highlight the importance of *Campylobacter*, it is important to underscore the fact that these studies all likely underestimate the true incidence of disease due to the surveillance methods utilized. For example, in the United States, active surveillance as part of FoodNet is established for 10 representative locations around the country

accounting for approximately 14% of the US population. Despite the 'active' nature of this surveillance, case identification is dependent on subjects seeking care. A 2011 report by the US Centers for Disease Control and Prevention highlighted the under-reporting inherent with this type of surveillance. For *Campylobacter*, it was estimated that for every 1 case of campylobacteriosis clinically diagnosed, over 30 cases occurred in the community.¹⁶

In travelers to LMICs, *Campylobacter* is a leading cause of travelers' diarrhea (TD) second only to diarrheagenic *Escherichia coli*.^{17–19} Geographically, the incidence of *Campylobacter*-attributable TD varies with by far the highest rates observed in travelers to Southeast Asia. In addition to being a common cause of travelers' diarrhea, *Campylobacter* is often associated with a more severe disease. In separate studies, Porter *et al* and Sanders *et al* found that approximately 50% of *Campylobacter*-attributed cases were unable to work as a result of their illness compared to only approximately 25% similarly affect cases attributed to other pathogens, highlighting the increased severity of illness in *Campylobacter* cases of TD.^{20,21} Clinically, *Campylobacter* cases tend to more commonly have fever, myalgia, arthralgia, and are more likely to have a sub-optimal response to treatment compared to TD cases of other etiology.²¹

In addition to the acute illness, *Campylobacter* infection is associated with numerous neurological, gastrointestinal and rheumatological post-infectious sequelae which further magnify the overall morbidity of infection.²² In particular, *Campylobacter* is the most common infection associated with Guillian-Barré Syndrome, a disorder in which the body's immune system attacks the peripheral nerve cells causing an acute, ascending flaccid paralysis, a process initiated by anti-ganglioside antibodies directed to the *Campylobacter* infection.^{23,24} The association between *Campylobacter* infection and several chronic gastrointestinal outcomes including irritable bowel syndrome, functional dyspepsia, inflammatory bowel disease and celiac disease was recently reviewed and highlights the significant costs and impact on quality of life measures associated with this pathogen.²⁵ *Campylobacter* is also one of several bacterial enteropathogens associated with reactive arthritis, a post-infectious sterile inflammation of joints and tissues that, in a significant proportion of cases, can be long lasting with significant associated morbidity.^{26,27}

When considering the overall burden of campylobacteriosis, in addition to the corresponding acute morbidity and mortality described above as well as the long-term post-infectious sequelae, a consideration must also be made regarding anti-microbial resistance (AMR). In a recent study of international travelers, rates of ciprofloxacin resistance appeared to increase over time with almost 75% of the strains resistant by 2014.²⁸ In addition to the rate of ciprofloxacin resistance, 4% were also resistant to erythromycin. The concern over AMR has led the US Centers for Disease Control and Prevention to designate antibiotic resistant *Campylobacter* as a serious threat to public health.²⁹ Additionally, the World Health Organization recently labeled *Campylobacter* as a high priority pathogen for novel antibiotic development due to the rates of ciprofloxacin resistance.³⁰ While the majority of *Campylobacter* cases do not require antibiotic treatment, the

rising rates of AMR threatens to yield few treatment options in those at high risk of more serious outcomes or with more severe disease.

Advances in understanding of pathogenesis

Current knowledge on *C. jejuni* infectious process has revealed that the bacteria colonize the intestine via penetration of the intestinal mucus layer, invade intestinal epithelial cells leading to damages and ultimately disease manifestations. A number of mechanisms have been attributed to *C. jejuni* virulence including, cell adhesion molecules, cytolethal distending toxin (CDT), lipooligosaccharide (LOS), antimicrobial efflux pumps, capsular polysaccharide (CPS), invasion proteins, flagella, N- and O-glycosylation,^{31–34} and a Type VI secretion system that has been recently discovered in some *C. jejuni* strains.³⁵ Unfortunately, the major mechanisms of virulence and human disease are currently unclear due to the lack of cell and animal models that accurately convey human infection and disease. Several small animal models have been used including new-born piglet,³⁶ ferret,³⁷ genetically modified mice^{38–40} and even insects.⁴¹ Nevertheless, each of those models require large *C. jejuni* doses and do not recapitulate the symptoms of human campylobacteriosis. Perhaps overcoming prior limitations, Giallourou *et al.* induced bloody and inflammatory diarrhea following *C. jejuni* infection in antibiotic-treated mice fed a low zinc diet.⁴² This novel animal model of campylobacteriosis may further elucidate mechanisms of *C. jejuni* virulence.

During the infectious process, *C. jejuni* is actively evading the host immune defenses through the action of various cell surface structures, including N- and O-linked glycosylation, multiple CPS and LOS serotypes and structures between strains, and molecular mimicry.³⁴ Much of the current research on *C. jejuni* pathogenesis has focused on these cell structure mechanisms, modifications, and phase variations with the hope of developing vaccines that are unaffected by mechanisms of immune system escape.

The *C. jejuni* CPS highly contributes to immune system evasion. *C. jejuni* CPS, which was originally thought to be lipopolysaccharide (LPS), was first discovered upon identification of a putative *kps* locus of the first published *C. jejuni* genome sequence.⁴³ Importantly, CPS was found to be the key antigenic determinant in the Penner heat-stable (heat-stable, HS) serotyping scheme.⁴⁴ The Penner serotyping system was the most widespread method of *C. jejuni* strain typing and based on antiserum raised against heat-stable *C. jejuni* antigens.⁴⁵ To date, 47 Penner serotypes have been described. Considering that CPS is a key determinant of the Penner serotyping scheme,⁴⁴ CPS represented a key antigenic determinant and hence a good potential vaccine target. Further studies revealed a key role of CPS in pathogenesis. Specifically, CPS was shown to promote resistance to complement-mediated cell death by normal human serum.⁴⁶ A recent study also indicated that CPS plays an essential role in promoting systemic infection and abortion in sheep which was demonstrated to be partially due to CPS-mediated serum resistance.⁴⁷ Importantly, *C. jejuni* CPS mutants were shown to be significantly attenuated in a ferret model of diarrheal

disease and nearly completely attenuated in a *Galleria mellonella* model of *C. jejuni* disease.^{46,48} Additionally, mutants lacking CPS expression were shown to be defective in chicken colonization, the major reservoir for human infection, although infected chickens do not develop diarrheal disease or other major disease symptoms.⁴⁹ CPS expression and structure may also influence *C. jejuni* adhesion to and invasion of host epithelial cells, which are both believed to be key steps in the *C. jejuni* infectious process, although the results have been inconsistent between studies.^{46,48-51} This inconsistency between studies was most likely been due to differences in the *C. jejuni* strain, CPS serotype, and/or the cell line used for these experiments. Taken together, these results suggest a major role of CPS in *C. jejuni* pathogenesis and immune evasion.

Phase-variable modifications play a key role in the mechanisms of CPS pathogenesis and immune evasion. Phase variation in *C. jejuni* occurs via slipped-strand mispairing, where high frequency introduction/deletion of C/G residues can occur during replication at stretches of 8 or more C/G residues. This mechanism introduces an early stop codon resulting in a truncated nonfunctional product.⁴³ One major phase-variable modification involved in *C. jejuni* pathogenesis is the unique O-methylphosphoramidate (MeOPN) modification of CPS. An 81-176 mutant defective in MeOPN biosynthesis was as sensitive to normal human serum killing as a non-capsulated mutant,⁵² highlighting the major role of the MeOPN modification in immune system evasion, although an 81-176 mutant deficient in MeOPN expression on CPS showed no significant defect in chicken colonization after 6 days of infection, although the long-term survival was not tested.⁴⁸ This result suggests that the MeOPN modification may be important to survival within humans and possibly other hosts but not chickens, at least in the short term. Intriguingly, indirect evidence suggests that the phase-variable MeOPN modification in the HS2 serotype strain NCTC11168 may actually reduce normal human serum resistance,⁵¹ although more detailed studies using specific mutants in the MeOPN transferase genes are needed to confirm this observation. In addition to immune evasion, the MeOPN phase variation may also play a role in pathogenesis since 81-176 mutants deficient in MeOPN biosynthesis or attachment to CPS surprisingly displayed increased Caco-2 cell invasion but not adherence vs. wild-type 81-176.⁴⁸ Therefore, cycling of MeOPN on 81-176 may play a multifaceted role in *C. jejuni* pathogenesis. Importantly, both the galactose 4-OH MeOPN transferase gene (*cjj1420*) and the galactose 2,6-OH MeOPN transferase gene (*cjj1435*) of 81-176 contain poly(C) tracts that can undergo slipped-strand mispairing, resulting in phase variable gene expression during infection.^{48,53} This mechanism likely promotes optimal *C. jejuni* virulence and fitness during infection.

Another major cell surface modification is LOS, the major structural component of the *C. jejuni* outer membrane. *C. jejuni* LOS consists of an endotoxin, core oligosaccharide, and a single O-chain oligosaccharide structure rather than the repeating oligosaccharide moiety observed in LPS. Importantly, a number of *C. jejuni* LOS O-chain oligosaccharide structures have similarity to ganglioside oligosaccharide

structures found on human and animal cells.^{54,55,56} More recent studies also identified strains displaying structural determinants at the non-reducing end of the LOS oligosaccharide similar to those found on human glycoconjugates including P blood group antigens, Type I N-acetyllactosamine, and α 2-3-sialylated Type I N-acetyllactosamine.⁵⁷ This molecular mimicry may be useful for *C. jejuni* evasion of antibody recognition by displaying self-antigens; moreover, ganglioside-like LOS structures are also subject to phase-variable expression,⁵⁸⁻⁶⁰ which may promote antibody and T cell escape. *C. jejuni* strains expressing ganglioside-like LOS structures are strongly associated with the autoimmune disorder Guillain-Barré syndrome (GBS), which is believed to occur via antibody generation towards the ganglioside mimics that then damage host tissues and ultimately GBS symptoms.⁵⁴ It is possible that *C. jejuni* LOS structural mimics may contribute to other autoimmune disease sequelae associated with *C. jejuni* such as RA and IBD, but this remains to be studied. For these reasons, LOS-based or whole cell vaccines against *C. jejuni* do not appear practical or fruitful, as discussed in more detail in the following sections.

C. jejuni also harbors N- and O-glycosylation systems. While protein glycosylation in bacteria is being more widely recognized and appreciated, *C. jejuni* is quite unique in that it displays numerous classes of glycoconjugates and glycosylation systems including protein N-glycosylation, protein O-glycosylation, LOS, CPS, and peptidoglycan.⁵⁴ The protein N-glycosylation synthesizes and attaches a unique heptasaccharide unit to target proteins that is not further modified,⁶¹ except that the terminal GalpNAc residue is sometimes modified with a phosphoethanolamine (PETN) by the *eptC* gene product.^{62,63} The role of this N-glycan PETN modification is currently unclear since *eptC* also adds PETN to LOS and the flagellar rod protein FlgG.^{63,64} Expression of this N-linked heptasaccharide has been shown to promote resistance to cell death by host digestive protease enzymes such as trypsin,⁶⁵ slightly reduce IL-6 production by human dendritic cells,⁶⁶ and promote *C. jejuni* adhesion to and invasion of epithelial cells as well as colonization of the mouse intestine.^{67,68} In all these cases though, it is unclear if the N-glycan heptasaccharide directly mediates these effects or if N-glycosylation is merely required for full activity of N-glycosylated protein virulence factors such as Peb1 and JlpA. Taken together, the N-linked glycosylation system of *C. jejuni* may play an important role in *C. jejuni* host survival and infection.

Another major *C. jejuni* virulence factor is flagella. *C. jejuni* cells express bipolar flagella that promote cellular motility, and flagellar motility contributes to *C. jejuni* virulence.⁶⁹ Additional roles of the flagella in pathogenesis, including host cell adhesion and as a virulence factor secretion system, have been previously reviewed.⁷⁰ *C. jejuni* flagellin, the major protein of the flagellar filament, is also modified by O-glycosylation. In fact, the protein O-glycosylation system of *C. jejuni* seems to be limited to glycosylation of the flagellin protein subunits of the flagella. The flagellin O-glycan structures are monosaccharide units consisting of one of two rare sugars, pseudaminic acid (Pse5Ac7Ac) or legionaminic acid (Leg5Am7Ac), which

can be further modified with small functional groups including acetate, acetamidine, and N-acetylglutamine (reviewed in^{54,70,71}). These monosaccharide functional group modifications may be important in virulence as strains defective in O-glycan biosynthesis or modification showed significantly reduced adherence to and invasion of epithelial cells⁷² anti-inflammatory dendritic cell responses,⁷³ colonization of chickens,⁷⁴ and incidence of diarrheal disease in ferrets.⁷² The mechanisms by which O-glycosylation contributes to virulence though is unclear since, depending on the locations of the glycosylation sites, the O-linked glycans in strain 81-176 may be involved in either flagellar assembly and motility or autoagglutination, the latter of which may be important in *C. jejuni* biofilm formation.⁷⁵ Importantly, the flagellum is one of the major *Campylobacter* antigens,^{76,77} with the O-glycosylated region of flagellin presumably being the most antigenic region.⁷⁸⁻⁸² The variable monosaccharide structures and degree of O-glycosylation may thus assist in antibody and T cell escape. Additionally, *C. jejuni* flagellin is relatively unique in that it is not recognized by human or chicken Toll-Like Receptor 5 with or without O-glycosylation, even though Toll-like Receptor-5 is known to recognize flagellins from other bacteria to initiate and promote immune responses.^{83,84} Therefore, the antigenic *C. jejuni* flagellum utilizes multiple mechanisms to evade the immune system. However, considering the strong antigenicity towards the *C. jejuni* flagellum, development of a *Campylobacter*

flagellar subunit vaccine has been of considerable interest, as discussed in more detail below.

Historical vaccine development and lessons learned

Development of an effective vaccine for the prevention of campylobacteriosis has been ongoing for over 20 years (Figure 2). The process has been hindered by multiple factors including the lack of small animal of disease, presence of LOS ganglioside mimics that can induce auto-immune diseases such as GBS, the lack of understanding of *C. jejuni* virulence factors, and the lack of known immunological correlates of disease protection. Poultry meat is considered the major source of infection, and much attention has been made in the veterinary field to develop a vaccine that would successfully control *C. jejuni* colonization in broilers.^{85,86} This review is focused on vaccines against *Campylobacter* that were processed into phase 1 human clinical trial. There is a multitude of *C. jejuni* antigens that were tested in animal models but were not tested in or even intended for humans. For example, there is a tremendous effort to reduce colonization of chicken in broiler setting via vaccination. Neal-McKinney et al. previously reviewed the control of *Campylobacter jejuni* colonization of poultry via vaccination.⁸⁷ One of the most recent approaches was made by Szymanski et al., using the conserved N-glycan heptasaccharide conjugated to Tox protein. This conjugate vaccine provided an impressive reduction of *C. jejuni* colonization of the chicken gastrointestinal tract.⁸⁸

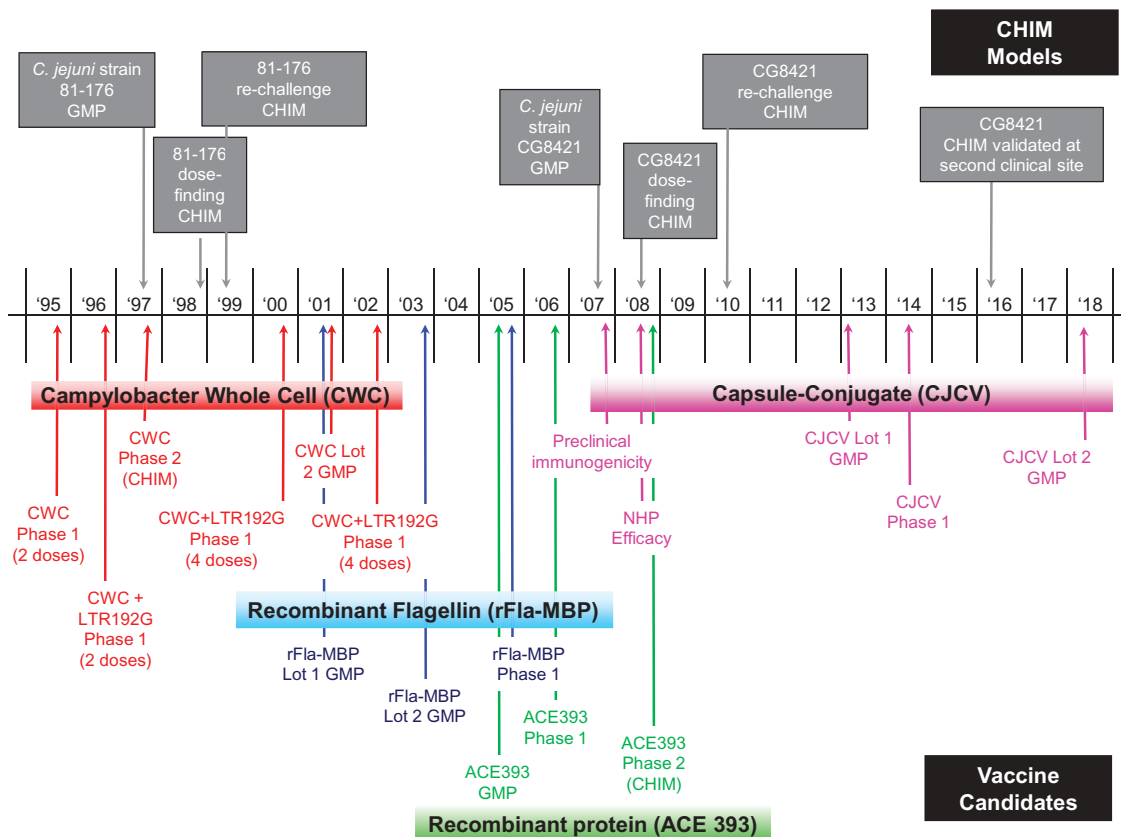


Figure 2. *Campylobacter* vaccine development history. Controlled Human Infectious Model (CHIM) Good Manufacturing Practice (GMP)

This promising vaccine candidate may thus be worth testing in humans to assess its potential against campylobacteriosis.

Killed whole cell vaccine

The first effort toward the development of a *C. jejuni* vaccine was started in the early 1990's using a killed whole cell approach. Inactivated whole cell bacteria is an attractive method of vaccination: They represent an economical process for large scale production and allows the inclusion of a myriad of antigens. Attenuated/killed whole cell vaccination has been successful for preventing disease induced by other enteric pathogens including *Salmonella typhi* and *Vibrio cholerae*. For *C. jejuni*, a killed whole cell vaccine represented an attractive approach that relieved costly and time-consuming fundamental research to understand mechanisms of protection as well as eliminated the need of the production of recombinant protective antigens.

The *C. jejuni* whole cell vaccine (CWC) was derived from the *C. jejuni* strain 81-176, isolated in 1981 from a child presenting acute diarrhea due to consumption of contaminated raw milk during a school field trip in Minnesota.⁸⁹ In pre-clinical studies using 0.2% formalin-killed 81-176 cells, robust prevention of *C. jejuni* colonization in mice was seen when delivered orally alongside the *E.coli* heat labile toxin (LT) as an adjuvant.⁹⁰ It was further demonstrated that the vaccination stimulated production of local and systemic IgA and IgG to *Campylobacter* antigens in rhesus monkeys that received the adjuvanted vaccine.⁹¹ These studies warranted the transition of the vaccine to safety and immunogenicity testing in a human phase 1 clinical trial.

The first human study of the CWC was performed using a vaccination regimen consisting of three vaccinations delivered 2 weeks apart at doses of 10^3 , 10^7 , or 10^9 cells, or 10^9 cells administered orally with 25 μg of LT(R192G) as adjuvant.⁹² The isogenic attenuated mutant of LT, LT (R192G), was used to reduce LT enterotoxicity but preserve adjuvanticity as demonstrated in animal studies. This first clinical evaluation demonstrated that the vaccine was safe in humans but failed to prevent campylobacteriosis during a homologous controlled human infection model (CHIM) with *C. jejuni* 81-176. The CWC approach thus needed further development.

In the meantime, a ferret animal model of disease was developed and demonstrated to mimic human campylobacteriosis symptoms.⁹³ Studies in the ferret model led to the modification of the dose, number of immunizations, and schedule for oral vaccination; specifically, the next phase 1 CWC human clinical trial used a 10^{10} cell dose with 25 μg of LT(R192G) adjuvant given orally 4 times, 2 days apart. The new vaccination regimen demonstrated to be superior by inducing a higher level of fecal IgA compared to the previous human vaccination, but the vaccine was unsuccessful to protect against disease in a Phase 2b CHIM when receiving the homologous strain.⁹² It has to be noted that the hefty infectious dose of 10^{10} CFU may have overwhelmed the host immune system the vaccine may have been more effective had a lower challenge dose been used. The CWC approach was abandoned in early 2000's and has not been further

refined. The major motive for no longer pursuing this CWC was that the strain *C. jejuni* 81-176 used in the vaccine was found to present sialylated LOS that resemble GM₂ and GM₃ human gangliosides,⁵⁹ which could potentially lead to the induction of GBS.

Subunit vaccines

Following the abandonment of the CWC approach, *C. jejuni* vaccinology efforts switched to the use of a recombinant *C. jejuni* antigens. Based on the findings of other successful enteric pathogen vaccines, the ideal antigen candidate should be surface exposed/accessible to the host immune system and able to elicit significant seroconversion of IgG as well as mucosal and serological IgA. In addition, the subunit vaccine should ultimately induce long-term immunological memory. In the early 2000's, the Naval Medical Research Center, Silver Spring, USA initiated the production of a recombinant *C. jejuni* flagellin vaccine.⁹⁴ Flagellin has well-documented proof of immunogenicity in various animal models and also in humans.^{94,95} Indeed, early CHIM studies demonstrated that volunteers challenged with *C. jejuni* 81-176 developed a robust immune response against the flagella.⁹⁴ In addition, the immunological level of response against the flagellin correlated with the protection against disease as demonstrated during re-challenge studies.⁹⁴ To thus generate a recombinant flagellin-based vaccine, the conserved region of FlaA from *C. coli* VC167 was fused with the maltose binding protein (MBP) of *E.coli*.⁹⁴ The vaccine (rFlaA-MBP) was tested for immunological response and protective efficacy in a nasal mouse model developed in house.⁹⁴ Encouraging results were made: intestinal secretory IgA response and protection against heterologous colonization and disease by *C. jejuni* 81-176 were established when the rFlaA-MBP was adjuvanted with LT (R192G).

The rFlaA-MBP vaccine was tested in a human clinical trial for safety and immunogenicity (ClinicalTrials.gov Identifier: NCT00124865). The vaccine was dispensed via intranasal delivery at one of four doses (25, 125, 625, or 1000 μg), with 3 total immunizations delivered at 2 week intervals. Interestingly, no adjuvant was administered, in contrast to the mouse model.⁹² The vaccine was deemed safe but only mildly immunogenic at the systemic and mucosal level. In part due to this disappointing result, the rFla-MBP *C. jejuni* vaccine was abandoned.

In the mid 2000's ACE BioSciences, now Zymenex, ventured into the development of a travelers' diarrhea vaccine.⁹⁶ This endeavor led to the development of a subunit vaccine, ACE393, a proprietary cell surface exposed *C. jejuni* protein. Following successful reduction of colonization in vaccinated mice, the vaccine was rapidly transitioned into a Phase 1 and extended Phase 1 for safety, immunogenicity and role in the prevention of disease (ClinicalTrials.gov Identifier: NCT00859716). The vaccine, consisting of 250 μg of recombinant protein and 500 μg Alhydrogel® (aluminum hydroxide adjuvant), was administered twice intra-muscularly 3 weeks apart. Volunteers were challenged with a newly established *C. jejuni* strains, CG8421, which lack sialylated LOS and by consequence unable to trigger auto immune sequelae

including GBS.⁹⁷ The vaccination regimen had low/no effect on prevention campylobacteriosis and has not been further pursued to this date.

Glycoconjugate vaccine

Protein-polysaccharide conjugate vaccines are superior to polysaccharide vaccines the protein carrier, provides the ability to induce T-cell responses towards the polysaccharide. Conjugated polysaccharide vaccines are immunogenic in young children⁹⁸ and induce long term immune protection compared to unconjugated polysaccharide vaccines.⁹⁹ Three licensed conjugated polysaccharide vaccines targeting encapsulated strains of the pathogens *Haemophilus influenzae* (type b), *Neisseria meningitidis*, and *Streptococcus pneumoniae* are available on the market. Based on the success of conjugated polysaccharide vaccines and the recent characterization of a polysaccharide capsule in *C. jejuni* described above, NMRC initiated a pilot vaccine study using the capsule of *C. jejuni* conjugated to CRM197 as protein carrier. The choice of CRM197 as protein carrier for the prototype vaccine was motivated for the reasons that CRM197, a non-toxic diphtheria toxin used in the pneumococcal conjugate vaccine, has been demonstrated to be safe, is commercially available, and is amenable to conjugation to oxidized polysaccharide.

For pre-clinical studies, the strain *C. jejuni* 81-176 was chosen. This strain harbors a serotype HS23/36 capsule that is composed of repeating units of the following core trisaccharide $[\rightarrow 3)\text{-}\beta\text{-D-GlcpNAc-(1}\rightarrow 3)\text{-}\alpha\text{-D-Galp-(1}\rightarrow 2)\text{-D-glycero-}\alpha\text{-D-althro-Hepp-(1}\rightarrow]_n$.^{100,101} As described above, the CPS structure also contains non-stoichiometric modifications including methyl phosphoramidate (MeOPN) occurring on the Galactose residue at the 2-OH, 4-OH, or 6-OH group.⁵³ Those MeOPN are believed to be immunodominant and required for vaccine efficacy. To prevent potential auto-immune reactions resulting from trace contamination with the 81-176 sialylated LOS structures, the 81-176 strain was genetically manipulated to prevent expression of ganglioside-like mimics.

The CPS was extracted and purified directly from the cell paste of *C. jejuni* 81-176. The glycoconjugate vaccine was then synthesized by CPS oxidation with sodium periodate to yield an aldehyde functional group at the non-reducing end, allowing the conjugation to the lysine residues of CRM197 via reductive amination.¹⁰²

Dose ranging and pre-clinical studies were performed in a mouse intranasal vaccination colonization model and validated via subcutaneous vaccination in a non-human primate (NHP) *Aotus nancymaae* model. NHP were vaccinated with 1, 5, or 25 μg of glycoconjugate with alum adjuvant administered in three doses of vaccine or PBS at 6-week intervals. The highest dose resulted in 100% protection against disease during homologous challenge in the NHP model 9 weeks post last vaccination.¹⁰² This encouraging result led to development of a cGMP grade vaccine that was tested for safety and immunogenicity in a phase 1 clinical trial that occurred in 2014 (ClinicalTrials.gov Identifier: NCT02067676). This study enrolled a total of 48 volunteers that received two intramuscular (IM) vaccinations, 4 weeks apart, of CJC1 of 2 μg , 5 μg ,

Table 1. Comparison of manufacturing parameters and vaccination regimen between *C. jejuni* CPS conjugate vaccine CJC1 and CJC2.

	CJC1	CJC2
Strain	81-176	81-176 o/o ^(a)
Growth medium	Animal-based	Non-Animal-based
Capsule extraction	Phenol	DOC ^(b)
Endotoxin removal	Long acetic acid treatment	Short acetic acid treatment
Oxidation	Sodium metaperiodate	Sodium metaperiodate
Conjugation	Sodium cyanoborohydride	Sodium cyanoborohydride
Protein Carrier	CRM197	CRM197
Adjuvant	Alum hydroxide	TBD
Number of dose	2 doses 4 weeks apart	TBD
Amount of CPS/dose	2, 5 and 10 mg	TBD

(a) *C. jejuni* 81-176 strain stably expressing both phase-variable MeOPN transferases.

(b) Sodium deoxycholate

or 10 μg with or without 125 μg Alhydrogel[®]. The vaccine was deemed safe but did not result in a significant serum immunological response (both IgA and IgG) against the CPS moiety. This disappointing result was retrospectively hypothesized to be due to the lack on the immunodominant MeOPN epitopes in CJC1 vaccine. Both the strain used and the manufacturing process can be incriminated. Unfortunately, the transferase enzymes responsible for the attachment of the MeOPN on the galactose residues are known to be affected by a mechanism of phase variation.⁵³

Based on this observation, a natural variant of *C. jejuni* 81-176 presenting both MeOPN transferases in the “phase on” configuration was selected and demonstrated to be stable during the cell production for CPS extraction. In addition, the extraction protocol was optimized and now utilizes a more gentle extraction procedure that minimizes MeOPN hydrolysis and loss. The current iteration of the *C. jejuni* CPS glycoconjugate vaccine, CJC2, is currently under manufacture and will be shortly tested in a Phase 1 clinical trial for safety and immunogenicity (Table 1).

Summary and looking towards the future

Momentum has been building in both the public and private sectors around research and development of new diarrheal disease interventions, including rotavirus, cholera, typhoid, ETEC, *Shigella*, and *Campylobacter* vaccines. Public funding for diarrheal diseases from high-income country governments and multinational organizations has increased substantially. Global agencies have made greater commitments to understanding diarrheal disease burden and the impact of specific pathogens. Opportunities to leverage private markets for the public good through implementation of tiered pricing schemes allow companies to achieve a return on investment in profitable markets (*i.e.* travelers and emerging economies), while providing products at lower cost in the developing world. While industry funding would likely be able to take a vaccine for the high-income country market, further development in low- and middle-income country markets would likely require funding and initiative from a range of sources, including vaccine-manufacturing partners in potential target markets, national governments, and global public health nonprofit organizations. The emerging epidemiology and understanding of campylobacter-attributable burden

(both acute and chronic) in both traveler and global populations, combined with promising development in vaccines, leads to the consideration of the possibility for a global vaccine against *C. jejuni*. Based on the epidemiological, immunological, and virulence studies reviewed here and described elsewhere, the targets and goals for such a *C. jejuni* vaccine are shown in Table 2.

A capsule-conjugate vaccine approach against *C. jejuni* is promising but several questions still need to be addressed. As mentioned previously, highly effective conjugate vaccines have been developed for other mucosal pathogens, one of which, *Streptococcus pneumoniae*, has more capsular types (~ 90) than *C. jejuni* (~ 35). There have been numerous prevalence studies of capsule serotypes in the developed world,^{77,95} but few studies from developing countries where the disease incidence is higher. Alternative vaccine approaches, such as recombinant protein subunits, have not yet demonstrated feasibility but are likely to advance with improved understanding of *Campylobacter* pathogenesis.

The CHIM for *C. jejuni* is likely to be utilized in future vaccine down/up-selection decisions and a brief review of its current status is warranted here. Briefly, the currently CHIM utilizes an HS23/36 strain, named CG8421, isolated from a US soldier in Thailand with acute febrile dysentery in 1999⁹⁷ The strain has been safely administered to 68 “naïve” subjects at 3 clinical sites at doses ranging from 2×10^4 to 1×10^8 colony forming units.^{103–105} In the most recent randomized, double-blind, placebo-controlled trial, the strain induced campylobacteriosis in 85.7% (24/28) of all subjects.¹⁰³ Importantly, microbial recrudescence has been observed with this strain, a phenomenon that may be specific to this model or may be reflective of natural *Campylobacter* infection.^{103,104,106,107} While the CG8421 appears to be sufficiently robust to assess current vaccine candidates, other vaccine strategies (or other capsule-types) may require the development of new *C. jejuni* CHIMs.

More research is needed on *Campylobacter*-host interactions and epidemiology to identify adequate coverage of a

capsule conjugate or protein subunit vaccine. The identification and characterization of a correlate of protection is important and could be informed indirectly through seroepidemiological studies. Favorable results from early phase clinical trials in adults from the high-income countries (e.g., travelers or other high-risk populations) would provide hope that such a vaccine might be effective in low- and middle-income country populations.

Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the authors.

Authors' contributions

All authors contributed to this manuscript equally.

Disclaimers

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Table 2. Target product profile of a capsule conjugated *Campylobacter* or other injectable vaccine for global use.

Variable	Traveler population to LMI Countries	Children up to 5 years of age in LMI Countries
Indication	Prevention of moderate to severe illness due to <i>Campylobacter jejuni</i> in travelers from high income countries	Prevention of moderate to severe diarrhea due to <i>Campylobacter jejuni</i> in children less than two years of age.
Target Population	Adults 18 – 65 years	Children up to 5 years of age.
Schedule and Route of Administration*	Optimal: 1 dose IM route. Acceptable: 2 dose IM route 28 days or less	Optimal: EPI schedule – 1 dose IM route. Acceptable: EPI schedule: 2 or 3 dose + booster IM route.
Safety	Safety and reactogenicity profile should be clinically acceptable.	Contraindications should be restricted to know hypersensitivity to any of the vaccine components
Efficacy*	70% efficacy against moderate to severe illness caused by all <i>C. jejuni</i> strains	Optimal: 70% efficacy against moderate to severe illness caused by all <i>C. jejuni</i> strains Acceptable: 50% efficacy against moderate to severe illness caused by <i>C. jejuni</i> strains in the vaccine
Duration of Protection	Optimal: lifetime Acceptable: 2 years	Optimal: To 5 years. Acceptable: 2 years, w/boosting possible to extend protection
Cost per regimen	\$75 – 100	\$1 – 3
Co-administration	With travel vaccines without interference	With EPI vaccines without interference
Vaccine volume	0.5 ml/dose	
Target Countries	US, Europe, Japan, Australia, New Zealand and other HDI countries	GAVI-eligible and LMIC
Onset of immunity	1–2 weeks after primary series	2 weeks after vaccine series
Indirect protection	No	Yes, ideally
Protection against chronic health sequelae	Yes (functional bowel disorders, reactive arthritis, GBS)	Yes (environmental enteropathy, GBS)

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