



Vaccine

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ABSTRACT

Campylobacter jejuni is one of the leading causes of bacterial diarrhea worldwide and is associated with a number of sequelae, including Guillain–Barre Syndrome, reactive arthritis, irritable bowel syndrome and growth stunting/malnutrition. Vaccine development against *C. jejuni* is complicated by its antigenic diversity, a lack of small animal models, and a poor understanding of the bacterium's pathogenesis. Vaccine approaches have been limited to recombinant proteins, none of which have advanced beyond Phase I testing. Genomic analyses have revealed the presence of a polysaccharide capsule on *C. jejuni*. Given the success of capsule-conjugate vaccines for other mucosal pathogens of global importance, efforts to evaluate this established approach for *C. jejuni* are also being pursued. A prototypical capsule-conjugate vaccine has demonstrated efficacy against diarrheal disease in non-human primates and is currently in Phase I testing. In addition to proof of concept studies, more data on the global prevalence of capsular types, and a better understanding of the acute and chronic consequences of *C. jejuni* are needed to inform investments for a globally relevant vaccine.

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1. Introduction

Campylobacter jejuni infection is the most common cause of bacterial gastroenteritis worldwide. The 2010 Global Burden of Disease study reported that *Campylobacter* accounts for the loss of 7.5 million disability-adjusted life years (DALYs), more than other globally prevalent enteric (*Shigella* (7.1 million DALYs), enterotoxigenic *Escherichia coli* (ETEC) (6.8 million DALYs) or non-enteric (*meningococcus* (5.2 million DALYs, dengue (0.8 million DALYs)) pathogens for which vaccines have already been or are being developed [1]. In high-income countries, *Campylobacter* is a significant cause of traveler's diarrhea and domestically associated food-borne infection [2–9]. The epidemiology of *Campylobacter* is different in low- and middle-income countries, where infection is considered almost universal in early childhood. Approximately 40–60% of children under the age of 5 will develop at least one

symptomatic infection, usually within the first year of life [10]. Furthermore, asymptomatic infections and prolonged carriage are common. Reliable studies defining disease burden in the developing world are challenging as the microaerophilic nature and fastidious growth requirements of this pathogen make culture diagnosis difficult, resulting in an underestimate of the incidence. The Global Enterics Multi-Center Study (GEMS), a prospective, multi-center, case-control study of acute moderate-severe diarrhea in children that used high-quality culture based methods of diagnosis found *Campylobacter* to be among the top 5 causes of acute diarrhea in 2 to 5-year-old children living in Bangladesh, Pakistan or India [11]. Other studies using culture independent (e.g., methods not relying on culturing the organism) and quantitative diagnostics have found increased burden of disease attribution to *Campylobacter* [12]. Most recently, pathogen attribution assessments using culture-independent methods from The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED Study) found *Campylobacter* spp. to be the third leading cause of diarrhea among infants 0–11 months old and the leading cause in children 12–24 months old. This study included all severity diarrheal disease among birth cohorts across 8 countries in the developing world [13]. The differential ranking of pathogens in the GEMS and MAL-ED studies are likely due to a combination of factors, including different study designs (cohort vs. case-control) and performance characteristics of diagnostics utilized (e.g., culture-based vs. culture independent methodologies).

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Campylobacter species are widely distributed commensal zoonotic organisms found in many types of livestock and domesticated animals. They are often associated with food-borne outbreaks, contaminated water and uncooked meats, particularly poultry. The infection typically presents, after a brief incubation period of 1–3 days, with acute watery diarrhea (sometimes bloody), abdominal pain and fever. In low resource settings, the clinical features may also include vomiting and signs of volume depletion. Susceptible patients in these settings are often underweight and/or malnourished. Volume resuscitation is the mainstay of therapy in all settings. Although antibiotics have demonstrated benefit during severe illness, they are generally not indicated and their use may actually contribute to the growing problem of antimicrobial resistance. Campylobacter infection is commonly self-limiting, but has been shown to be an independent risk factor for a growing list of chronic sequelae. The most severe is Guillain-Barré syndrome (GBS), an ascending paralysis of autoimmune origin considered to be the leading cause of acute flaccid paralysis worldwide. *C. jejuni* is the most frequent pathogen associated with GBS [14]. As disease burden studies have relied on culture-based diagnostics, they have likely underestimated the attribution of Campylobacter infection to GBS. In addition to GBS, a number of studies have identified other chronic health effects of Campylobacter infection, including but not limited to stunting, microbiome changes, functional bowel disorders and reactive arthropathies [15]. Most recently, a Peruvian birth cohort study found that both symptomatic and asymptomatic Campylobacter infections were associated with growth reductions over the 9 months following infection and that the magnitude of growth retardation was correlated with the severity of infection [16]. Despite these studies, there remains a need for more global research on the burden of campylobacteriosis, as called for in a joint publication by the WHO, the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) [17]. Large longitudinal cohort studies, like MAL-ED, have responded to this call and promise to provide better disease burden data (e.g., DALYs associated with acute and chronic consequences) in the coming years [18].

2. Biological feasibility for vaccine development

Currently, there are no vaccines approved by any global regulatory authority to prevent Campylobacter-associated illness. Biological feasibility of a Campylobacter vaccine is supported by data from epidemiologic and human challenge studies. The epidemiology of Campylobacter infections in the developing world is characterized by falling incidence of clinical disease with increasing age, particularly after 5 years of age when infections become mostly asymptomatic [19,20]. These observations suggest acquired protection to infection over time and repeated exposures. In addition, human challenge studies have demonstrated that previously infected subjects can be protected from disease following challenge with the homologous strain of bacteria [21,22]. Vaccine strategies against *C. jejuni* are limited by an incomplete understanding of its pathogenesis, protective epitopes, antigenic diversity, as well as its association with post-infectious syndromes such as reactive arthritis, IBS and GBS. Human challenge studies indicate that although infected subjects are subsequently protected from disease after challenge, the bacteria succeed in colonizing and inducing local and intestinal antibody responses [21,22]. It is not known what degree of infection or illness increases the risk of chronic sequelae or whether colonization alone is itself a risk, but it highlights important considerations regarding clinically relevant endpoints: prevention of disease, prevention of infection or prevention of colonization, or a combination thereof.

3. General approaches to vaccine development for low- and middle-income country markets

Most strains of *C. jejuni* produce lipo-oligosaccharides (LOS) that contain N-acetyl neuraminic acid moieties that are structural mimics of human gangliosides. Antibodies directed against these ‘molecular mimics’ can cross-react with human peripheral nerves, the pathogenic basis of GBS. Therefore, whole cell oral vaccines, which might seem logical for an enteric pathogen, may not be the best approach for *C. jejuni*. The concerns about GBS have generated increasing interest in alternative approaches that will avoid the use of bacterial molecules that mimic those of humans. Genomic research has revealed that *C. jejuni* expresses a polysaccharide capsule (CPS) [23] and has given rise to efforts to develop a CPS-conjugate vaccine, similar to those that have been licensed for other encapsulated, mucosal pathogens. Although molecular details are lacking, invasion of intestinal epithelial cells is an important early step in pathogenesis, and the polysaccharide CPS appears to be critical for this process [24], supporting the hypothesis that anti-capsular antibodies will confer protection against *C. jejuni* disease [25]. Furthermore, there is no evidence that any CPS structures feature ganglioside mimics, alleviating concerns of GBS and distinguishing it from approaches containing the LOS core structures. Conjugate vaccines have been widely used for more than 25 years and have been safe and highly successful in preventing disease caused by a variety of encapsulated namely type B *Hemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae*. A prototype, monovalent conjugate vaccine against *C. jejuni* has provided 100% protection against diarrheal disease in non-human primates [25]. However, a similar challenge for Campylobacter vaccine development using this approach will be developing a safe, immunogenic formulation that covers the most relevant capsule types for the target population.

Beyond a CPS-based approach, recombinant protein subunit antigens are also being explored. A recombinant form of an ABC transporter, PEB1 has recently been shown in pre-clinical studies to be immunogenic and protective in a non-diarrheal disease mouse model [26]. Campylobacter flagellin had also been studied as a potentially effective immunogen, but it is heavily glycosylated and the surface exposed glycosylations vary among strains. Moreover, a recombinant non-glycosylated form of *C. jejuni* flagellin was poorly immunogenic in Phase I trials. A better understanding of pathogenesis and virulence factors is first needed to advance subunit protein products. While more high-quality data are needed, it is clear that *C. jejuni* is widely distributed and causes significant global morbidity and mortality. Given the past performance of CPS conjugate vaccines, it is conceivable that a highly immunogenic and safe vaccine could be manufactured for use in all relevant markets. An effective *C. jejuni* vaccine would benefit children living in developing countries, but would also appeal to travelers. As previously stated, efforts are needed to understand CPS type valency distribution in the developing world, and the attributable burden of acute and chronic consequences of infection through better epidemiological studies. With such data, a convincing case for a *C. jejuni* vaccine in these target populations could be made. Furthermore, with the potential of this vaccine having broader appeal to the developed world (both in travelers and domestic settings), sufficient markets may allow for tiered pricing as has been seen with other licensed vaccines (e.g., rotavirus vaccines).

4. Technical and regulatory assessment

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

Table 1
Development status of current vaccine candidates.

Candidate name/identifier	Preclinical	Phase I	Phase II	POC	Phase III
<i>Campylobacter jejuni</i> capsule conjugate (US DoD)		X			
PEB1 DNA prime/protein boost (China)	X				

POC = Proof-of-concept trial.

developed for other mucosal pathogens, one of which, *S. pneumoniae*, has more capsular types (~90) than *C. jejuni* (~35) [27]. There have been numerous prevalence studies of capsule serotypes in the developed world, but few studies from developing countries where the disease incidence is higher (19). The complexity and cost of classical serotyping has limited its usefulness, but a recently developed multiplex PCR assay that determines capsule type offers the potential of a more rapid and affordable diagnostic [27]. Comparative studies have shown a strong correlation between the two methods and are beginning to ascertain capsule-type distribution worldwide [28] (and Poly et al). Alternative vaccine approaches, such as recombinant protein subunits, have not yet demonstrated feasibility but are likely to advance with improved understanding of campylobacter pathogenesis. Relevant non-human primate (NHP) disease and human challenge models will facilitate early evaluation of vaccine candidates and lower risk during clinical development.

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. Using the capacity built for rotavirus vaccine testing, there are sufficient field sites, experience, and regulatory pathways to take a *C. jejuni* vaccine through safety studies and pivotal trials in low resource settings. While standardized and accepted endpoints exist for rotavirus-associated illness, acute gastroenteritis endpoints for illness associated with *Campylobacter* in the low resource settings are needed. The incidence of *C. jejuni* disease is high enough to support a trial with a feasible sample size. However, the formulation of a *C. jejuni* vaccine needs to be considered carefully to ensure acceptability and affordability in developing countries. In addition, the Expanded Programme on Immunization vaccine schedule is already crowded, thus, integration of another vaccine into the schedule will be complex.

5. Status of vaccine R&D activities

C. jejuni vaccine candidates that have been or are in clinical development include killed whole cell, protein subunit and capsule-conjugate products. A recombinant protein vaccine (ACE 393) was tested in a Phase II human challenge study but failed to demonstrate protection. Killed whole-cell and recombinant flagellin subunit vaccine constructs have also been advanced to human clinical trials but, again, both failed to demonstrate protection in a Phase II human challenge trial. Currently, the capsule-conjugate vaccine is the leading vaccine under development, though a DNA vaccine approach is in preclinical development (Table 1). A prototypical monovalent CPS conjugate vaccine using CRM197 as the protein carrier has been evaluated in a number of preclinical studies

and found to be highly immunogenic in mice, with significantly elevated anti-capsular IgG titers that persisted for >26 weeks (Guerry, unpublished). Since mice do not develop diarrhea when orally challenged with *C. jejuni*, this vaccine candidate has been evaluated for efficacy against diarrheal disease in an NHP model (*Aotus nancymae*, a new world owl monkey species). In this model, 2.5 µg of conjugated polysaccharide was given in three doses at 6-week intervals by subcutaneous injection and provided 100% protection from disease after infection with an orogastric challenge of the homologous strain nine weeks after the last vaccine dose [29]. A Phase I trial of this product is currently underway (clinicaltrials.gov, NCT02067676). There is a human challenge model with a *C. jejuni* strain that naturally lacks ganglioside mimicry in its LOS, which can be used in proof of concept clinical trials [30]. Important to note, while not currently being pursued as a vaccine candidate, *in vitro* and pre-clinical studies suggest that antibodies elicited by cholera toxin, but not enterotoxigenic *E. coli* heat-labile toxin, cross-react with a single epitope of the major outer membrane protein of *Campylobacter* and, thus, may afford some level of cross protection [31–34]. Lastly, several vaccine candidates are also in development for the poultry industry, but are outside the scope of this review and hence are not discussed.

6. Likelihood for financing

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 and Shigella 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*. The GAVI Alliance has indicated an interest in enteric vaccines. A combination vaccine with one of the other major enteric pathogens (e.g., shigella conjugate or typhoid conjugate) could enhance feasibility. Studies are also ongoing to evaluate conjugation of *C. jejuni* capsular polysaccharides with subunit proteins from ETEC (Guerry et al., unpublished). A *Campylobacter* vaccine would likely have a dual market (i.e., both developed and developing countries), providing scale-up advantages for commercial development and global distribution.

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Conflict of Interest

Neither of the authors have any relevant conflict of interest to disclose.

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