Status of Vaccine Research and Development for Campylobacter
Prepared for WHO PD-VAC

I. About the Disease and Pathogen

Basic information on pathogen, including transmission, estimated global disease burden for those at risk, for morbidity and for mortality, including uncertainties/data gaps, geographical distribution, economic burden if available, age groups affected and target groups for vaccination. Existing preventive, diagnostic and treatment measures and their limitations.

*Campylobacter jejuni* infection is considered to be the most common cause of bacterial gastroenteritis world-wide and a major global health problem. The 2010 Global Burden of Disease study reported 7.5 million disability-adjusted life years attributed to Campylobacter compared to Shigella (7.1 million) and ETEC (6.8 million) (1). In developed countries, Campylobacter is a significant cause of travellers’ diarrhea, after ETEC. The epidemiology of Campylobacter is different in the developing world, where the infection is considered almost universal in early childhood, with estimates that 40-60% of children under the age 5 will develop at least one symptomatic infection, usually occurring during the first year of life (2). Furthermore, asymptomatic infections and prolonged carriage are common. Reliable studies defining the burden of disease in the developing world are challenged by the microaerophilic nature and fastidious growth requirements of this pathogen, and often lead to an underestimate of the incidence. However, high quality studies are starting to emerge and highlight the further importance of *C. jejuni*. In the Global Enterics Multi-Center Study (GEMS), a prospective, multi-center, case-control study of acute moderate-severe diarrhea in children, using culture based techniques; Campylobacter was found to be in the top 5 causes in Bangladesh, Pakistan and India among 2-5 year olds (3). Other additional studies using culture independent techniques and quantitative diagnostics have supported increased burden of disease attribution to Campylobacter (4).

Campylobacter species are widely distributed globally and are commensals of many food animals as well as domesticated cats and dogs. Considered a leading zoonosis, it is often associated with food-borne outbreaks with contaminated water and uncooked meats, particularly poultry. The infection presents after a brief incubation period of 1-3 days with acute watery diarrhea (sometimes bloody), abdominal pain and fever. In developing world settings, disease features most commonly reported are watery stool, fever, abdominal pain, vomiting, dehydration, and presence of fecal leukocytes; patients are also often underweight and malnourished which may be secondary to repeated infections or a marker of a poor nutrition state. Rehydration is the mainstay of therapy, and although antibiotics have been shown to be beneficial in severe illness, they are not generally indicated and resistance acquisition to common antibiotics is an increasing problem.

The infection is commonly self-limiting, but persistent disease can occur and in addition, a number of chronic sequelae have been identified. Equally concerning in both the developing and developed world settings is Campylobacter’s association with life-threatening cases of Guillain–Barre’ syndrome (GBS). GBS, an ascending paralysis of autoimmune origin, is considered the leading cause of acute flaccid paralysis worldwide (given the success of polio eradication), and *C. jejuni* is the most frequent pathogen associated with GBS (5). Though as with acute detection, these studies have relied primarily on culture-based techniques and likely underestimate accurate attribution of GBS triggered by campylobacter infection. Aside from GBS, the chronic health effects of campylobacter infection in the developing world are emerging. Studies have identified stunting, microbiome changes, functional bowel disorders, reactive arthropathies and other chronic health disorders in developed world populations (4,7,8). The known and potential burden of campylobacteriosis globally is an area where more research is needed, a fact recently highlighted in a WHO joint publication in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) (6). Studies such as the
II. Overview of Current Efforts

A. Biological feasibility for vaccine development

Evidence that vaccine development is biologically feasible including from development of naturally acquired immunity, from vaccine development for related pathogens, from animal models or in vitro data

Currently, there are no vaccines approved by any global regulatory authority to prevent Campylobacter-associated illness. Biological feasibility of vaccine development is supported by data from epidemiologic and human challenge studies. The epidemiology of Campylobacter infections in the developing world is characterized by de-escalating rates of clinical disease with increasing age, and after where infections are more likely to be asymptomatic after the age of 5 years (9). This, along with the association of decreasing rates of infection with rising titers of Campylobacter-specific antibodies, supports the development of some form of immunity to Campylobacter infections (10). In addition, human challenge studies have demonstrated that subjects who were infected were protected from disease following challenge with the homologous strain of the bacteria (11).

Vaccine strategies against C. jejuni are limited by an incomplete understanding of its pathogenesis, development of immunity and associated protective epitopes, and the association with post-infectious syndromes of reactive arthritis and GBS. The bacteria also has tremendous antigenic diversity, with current serotyping schemes identifying a large number of serotypes; although there is evidence that a limited number of serotypes dominate the majority of infections in different regions of the world. Clearly the association of the agent with reactive or post-infectious arthritis and particularly with GBS, poses a challenge for vaccine developers. The human challenge studies indicate that although infected subjects can be subsequently protected from disease after challenge, the bacteria does succeed in colonizing and inducing local and intestinal antibody responses. It is not known what degree of infection or illness increases the risk of GBS, or whether colonization alone is itself a risk, but it highlights important considerations regarding what will be the clinically beneficial target endpoint for the vaccine – prevention of disease, prevention of infection or prevention of colonization – or combination thereof.

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

What are the scientific approaches and indications and target/age/geographic groups being pursued? What public health needs will these vaccines meet if successfully developed? Where are there several different possible indications/target groups, how much consensus is there as to prioritization between these for vaccine development in LMIC.

Most strains of C. jejuni produce lipooligosaccharide (LOS), which can be decorated with sialic acid (Neu5Ac) 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 although whole cell approaches using the oral approach seems logical for an enteric pathogen, this well-documented association between C. jejuni and GBS weighs heavily against whole cell Campylobacter vaccine strategies, and has fostered increasing interest in alternative approaches that can be hopefully tailored to avoid the potentially troubling bacterial structures. The genomics-derived discovery in 2000 that C. jejuni expresses a polysaccharide capsule (CPS), which is highly unusual for an enteric pathogen, has given rise to an intense effort to develop a CPS-conjugate vaccine, similar to those that have been licensed for other encapsulated, mucosal pathogens (9). Although molecular details are lacking, invasion of intestinal epithelial cells is a critical early step in pathogenesis, and the polysaccharide CPS appears to be critical for this process, supporting the hypothesis that anti-capsular antibodies will confer protection against C. jejuni disease (12). 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 over 25 years and have been safe and highly successful in preventing disease caused by bacteria that have in common a polysaccharide capsule, namely type B *H. influenzae*, *N. meningitidis* and *S. pneumoniae*. A similar challenge for Campylobacter vaccine development using this approach will be developing an immunogenic, efficacious formulation for the target population that covers the most relevant serotypes. Beyond a CPS-based approach, recombinant subunit protein antigens are also being explored. Amino acid ABC transporter, permease protein PEB1, has recently been described in pre-clinical studies to demonstrate good immunogenicity and protection in a non-disease mouse model (13). Campylobacter flagellin had been a target of interest as studies have indicated that it may play a role in protection. However, *C. jejuni* flagellin is heavily glycosylated and the surface exposed glycosylations vary among strains, making it a poor choice for a vaccine. Moreover, a recombinant form of *C. jejuni* flagellin was poorly immunogenic in phase 1 trials. A further understanding of pathogenesis and identification of virulence targets is likely needed to advance the subunit approaches.

While more high-quality data are needed, it is clear that *C. jejuni* has a global distribution that causes significant morbidity and mortality. Given past performances 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 have appeal for the traveller market. 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 vaccines on the market (e.g. rotavirus vaccines).

### III. Technical and Regulatory Assessment

*Highlight perceived positive/negative aspects in clinical/regulatory pathways e.g. well established product development and regulatory pathway to licensure, accepted immune correlates and/or functional assays, accepted surrogate efficacy endpoints, existence of well accepted animal or challenge models, agreed trial designs and endpoints. Possibilities to develop case for correlates/surrogates should be included.*

The feasibility of a capsule conjugate vaccine approach against this pathogen is intriguing, but several questions 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*. There have been numerous studies of prevalence of capsule serotypes in the developed world, but few studies from developing countries where the disease incidence is higher. The complexity and cost of classical serotyping has limited its usefulness, and a recently developed multiplex PCR method for determination of capsule type offers the potential of a more rapid and affordable method. Comparative studies have shown a strong correlation of the two methods and studies are beginning to ascertain capsule-type distribution worldwide (14).

Alternative vaccine approaches (recombinant protein sub-unit) have not yet demonstrated feasibility but are possible if there are advancements in our understanding of campylobacter pathogenesis. A relevant non-human primate (NHP) disease model and human challenge model should facilitate early evaluation of vaccine constructs and lower risk for full development.

More research is needed on campylobacter-host associations and epidemiology to identify adequate coverage of a capsule conjugate or sub-unit protein vaccine approach. Further defining a correlate of protection that is also present in a developed-world setting is important and could be supported indirectly.
by seroepidemiological approaches. Favorable results from early phase clinical trials in adults from the developed world (e.g., travelers or other high-risk populations), would provide hope that such a vaccine approach might be effective in low- and middle-income country populations.

Using the example of rotavirus vaccines, there are sufficient field sites, experience, and regulatory pathways to take a C. jejuni vaccine through safety studies and pivotal trials in the developing world. Acute gastroenteritis endpoints are well defined and accepted in this population. The incidence of C. jejuni disease is high enough to support such a trial with reasonable numbers.

Formulation of a C. jejuni vaccine needs to be considered to achieve acceptability (and low cost) in developing countries. In addition, the Expanded Programme on Immunization vaccine landscape is already quite crowded, and appropriate integration of another vaccine into the schedule would need to be considered.

IV. Status of Vaccine R&D Activities
Summarize status of vaccine design, pre-clinical and clinical trial activity, including platforms, vectors, and adjuvants. Note academic, government, biotech and industry entities engaged. Summarize antigenic targets (if subunit approaches). Section on major advances in last 3-5 years, including key opportunities highlighted by recent science developments in the area.

C. jejuni vaccines including killed whole cell, sub-unit protein, and most recently capsule-conjugate have been developed and advanced into clinical trials. A recombinant protein vaccine (ACE 393), reached a phase 2 human challenge study where it failed to demonstrate protection. A killed whole-cell also failed in a phase 2 human challenge, and a recombinant flagellin subunit vaccine completed phase 1, but failed to advance to phase 2.

Currently, the capsule-conjugate vaccine is the leading vaccine under development, though a DNA vaccine approach is in preclinical development (Table 1). A prototype 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, and resulted in significantly elevated anti-capsular IgG titers that persisted for >26 weeks. Since mice do not develop diarrhea when orally challenged with C. jejuni, the prototype vaccine has been evaluated for efficacy against diarrheal disease in a NHP model of disease in Aotus nancymaae, a new world owl monkey species. In this model, 2.5 micrograms of conjugated polysaccharide given in three doses at 6-week intervals by subcutaneous injection, provided 100% efficacy against disease upon orogastric challenge with C. jejuni strain 81-176 nine weeks after the last vaccine dose.

A phase 1 first in human trial is currently underway (clinicaltrials.gov, NCT02067676). There is a human challenge model of C. jejuni diarrheal disease that utilizes a strain that naturally lack ganglioside mimicry in its LOS which can be used in phase two studies assessing proof of concept for efficacy in humans.

Several vaccine candidates are in development to be used in the poultry industry and are not considered here.

Table 1: Development Status of Current Vaccine Candidates (POC = Proof-of-concept trial)

<table>
<thead>
<tr>
<th>Candidate Name/Identifier</th>
<th>Preclinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>POC</th>
<th>Phase III</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. jejuni Capsule Conjugate (US Department of Defense)</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEB1 DNA Prime/Protein boost (China)</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
References

4. Platts-Mills JA, Kosek M. Update on the burden of Campylobacter in developing countries