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.

Enterotoxigenic Escherichia coli (ETEC) remains among the most common bacterial causes of diarrhea-associated morbidity and mortality (1,2). ETEC is often the first bacterial illness that children experience in endemic areas, with infants and young children experiencing two to five diarrhea episodes due to ETEC during their first three years of life. Recent studies in sub-Saharan Africa and South Asia conducted under the Global Enteric Multicenter Study (GEMS) have reaffirmed the continuing importance of ETEC as one of the top four causes of moderate-to-severe diarrhea (MSD) among children less than five years of age in both regions (3). GEMS data also indicate that ETEC-attributable MSD is associated with a significant increase in the risk of death and stunting.

In 2010, annual mortality from illness due to ETEC was estimated at 157,000 deaths (9 percent of all deaths attributed to diarrhea) and approximately 1 percent of all deaths in children 28 days to 5 years of age. A meta-analysis of hospitalization and stool culture data project that ETEC may contribute to an additional 89,000 deaths per year among age-groups older than five years in Africa and South Asia (4). In addition to mortality estimates, 2010 ETEC Disability-Adjusted Life Years (DALYs) were estimated at 8.5 million (10 percent of all diarrhea DALYs), and Years Lived with Disability (YLDs) were estimated at one million (13 percent of all diarrhea YLDs) (5,6). The analysis also estimated that, in 2010, ETEC was much more common in these age-groups than cholera and typhoid combined, with 44 million cases of ETEC versus 9 million typhoid (approximately 6 million) and cholera cases (approximately 3 million), with school-age children being at the highest risk for illness (4). Moreover, another meta-analysis describing the etiology of diarrhea among individuals in older age groups suggests that ETEC may be associated with 10 to 14 percent of hospitalized cases and 6 percent of all diarrheal illness in outpatient/community settings.

In addition, ETEC is also the most frequent bacterial cause of diarrhea among travelers to Africa, Asia, and Latin America, including military personnel deployed to these areas. ETEC is estimated to cause approximately 10 million episodes of travelers’ diarrhea each year (1,2). Recent data also strongly suggest that ETEC infections in travelers can increase the risk of subsequent functional bowel disorders. In fact, 10 to 14 percent of travelers convalescing from ETEC-associated travelers’ diarrhea may go on to develop irritable bowel syndrome, thus further highlighting the importance of disease prevention and the potential benefit of having effective vaccines (2).

In the classical paradigm for ETEC pathogenesis, these bacteria must first colonize the small intestine where they employ plasmid-encoded fimbrial colonization factors (CFs) or coli surface antigens (CS) to bind enterocytes in the upper small intestine (1,2). Here they produce heat-stable (ST) and/or heat-labile (LT) enterotoxins, and their close association with enterocytes via their CF/CS antigens promotes transfer of ETEC enterotoxins that stimulate the release of fluid and electrolytes from the intestinal epithelium, resulting in watery diarrheal illness (1,2). Different strains of ETEC express antigenically distinct types of fimbriae, and immune responses against these fimbriae are thought to be protective through inhibition of binding of the bacteria to the intestinal epithelium. Immune responses to the different fimbrial types do not appear to be cross-protective. These plasmid-encoded traits are considered to be the key virulence factors, and have therefore been intensively studied over the last three decades. While more than 25
unique CF/CS types and putative factors have been characterized so far, in only about 50 percent of ETEC clinical isolates have any of these known CFs been identified. Therefore, standard bacterial culture methods and supportive laboratory-based secondary assays used in clinical microbiology laboratories around the world likely underestimate the true incidence of ETEC. This has stimulated renewed interest in exploring more novel adhesins and other conserved ETEC proteins contributing to virulence that may have gone undetected using standard molecular biology and immunological approaches (2,7).

ETEC is transmitted by the fecal-oral route associated with the consumption of contaminated water or food. Most infections are self-limiting in healthy individuals and require no specific treatment. Dehydration and malnutrition can occur in young children and developing country settings. Although antibiotics can in some cases limit the duration of illness and discomfort, they are not indicated and like most enteric pathogens, multi-drug resistance is becoming more common (1,2). Since it is an enterotoxin non-invasive disease of the small intestine, rehydration therapy to prevent dehydration is the mainstay of treatment. However, given growing concerns about longer-term effects of ETEC illness, prevention remains an important area to address.

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

There are no licensed vaccines for ETEC. However, field studies and human challenge studies indicate that protective immunity to ETEC develops after natural or experimental infection, which means that vaccine development should be feasible. In addition, in ETEC-endemic areas, age-specific attack rates for symptomatic ETEC infection decline after three years of age (1,2). And, in human challenge studies, subjects who recovered from ETEC diarrhea were protected against disease when challenged a second time with the same strain. Finally, active immunization with candidate vaccines has led to protective immunity in limited challenge studies and field efficacy trials.

B. General approaches to vaccine development for low- and middle-income 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 there are several different possible indications/target groups, how much consensus is there as to prioritization between these for vaccine development in LMIC.

ETEC vaccine development efforts have focused on the induction of anti-toxin and anti-colonization immunity, including using whole cell inactivated or live attenuated, purified antigens, subunit and peptide approaches. Specifically, studies in animals and human subjects indicate that LT and CF/CS antigens contribute to protection against ETEC and have potential for use in a vaccine. LT toxin is structurally, functionally, and immunologically related to cholera toxin (CT); in particular, the LTB and CTB subunits are closely related. Whereas LT is strongly immunogenic, ST is not immunogenic unless coupled to a carrier protein; to date, no safe ST toxoid is available for use in humans. Some CFs are more prevalent than others, e.g., CFA/I, CS3, CS5, and CS6, accounting for 50 to 80 percent of all CF-positive clinical ETEC isolates. In addition, some CF/CS antigens are immunologically related to these more prevalent CFs. Clinical trials both in travelers and in endemic populations have also shown that CTB may provide significant protection against LT/ETEC-associated disease. However, it has been suggested that an LT-like toxoid may provide stronger protection against LT/ETEC, at least in young, immunologically naïve children in comparison to CTB. Active immunization studies in human volunteers followed by experimental challenge with wild-type ETEC strains have also shown that CF antigen alone or in combination with LTB can provide protection. In addition, the application of new “Omics” technologies
has identified a number of novel proteins that also may contribute to toxin delivery or colonization and thus, may also have vaccine potential, including flagelin, EtpA, EatA, EaeH, and YghJ (7).

Since ETEC infections are confined to the mucosal surfaces in the gut, immune protection is most likely provided by locally produced secretory IgA antibodies against major protective antigens. Hence, when assessing the relative immunogenicity of vaccine candidates, it is important to evaluate antigen-specific immune responses induced at the intestinal mucosa or by measuring intestinally derived antibody responses. Data from human subjects and animals indicate that oral and parenteral ETEC vaccine candidates may benefit from adding the new double-mutant LT (dmLT) adjuvant to these vaccine formulations (2). The dmLT is unique in the ETEC vaccine development context in that it can function as both an antigen and adjuvant in vaccine preparations, potentially facilitating vaccine dose-sparing and improving efficacy (2).

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 availability of an established ETEC challenge model provides an opportunity to assess how anti-CF/CS responses and responses to LT toxin may contribute to protection (2,7). However, there are no well-established correlates of protection or functional assays for predicting ETEC vaccine efficacy (1,2,7). The association between these responses and reduced risk of illness has not been clear in all studies, and this variability suggests that the responses may not have been measured in the best context. The functional aspects of antibodies against these antigens might be a better or more consistent marker for protection, and other antigens that are currently are not being measured may also contribute to protection. Therefore, the development of better functional assays for assessing ETEC immunity is an important technology gap that needs to be addressed.

High throughput assays are also needed to more easily address toxin-neutralizing antibody responses for both LT and ST, in addition to better HAI assays for measuring functional serum or fecal antibody responses to CFs in order to better understand how a vaccine induces responses that block intestinal adherence and colonization. Similarly, a bactericidal assay, much like the one currently in use to measure vibriocidal antibody responses, may also be a valuable step forward for ETEC vaccines. Finally, it should be noted that the field has yet to integrate a significant systems biology component into protection studies or to fully apply “Omics” technologies to further facilitate ETEC vaccine antigen discovery and defining immune profiling, which may be better predictors of protection. These new technologies represent important new tools that could help further accelerate ETEC vaccines toward licensure.

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.

Table 1 provides a summary of the development status of current ETEC vaccine candidates. As indicated above, most ETEC vaccine candidates currently under development using cellular or subunit based vaccine approaches have focused on the induction of anti-LT and anti-CF/CS antibodies at mucosal and systemic sites.

The leading cellular vaccine candidates include inactivated and live attenuated approaches, ETVAX and
ACE527, respectively, both of which are being developed by PATH (2). PATH is working in partnership with manufacturing partners for each vaccine: Scandinavian BioPharma (SBH) for ETVAX and China National Biotec Group (CNBG) for ACE527. Both candidates have been found to be safe and immunogenic in Phase 1/2 trials. In addition, both were given with the dmLT adjuvant, which may help improve immunogenicity and protective efficacy, even when given at lower doses than the vaccine alone. Of the two cellular vaccines, the ETVAX/dmLT candidate is furthest along, with a descending-age safety and immunogenicity study projected to begin at ICDDR, B in Bangladesh in late 2014. The ACE527/dmLT candidate demonstrated significant protection (PE) in a Phase 2b challenge study (PE of 58.5 percent against ETEC diarrhea of any severity), but it needs further process development work to allow for co-formulation of its three vaccine strains plus dmLT before studies can begin at a developing-country site. Progress has also been made by the Center for Vaccine Development (CVD) at the University of Maryland, Baltimore toward achieving stable attenuated Shigella-ETEC antigen hybrids that could become a combined vaccine, since constructs would express both Shigella-specific LPS serotype “O” antigens, as well as CF/CS antigens and LTB (constructed in GuaBA Shigella mutants) (1,2). In a similar vein, Prokarium has a combined ETEC-typhoid vaccine candidate in preclinical development (Typhetec) that utilizes an attenuated typhoid vaccine train to vector an LT-ST fusion protein.

Other ETEC vaccine candidates based on subunits, toxins, or novel antigens are also under development. An innovative, new subunit ETEC candidate, using the tip proteins from fimbriae and administered intradermally with an LT-based adjuvant (mLT), induced strong immune responses at systemic and mucosal sites (2). The US Naval Medical Research Center (NMRC) is currently evaluating the efficacy of this approach in a Phase 2b immunization and challenge study. Using a more classic approach, Kansas State University (KSU) and Johns Hopkins Bloomberg School of Public Health (JHBSPH) have developed a multicomponent fusion protein that will deliver the most common CFs as well as an LT-ST hybrid toxoid (2). In addition, substantial progress has been made recently in the identification and testing of mutant ST toxoids that could be added to cellular or subunit vaccine approaches to improved coverage and efficacy. This work is being carried out by the EntVac Consortium, which is composed of investigators from University of Bergen, CVD, Tulane University, Virginia University, Kansas State University, and South Dakota State University. PATH, GLOBVAC, and STOPENTERICS are also supporting this effort. Novel toxoids and application of new “Omics” technologies and other gene-based approaches also offer great promise for yielding new vaccine antigens that may provide broader protection against ETEC, as well as to facilitate combined vaccine approaches (2,7). However, these have yet to move beyond preclinical animal studies.

Table 1: Development Status of Current ETEC 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><strong>Cellular candidates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactivated tetravalent whole cell supplemented with LTB-CTB hybrid toxoid; may include dmLT adjuvant (ETVAX) [PATH; SBH]</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>aroC, omp F, and Omp C-based live attenuated; may include dmLT adjuvant (ACE527) [PATH; CNBG]</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>ZH9 attenuated typhoid vaccine expressing LT-ST toxoid (Typhetec) [Prokarium]</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Subunit candidates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-adhesin based subunit vaccine [NMRC; PATH]</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-adhesin-toxoid fusion (MEFA) [KSU; JHBSPH]</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti-toxin candidates</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>dmLT [PATH]</td>
<td>X</td>
</tr>
<tr>
<td>LT-ST fusion/LTB-ST conjugate [EntVac Consortium; GLOBVAC; STOPENTERICS; PATH]</td>
<td>X</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Novel antigen candidates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flagellin; EpA; EatA; EaeH; YghJ</td>
</tr>
</tbody>
</table>

Novel antigens are being explored by a number of investigators from: Washington University in St. Louis; University of Maryland; University of Virginia; University of Bergen; Sanger Institute; Johns Hopkins Bloomberg School of Public Health; and Antigen Discovery, Inc.

References