DRAFT WHO Preferred Product Characteristics for Vaccines against Enterotoxigenic *Escherichia coli*

Written comments proposing modifications to this text must be received by **14 May 2020** and entered in the Comment Form (available separately), and should be addressed to the Responsible Officer: Dr Birgitte Giersing at giersingb@who.int.
TABLE OF CONTENTS

Acknowledgement
Abbreviations and glossary

Executive summary

1. Background and purpose of WHO PPCs
2. Development of an ETEC vaccine for LMICs – a strategic priority for WHO
3. Background of ETEC Diarrhoea
   3.1. ETEC Infection and Diarrhoea
   3.2. Mode of Transmission and Pathogenicity
   3.3. Prevention and Treatment of ETEC Diarrhoea
4. Full Value of Vaccines Assessment (FVVA) for ETEC Vaccines
5. Burden of ETEC Diarrhoea
   5.1. IHME Global Burden of Disease (GBD) study Mortality Estimates
   5.2. The MCEE Group Mortality Estimates
   5.3. Diarrhoeal diseases and ETEC Morbidity Burden
6. ETEC Vaccine Development
   6.1. ETEC Vaccine Feasibility
   6.2. ETEC vaccine pipeline: Clinical stages
   6.3. ETEC vaccine pipeline: Preclinical stages
   6.4. ETEC Vaccine Immunogenicity and Efficacy Considerations
   6.5. ETEC Vaccine Safety Considerations
   6.6. ETEC Vaccine Formulation and Delivery considerations for use in LMICs
7. PPCs for ETEC vaccines
8. Main Research Gaps in ETEC vaccine Development and Implementation:
9. References
Acknowledgements (to be updated)

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AMR</td>
<td>Anti-microbial resistance</td>
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<td>CoP</td>
<td>Correlates of protection</td>
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<td>CHIM</td>
<td>Controlled Human Infection Models</td>
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<td>CF</td>
<td>Colonization Factor</td>
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<td>DALYs</td>
<td>Disability Adjusted Life Years</td>
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<td>EPI</td>
<td>Expanded Programme on Immunization</td>
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<td>ETEC</td>
<td>Entero-toxigenic E. Coli</td>
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<td>EED</td>
<td>Environmental Enteric Dysfunction</td>
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<tr>
<td>FVVA</td>
<td>Full Vaccine Value Assessment</td>
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<td>GBD</td>
<td>Global Burden of Disease</td>
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<td>HIC</td>
<td>High-income country</td>
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<td>IVB</td>
<td>Immunizations, Vaccines &amp; Biologicals</td>
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<td>IHME</td>
<td>Institute For Health Metrics and Evaluation</td>
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<td>LMIC</td>
<td>Low and middle-income country</td>
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<td>LT</td>
<td>Heat Labile Toxin</td>
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<td>MCEE</td>
<td>Maternal Child Epidemiology Estimation</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PDVAC</td>
<td>Product Development for Vaccines Advisory Committee</td>
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<td>PPCs</td>
<td>Preferred product characteristics</td>
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<tr>
<td>PQ</td>
<td>Pre-qualification</td>
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<td>R&amp;D</td>
<td>Research and Development</td>
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<td>SAGE</td>
<td>Strategic Advisory Group of Experts on Immunization</td>
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<td>ST</td>
<td>Heat Stable Toxin</td>
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<td>TPP</td>
<td>Target Product Profile</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Executive summary

Diarrhoeal diseases are among the leading causes of morbidity and mortality worldwide. Among children younger than 5 years, it is estimated that diarrhoea is responsible for about 446,000 deaths (390,894–504,613), which are geographically concentrated in sub-Saharan Africa and South Asia.

Enterotoxigenic *Escherichia coli* (ETEC) is one of the leading bacterial causes of diarrhoea, especially among children in low resource settings and among travelers and military personnel from high-income countries. It is estimated that ETEC causes about 220 million diarrhoea episodes globally, with about 75 million episodes in children under 5 years of age, and between 18,700 (Global Burden of Disease study estimates) and 42,000 (Maternal Child Epidemiology Estimates) deaths in children younger than 5 years. In this 0-5 year age group, an intervention that is able to reduce mortality as well as the morbidity burden of ETEC diarrhoea effectively will impact the long-term consequences of infection related to malnutrition, that lead to poor physical and cognitive development as well as increased risk of death due to other infectious diseases. Such an intervention could avert 4.2-6.0% of under-5 diarrhoea deaths and offer significant but currently under recognised public health value to older children, adolescent and adults by contributing to improved social and economic development.

ETEC may be the first enteric illness encountered by many infants in low- and middle-income countries (LMICs), and the broad range of pathotypes suggest that more than one infection may be needed to acquire broadly protective immunity. Although diarrhoeal mortality rates for ETEC and other pathogens are declining due to improvements in economic development and availability of safe water and sanitation, these reductions have not been paralleled by significant declines in diarrhoea associated morbidity, which continue to impact negatively on infant and child health in many LMICs. In addition to potential direct (individual) effects on ETEC mortality and morbidity, an ETEC vaccine is also likely to have indirect effects such as decreasing anti-microbial resistance (AMR), increasing herd (community level) immunity, as well as healthcare cost savings through prevention of malnutrition and improving child physical and cognitive development. Protection from all cause diarrhoea may also be observed, a phenomenon that has been seen with rotavirus vaccines usage. Although several candidate ETEC vaccines have been tested and are in the pipeline at different stages of product development, currently no licensed vaccines against ETEC diarrhoea exist.

Prevention and treatment options to address diarrhoeal illness from ETEC are available, and are important for averting and reducing the health impact of the high the burden; however, they are not always practical to implement and sustain in many low resource settings. Consequently, the need to develop better and
more equitable prevention and control measures for diarrhoeal diseases, like vaccines, remains a public health priority for WHO, particularly for children in LMICs. An ETEC vaccine developed for this target population would also be of benefit to international travellers to endemic areas that suffer from a high burden of enteric illness associated with ETEC, as well as to age-groups above 5 years of age in LMICs that bear a significant burden of ETEC associated illness. In addition, an investment case for ETEC vaccine development suggested that younger age-groups in emerging middle-income countries may be an additional target for ETEC vaccine use.

Preferred product characteristics (PPCs) provide strategic guidance on World Health Organization (WHO) preferences for new vaccines, particularly from a LMIC perspective. WHO PPCs are intended to help advance development of an ETEC vaccine that is suitable for the use in the primary target population and in contexts where it is most needed, and to raise awareness of potential considerations for future policy recommendations. To frame the development of ETEC vaccine PPCs, the WHO convened global stakeholders to assess the priority public health needs, particularly in endemic areas. The outcome of this consultation was a consensus statement that the primary strategic goal is to develop a safe, effective and affordable ETEC vaccine that reduces mortality and morbidity due to moderate to severe diarrhoeal disease in infants and children under 5 years of age in LMICs. Participants considered critical vaccine attributes in the context of this strategic goal. These discussions are the foundation for this ETEC vaccine PPC guidance.
1. Background and purpose of WHO PPCs

The mission of WHO’s department of Immunization, Vaccines and Biologicals (IVB) is to accelerate the development and uptake of safe and effective vaccines and related technologies that could have global public health impact. Priority areas for IVB include developing guidance and co-ordinating activities that enable: 1) prioritization and acceleration of vaccine candidates towards licensure; and 2) identification and generation of evidence to inform policy recommendations for candidate vaccines as they progress to advanced stages of development, in order to avoid a delay between licensure and vaccine implementation.

Vaccine PPCs, published by WHO IVB, are intended to encourage innovation and promote development of vaccines for use in settings most relevant to the global unmet public health needs. They describe preferred parameters pertaining to vaccine indications, target populations, use case, and immunization strategies, as well as data that should be collected for safety and efficacy evaluation and policy consideration.¹ PPCs are pathogen-specific and do not specify minimally acceptable characteristics; they are intended to provide early guidance to inform optimal characteristics for candidate specific target product profiles (TPPs). Selected disease areas for vaccine PPC development are identified by WHO’s Product Development for Vaccines Advisory Committee (PDVAC), based on the unmet public health need for a vaccine, interest and demand for a vaccine from LMIC stakeholders, and technical feasibility. They may be updated in the event of product or technology innovations, or any other change in the identified need or R&D landscape.

The primary target audience for WHO PPCs is any entity intending to eventually seek WHO policy recommendation and prequalification (PQ) for their products. Communication of WHO preferences can be useful to all those involved in vaccine development, including academic groups, funders and manufacturers. As such, the various ETEC vaccine candidates will likely benefit from guidance regarding WHO and LMIC preferences as they approach upcoming stage gates for future investment and strategic decisions, particularly regarding field efficacy testing and SAGE policy recommendations for introduction. However, it is important to note that a vaccine that offers the preferred characteristics and is intended for use in LMICs will also undergo evidence-based assessment by WHO’s Strategic Advisory Group of Experts (SAGE) on Immunization.² As such, WHO PPCs offer early guidance and complement but do not supersede existing WHO processes for vaccine development and evaluation for a particular vaccine class or product.
2. Development of an ETEC vaccine for LMICs – a strategic priority for WHO

The immunization agenda 2030 (IA2030) is a global stakeholder strategy for the decade of 2021-2030, to Leave No-one Behind. It includes primary goals to i) reduce mortality and morbidity from vaccine preventable diseases across the life course, and ii) decrease disease burden by increasing access to and uptake of new vaccines. ETEC vaccine development has been a WHO priority for the last 20 years and a guidance document published in 2006 has helped to guide development efforts. The WHO priority strategic objective for ETEC vaccine development is to develop a safe, effective and affordable ETEC vaccine that reduces mortality and morbidity due to moderate to severe diarrhoeal disease in infants and children under 5 years of age in LMICs. This priority goal is reflective of the public health stakeholder input and scientific community understanding of the predominant burden of ETEC infections and their adverse long-term sequelae, that decrease the potential socio-economic prosperity of future generations in some of the most impoverished areas of the world. Other groups that would benefit from the availability of an effective vaccine include infants and young children in emerging middle-income countries; older children, adolescents and adults in ETEC endemic LMICs, international travellers and military personnel deployed to endemic areas.

The development of vaccines against ETEC infections has been hampered by technical challenges, insufficient support for coordination of research and development (R&D) efforts, and a poorly defined market to motivate investment in product development. In response to the urgent need for a vaccine in LMICs, and to provide guidance for the number of candidates in product development, IVB’s PDVAC recommended the development of PPCs for ETEC vaccines. The development of PPCs is particularly pertinent, considering the characteristics and status of vaccine candidates in development. At this time, the most advanced candidates, based on a combination of inactivated ETEC strains or live, attenuated strains are in phase I/II/IIb field trials in adults, with the inactivated candidate in parallel clinical studies in infants (see section 5 for more details). Any approach that supports the development of a combination vaccine could improve cost-effectiveness, due to simpler delivery logistics, particularly in low-resource settings. This is especially relevant to consider in the case of parenteral candidates, given the increasingly congested vaccination schedule for infants and young children, and the availability of attractive candidates, like parenteral typhoid vaccines and the progress being made on parenteral Shigella vaccines. For oral candidates, the co-formulation options are more limited with rotavirus, cholera or Shigella vaccines.
Any enteric vaccines may have a significant impact on antibiotic use since antimicrobial therapy is usually given to address serious syndromic presentations (i.e. cholera-like watery diarrhoea or dysentery) independent of a specific diagnosis. Antibiotic exposure among children under 5 years of age in LMICs is relatively high with prescription levels estimated as five time higher than those observed in high income countries. Diarrhoea and enteric diseases are among the leading drivers of antibiotic use. In a recent study in six African countries, plus Nepal and Haiti, 50% of children presenting with diarrhoea to a health care-facility were prescribed an antibiotic.
3. **Background of ETEC Diarrhoea**

3.1. **ETEC Infection and Diarrhoea**

Among the six recognized diarrhoeagenic categories of *Escherichia coli* ETEC is the most common, particularly in LMICs. ETEC is one of the first symptomatic enteric illnesses for children, causing several million cases of diarrhoea each year, mostly in under five-year olds. Infection with ETEC can cause profuse watery diarrhoea and abdominal cramping. Fever, nausea with or without vomiting, chills, loss of appetite, headache, muscle aches and bloating can also occur but are less common. Illness develops 1-3 days after exposure and usually lasts 3-4 days. Some infections may take a week or longer to resolve. Without adequate treatment this can lead to severe dehydration, electrolyte imbalance, and eventually death.

Repeated ETEC infections - with and without symptomatic episodes- are common among children in LMICs, in part because of the multiple pathotypes (enterotoxin and colonization factor combinations) associated with the disease. However, the decrease in the incidence of symptomatic illness with increasing age shows that protective immunity develops suggesting biological plausibility for protection by vaccination. The incidence of ETEC diarrhoea in low-income countries rises rapidly between 6-9 months, peaking in the first 2 years of life. Given the antigenic diversity among ETEC pathotypes, ETEC can also be a significant cause of diarrhoeal illness in older children and adults, particularly in South Asia and Africa, and may contribute to periodic outbreaks or epidemics of watery diarrhoea effecting a broad range of age-groups. ETEC is also the most common cause of diarrhoea in travellers, affecting individuals from high-income countries who visit endemic areas in LMICs. A systematic review suggests that ETEC was detected in 30-4% of cases of diarrhoea in travellers, with the highest rates seen in those travelling to areas with a high prevalence of ETEC.

Beyond its potentially devastating and immediate impacts on health, ETEC diarrhoea can also have long-term implications, and adverse consequences on growth and development, and increased risk of death due to other infectious diseases such as pneumonia, measles, and malaria. Repeated infections and symptomatic episodes due to these pathogens can induce or exacerbate stunting and other forms of malnutrition, reduce immune function, and increase the propensity for a subsequent inflammatory bowel disease. These infections can also hinder cognitive development, with adverse consequences on school attendance and performance as well as economic status.
3.2. Mode of Transmission and Pathogenesis

ETEC is primarily transmitted via food and water contaminated by faeces, causing secretory diarrhoea mediated by adherence to the gut and enterotoxin production within the small intestine. These toxins induce the hypersecretion of fluids and electrolytes, which cause the typical watery diarrhoea.\(^{33}\)

ETEC belongs to a heterogeneous family of lactose-fermenting *E. coli*, belonging to a wide variety of O antigenic types. Specific virulence factors such as enterotoxins and colonization factors differentiate ETEC from other categories of diarrhoeagenic *E. coli*. ETEC produces colonization factors (CFs), which are proteinaceous surface polymers (pili/fimbrial or nonfimbrial), that facilitate adherence to the intestinal mucosa and allow the organisms to readily colonize the small intestine and thus cause diarrhoea.\(^{34, 35}\) The clinical symptoms of ETEC diarrhoea can mimic those of cholera, and mild to severe disease has been observed; infection may be asymptomatic, mildly or severely symptomatic.

Experimental infections in animals and humans have shown that CF-positive bacteria, but not spontaneous CF-negative derivatives, better colonize the intestinal mucosa and induce diarrhoea.\(^{36, 37}\) Although numerous (>25) CFs have been identified, and additional, so far unidentified CFs may exist, seven (CFA/I and CS1-CS6) are generally more prevalent than others.\(^{20, 38}\) Recent animal and human data have also identified LT toxin and the protein EtpA as secondary adhesions for ETEC strains.\(^{39}\) Many ETEC isolates have no detectable CFs\(^{35}\), perhaps due to lack of detection methodologies. Nonetheless, various studies, including human challenge trials, have led some researchers to conclude that antibodies against CFs alone are sufficient for protection against ETEC-mediated disease.\(^{40, 41, 42}\)

After adherence to the intestinal mucosa, ETEC produces one or both of two enterotoxins, the larger heat-labile enterotoxin (LT) and the smaller heat-stable enterotoxin (ST). There are two variants of ST, ST\(_p\) and ST\(_h\), initially discovered in pigs and humans respectively, which have identical actions and are both found in human infections. Both toxins have been fully characterized, cloned, and sequenced.\(^{43, 44, 45, 46}\) Additionally, LT has been shown to be highly immunogenic in humans and anti-LT antibody responses have been shown in CHIMs and field studies to contribute to protection against ETEC and to impact on diarrhoeal disease severity.\(^{33, 47, 48}\)

There are three genotypes of ETEC based on the presence of toxin genes responsible for production of heat stable toxin (ST-ETEC), heat labile toxin (LT-ETEC), or both. The ST genotype can be further divided into the ST\(_a\) and ST\(_p\) that differ by a single amino acid.\(^{15, 22}\) The relative proportions of LT, ST, and LT/ST toxin-producing ETEC vary from one geographic area to another in patients with ETEC diarrhoea, or asymptomatic carriers. ETEC strains expressing only LT have been considered less important as pathogens, especially since they are more frequently isolated than the other two toxin types from healthy persons
than from patients. However, the recent observation from the multicentre community based cohort study, the Malnutrition and Enteric Disease Study (MAL-ED) showing an association between infection with LT only ETEC strains with persistent diarrhoea also need to be further examined given that persistent diarrhoea can frequently be a prelude to malnutrition, stunting and Enteric Enteropathy Dysfunction (EED). In addition recent, CHIMs studies have shown that LT only strains, that also express a colonization factor may be more efficient pathogens.

The pathologic pathways by which such infections may lead to chronic health consequences are complex and incompletely understood. It is hypothesized that enteropathogen infection may directly damage intestinal barrier and absorptive function and/or cause intestinal inflammation that further distorts the intestinal barrier and absorptive function or have systemic effects on metabolism and even cognitive development. Animal model and in vitro data suggest that LT toxin may contribute to intestinal barrier dysfunction and EED, and both animal studies and human CHIM studies have shown ETEC strains are capable of inducing gut inflammation. A healthy intestinal tract is especially critical in the first few formative years of life, as the predominant brain and synapse development in humans occurs in the first 2 years after birth. Hence, the absorption of key nutrients during this time is critical to assure the optimal growth and development of the body, brain, and neuronal synapses that determine human capacity.

Enteric infections may also lead to changes in the host microbiome and trigger complex immunologic responses that result in autoimmune dysfunction. Other factors have been suggested as potential mediators of these sequelae, including nutrient deficiencies, other infectious diseases, environmental exposures, lack of adequate stimulation and/or learning opportunities, maternal depressive symptoms, and host genetics.

### 3.3. Prevention and treatment of ETEC Diarrhoea

Proven, lifesaving interventions to prevent and treat diarrhoeal disease, including illness caused by ETEC, already exist. They include prevention methods, such as improved sanitation and hygiene, access to safe drinking water, exclusive breastfeeding, optimal nutrition, and vaccines against other pathogens (e.g., rotavirus and measles). While it is likely that living conditions in LMICs will improve with economic progress, the timelines are unpredictable and likely too slow to achieve the United Nations sustainable development goals and the immunization agenda 2030. As of 2015, an estimated 844 million people still retrieve water directly from surface water sources or use unprotected wells and springs. In addition, 2.3 billion people lack facilities for excreta disposal and 892 million people practice open defecation. Global access to improved sanitation and clean water is a long-term goal and represents the ideal solution.
to preventing transmission of ETEC and other faecally-transmitted organisms. However, this will be difficult to achieve and sustain in the near term, given the financial and logistical constraints in low-resource regions. Breastfeeding is also one of the most effective prevention interventions for diarrhoeal diseases and it provides a wide array of proven benefits to infants and young children, however, breastfeeding during a child’s first hour of life, exclusively for six months of age, and for two years overall is well short of universal.

The correction and maintenance of hydration is always most important for case management. Provision of adequate nutrition is critical in children in low resource settings, where all diarrhoeal diseases are frequent. The guidelines for therapy of all diarrhoeas have been widely disseminated by the World Health Organization and the Infectious Diseases Society of America. It has been found that Zinc treatment can speed recovery time and in July 2019, WHO included a new listing for co-packaged oral rehydration salts (ORS) and zinc sulfate in its Model List of Essential Medicines (EML) and Model List of Essential Medicines for Children (EMLc). While the available treatment strategies have been increasingly used successfully over the past decades, there are notable limitations and issues with coverage and sustainability. Therefore, vaccination is considered one of the most equitable preventive interventions.

As already indicated, the rise of antibiotic-resistant enteric bacteria has made the prevention of infectious diarrhoea, and the need for an effective vaccine, an even greater public health priority. WHO is evaluating the value of vaccines (including ETEC) against AMR. Wellcome conveyed its concerns over the increasing levels of multiple antibiotic resistant organisms among enteric pathogens in a recent report, and recommended that development of vaccines against enteric E. coli pathogens, like ETEC be accelerated.
4. Full Value of Vaccines Assessment (FVVA) for ETEC Vaccines

The value proposition of a vaccine candidate defines its epidemiologic, product development, economic, market, policy, financing, delivery and regulatory environments to guide investment in that product. Value propositions seek to identify and engage the major stakeholders and beneficiaries who may value the product differently, and articulate how the envisaged product will address their unmet need, as well as identify gaps in evidence to justify the product’s development and uptake. One of the fundamental elements that will inform the ETEC vaccine value proposition is a robust assessment of the current and future mortality and morbidity-related economic burden of disease. Therefore, it is imperative to capture the full burden resulting from ETEC illness. Current mortality burden estimates are sometimes inconsistent and divergent, and incidence data are inaccurate at best, or vary widely by region and season. Co-infecting enteric pathogens, subclinical infections, antigenic diversity, and variability of diagnostic methods can complicate the determination of diarrhoeal aetiology for children in LMICs. Epidemiologic studies are hampered by methodological limitations and narrowly focused study populations. Furthermore, diagnostic and modelling methods are continually undergoing optimization, resulting in variation of the mortality estimates for each iteration. A cost-effectiveness model for two ETEC and Shigella vaccine candidates was developed to evaluate vaccine impact on long-term sequelae of diarrhoeal diseases over the first ten years after vaccine introduction in children under five years old living in 79 low and low-middle income countries. However, to date, burden estimates have not included comprehensive estimates of disability adjusted life years (DALYs) specifically assessing long-term sequelae such as stunting, malnutrition, and cognitive impairment, as well as an increased risk of death due to other infectious disease causes.

In many settings, diarrhoea diagnosis and case detection are not possible or are inadequate. This creates significant data gaps in many geographies, therefore data from surrounding regions are extrapolated to generate regional and global burden estimates. An additional contributor to uncertainty intervals in the mortality estimates is the geographic heterogeneity that exists for ETEC disease burden. A recent study explored how accounting for subnational and economic heterogeneity in Shigella and ETEC disease burden affects projected vaccine impact and cost-effectiveness of ETEC and Shigella vaccines after introduction in four sub-Saharan African countries, using dynamic models for provincial areas and socioeconomic status. It suggested that impact and cost-effectiveness were more favorable if vaccinations reach the most vulnerable children in underserved provinces. This may impact the delivery strategy for
an ETEC vaccine, prioritizing introduction in high burden areas at sub-regional or sub-national levels. To inform investment in ETEC vaccine development, as well as to determine the potential market size and implementation strategy, the epidemiology of ETEC needs to be characterized at regional and national levels, and for this, surveillance data are essential. Modelling of indicators for high ETEC prevalence from existing longitudinal cohorts can be helpful as the most vulnerable populations are not likely linked to centers of excellence in diarrhoeal disease research.

While both the traveler and military populations represent substantial market segments that contribute to the value proposition for ETEC vaccine, the target product profiles for vaccines that are developed for these predominantly high-income populations may not be compatible with the programmatic requirements for a vaccine to be suitable for pediatric use in LMICs. These constraints relate to attributes such as storage requirements, ease of administration, number of doses, duration of protection and fit within the established immunization schedule. In addition, whilst both HIC travelers and infant populations in LMICs may be considered immunologically naïve and therefore their responses to vaccination may be similar, the protective immune responses in infants has been observed to decline with age de-escalation for many enteric vaccines. However, vaccines that are targeted primarily to infants and young children in LMICs will likely also be effective travelers’ vaccines. Therefore, in order to maximize potential global reach and impact on reducing disease and transmission, travelers’ vaccines must be developed with the endemic use indication in consideration.

Endemic-country awareness of the true impact that ETEC disease has or may have on a country’s population is fundamental to informing health-policy decisions. Policymakers in some endemic nations are unaware of the significance of ETEC and the burden of diarrhoeal illness. Considering that the first vaccine may be 5-10 years from licensure, the level of awareness must improve in the near term to create a pull for these vaccines, otherwise the potential impact of an ETEC vaccine may be limited because of low uptake due to inadequate information and advocacy.
5. Burden of ETEC Diarrhoea

Global Burden of enteric diseases, including ETEC estimates, are currently being modeled by 2 groups; the Institute for Health Metrics and Evaluation (IHME) and Maternal Child Epidemiology Estimation (MCEE). Each disease burden model has its strengths and limitations. Factors such as inclusion/exclusion criteria, model inputs and adjustments, assessment of pathogenicity, geographical representativeness, and country or regional extrapolation affect conclusions about the attributable burden. Neither the MCEE or IHME burden estimates accounted for differences in the ST, LT, ST/LT toxin genotypes, despite observations that strains producing ST alone or in combination with LT produce more severe disease.

5.1. Institute For Health Metrics and Evaluation (IHME) Global Burden of Disease (GBD) study Mortality Estimates

According to the IHME’s Global Burden of Disease (GBD) study estimates, diarrhoea accounts for more than 1 million deaths and about 4% of the total global DALYs per year across all age groups. ETEC was the eighth leading cause of diarrhoea mortality in 2016 among all age groups, accounting for 51,186 (26,757–83,064) deaths and about 3.2% (1.8–4.7) of diarrhoea deaths. Among children younger than 5 years of age, ETEC was responsible for an estimated 18,700 deaths (9900–30,659), about 4.2% (2.2–6.8) of diarrhoea deaths. The greatest estimated number of under-5 deaths due to ETEC was in eastern sub-Saharan Africa with 5,485 deaths (2889–8941).

5.2. The Maternal Child Epidemiology Estimation (MCEE) group Mortality Estimates

The MCEE group (previously known as CHERG), published estimates of pathogen-specific global mortality for children under-5 years of age, for the year 2011, using aetiologic data from hospital inpatient studies as a proxy for the pathogen distribution. MCEE estimated 712,000 ETEC diarrhoea deaths in all age groups and 42,000 ETEC diarrhoea deaths in children younger than 5 years of age.

5.3. Diarrhoeal Diseases and ETEC Morbidity Burden Estimates

The limitations and divergence in the IHME and MCEE mortality estimates pose challenges for vaccine developers, funders and policy makers in prioritizing the relative importance of intervention strategies against ETEC. The drivers for these different estimates are being investigated by a WHO working group on burden of enteric diseases. However, the potential value of an ETEC vaccine should not just account
for the impact on death; the burden estimates and associated vaccine impact should also incorporate the benefits that a vaccine could have on reduction of the long term and indirect morbidity effects, as well as reduction the use of antibiotics for treatment. Furthermore, the likely impact of ETEC on individual health, cognitive function, and productivity in endemic countries would also benefit from further study.

Frequent episodes of diarrhoea as a result of repeated infections with enteric pathogens lead to malnutrition, and the chance to have “catch-up” growth is linearly ablated. Infection with specific enteric pathogens such as ETEC can affect growth even in the absence of overt diarrhoea. It has been suggested these repeated episodes in the first 2 years of life can lead to a loss of up to 10 IQ points and 12 months of schooling by age 9 years. The cost of the vicious cycle of enteric infections and malnutrition (explained in section 3.2) and their potential lasting impact is so great that multiple, likely synergistic approaches to interrupt it must be taken. Not taking these consequences into account would thus be a serious oversight in accruing clinical and epidemiologic evidence to address this substantial burden.

In an effort to quantify a proportion of this morbidity burden for diarrhoeal diseases, IHME conducted a study to quantify the long-term sequelae due to growth faltering, using DALYs, which are the sum of the number of years of life lost due to premature mortality (YLL) and the number of years lived with disability (YLD). DALYs are now widely used in public health practice to assess and monitor population health and to set health priorities in a given country. The study showed that the global burden of diarrhoea is substantially underestimated when only incidence and mortality are considered, and that accounting for long-term sequelae associated with growth impairment increased the number of diarrhoea DALYs lost among children younger than 5 years by about 40%. After inclusion of these long-term sequelae, diarrhoea moves from the fifth-leading to the third-leading cause of DALYs among children younger than 5 years, surpassing malaria and neonatal encephalopathy in the number of DALYs in this age group. To date, there is no ETEC specific analysis to account for the additional DALYs burden due to long term sequelae. If available, this would help to refine the pathogen specific burden estimates and the full value of a vaccine for ETEC. Indeed, as noted above about the importance of DALYs estimates, the blinded controlled study of a vaccine for ETEC, with height-for-age z-scores (HAZ) measurements, can uniquely help assess and document the magnitude of ETEC’s role in this common additional DALY burden, with or without overt diarrhoea.
6. ETEC vaccine development

6.1. ETEC Vaccine feasibility

Evidence from field and human challenge studies indicate that protective immunity to ETEC develops after natural or experimental infection, suggesting that vaccine-induced ETEC immunity should be feasible.\textsuperscript{87, 88} In ETEC-endemic areas, age-specific attack rates for symptomatic ETEC infection decline after three years of age.\textsuperscript{89, 90, 91} Studies with candidate vaccines have also led to protective immunity in limited challenge studies and field efficacy trials.\textsuperscript{89, 90, 91, 92, 93, 94} In human challenge studies, subjects who recovered from ETEC diarrhoea were protected against disease when challenged a second time with the same strain.\textsuperscript{48, 92, 91} In addition, both field studies and human challenge studies indicate that antibodies against colonization factors and LT toxin can play a role in protection.\textsuperscript{48, 95, 96, 97}

Although the development of a vaccine is believed to be biologically feasible, several challenges remain. ETEC strains are highly diverse antigenically, expressing a multitude of colonization factors and surface antigens, toxins, and other virulence proteins. Therefore, in order to provide strain coverage, ETEC vaccines need to be multi component formulations. On an encouraging note, recent genotyping studies on ETEC strain collections from various geographic locations indicate that vaccines providing coverage for the most common colonization factors, CFA/I, CS3, CS5 and CS6 along with related antigens, like CS7 and other class 5 fimbriae should cover 80-90% of the ETEC strains associated with diarrhoea in LMICs and in travelers.\textsuperscript{20, 98} Recent studies have also suggested that including conserved ETEC antigens in vaccine formulations may also help improve coverage particularly for those strains that may lack identifiable colonization factors.\textsuperscript{39, 80}

Vaccines intended for use in pediatric populations in LMICs must also be formulated and delivered in such a way that their costs are reasonable, and their tolerability and immunogenicity are assured.\textsuperscript{99, 75, 76} The latter criterion is a particular challenge for vaccines used in low resource settings as sub-optimal performance in terms of efficacy and effectiveness, especially with oral vaccines, has been demonstrated in many countries in Asia and Africa.\textsuperscript{72, 73, 74} Several reasons for this phenomenon were suggested, including the underlying gut enteropathy, co-infections and malnutrition.\textsuperscript{25, 74} These aspects are likely to be less of a challenge in the development of a traveler’s vaccine.\textsuperscript{100}

To achieve optimal public health impact, novel routes of delivery need to be explored, as well as mucosal adjuvants that may help overcome the detrimental effects of gut enteropathy and overcome the barriers of the full efficacy of an oral vaccine.
6.2. ETEC vaccine pipeline: Clinical stages
Current ETEC vaccine development efforts have focused on inducing antitoxin and anti-colonization immunity, as studies indicate that antibodies against both antigen types can contribute to protection. The leading whole cell based vaccine candidates is an oral quadrivalent inactivated vaccine, consisting of four *E. coli* strains overexpressing the most prevalent colonization factors (CFA/I, CS3, CS5, and CS6) and a toxoid (LCTBA) administered with a double-mutant heat-labile enterotoxin (dmLT) as an adjuvant. The development of another candidate that comprises three genetically attenuated strains of *E. coli*, with antigen profiles covering a wide range of ETEC surface colonization factor antigens (CFA/I, CFA/II [CS1, CS2, CS3] and CFA/IV [CSS, CS6]) and also expressing LT-B, the inactive subunit of LT, has been discontinued due to funding constraints, but both have both been found to be safe and immunogenic in Phase I/II, trials. The quadrivalent oral inactivated vaccine has progressed to descending-age studies in Bangladesh down to 6-11 months of age, with fractional doses (1/4th or 1/8th of the adult dose). A Phase I age descending trial in Zambia in healthy adults and children (PACTR201905764389804) is underway and a Phase IIb field pediatric efficacy trial is planned.

6.3. ETEC vaccine pipeline: Preclinical stages
There are several candidates in preclinical development for both oral and parenteral administration. Of particular interest are the platforms that support a combination of ETEC antigens with other enteric vaccines. As this PPC is being drafted, live attenuated combined Shigella -ETEC candidates are poised to enter the clinic. Co-administration or co-formulation of enteric vaccines that can target the same age group, for example, ETEC-cholera or ETEC-cholera-typhoid would be very beneficial. Additionally, the application of new “Omics” technologies has identified a number of conserved novel proteins that may also contribute to toxin delivery or colonization and thus may also have vaccine potential, since they tend to be shared across ETEC pathotypes. These antigens include flagellin, EtpA, EatA, EaeH, and YghJ. The potential inclusion of selected antigens from this group in future vaccines may also help broaden vaccine protection against a wider range of ETEC strains and drive antibody responses to further interfere with intestinal colonization and LT toxin delivery.

6.4. ETEC Vaccine Immunogenicity and Efficacy Considerations:
As indicated by the diversity of the vaccine pipeline, efforts to improve vaccine immunogenicity are ongoing, and include formulation with adjuvants or investigation of new delivery routes that may potentially facilitate vaccine dose sparing and improve efficacy. Controlled human infection models (CHIM) for ETEC are well developed and provide a tool to demonstrate early proof of concept.
for candidates, to potentially compare relative performance of difference candidates and to investigate correlates of protection.

However, CHIM studies will not be sufficient for licensure or to inform introduction decisions, so vaccine developers will need to undertake a field efficacy study to support the pediatric indication. To enable discovery of new correlates of protection, archiving specimens from field studies to be readily available for future studies should be considered.

A major challenge is the lack of consensus on how to define diarrhoeal diseases and ETEC diarrhoea severity in community-based studies in low- and middle-income countries. This limits comparability between candidates and studies in the endemic settings. The Vesikari score\textsuperscript{106} was designed for use in rotavirus vaccine trials, and although useful in that context,\textsuperscript{107} it may be less so in community-based studies where there are multiple etiologies. Other scores have been proposed,\textsuperscript{108,109,110} and one of the scores ‘CODA’,\textsuperscript{111} was validated in a large multisite study (MALED), providing more confidence than scores that have not been validated, or only validated in a single site, however it has not been broadly implemented.

Human challenge model data can help to develop a scoring system; however, it may not be suitable for field trials of both travelers and young children in LMICs. It may also not be practical to develop an ETEC specific severity score; thus, consensus on a definition for moderate to severe diarrhoea attributable to ETEC may be more realistic. This has been proposed for candidates in clinical trials involving infants in LMICs.\textsuperscript{112} However, given the desire to demonstrate vaccine impact across the spectrum of ETEC disease, there is a need to reach consensus on a severity score that is validated, predict hospitalization, independent of health care seeking, and is amenable for field use, and more research in this area needs to be encouraged and supported. The most promising scoring systems need to be comparatively evaluated in clinical and epidemiological studies that are being planned in support of ETEC vaccine development and tested with the goal of validating 1-2 scores for use in future Phase III efficacy trials.

No clear efficacy threshold has been defined for achieving a minimal public health benefit for ETEC vaccines. Acceptable thresholds for efficacy can be informed by updated vaccine impact models,\textsuperscript{70, 69} inclusive of those that demonstrate indirect effects, and by market research with key stakeholders. A cost-effectiveness model\textsuperscript{69} suggested that introducing ETEC or *Shigella* vaccines, each with 60% efficacy could prevent a substantial number of direct ETEC and *Shigella* diarrhoea and stunting deaths, in addition to a favorable Incremental cost-effectiveness ratio.
6.5. ETEC Vaccine Safety considerations
ETEC vaccines are expected to be well tolerated and to have a safety profile at least as favorable as those for current WHO-recommended pediatric vaccines. Attenuated vaccines may have the greatest challenge for safety in infants and young children, but may be the most immunogenic\textsuperscript{113}, whereas non-living (both killed and subunit approaches) may be better tolerated with more predictable immunogenicity profiles since the antigen dose given to each vaccine recipient can be better standardized and controlled.

6.6. ETEC Vaccine Formulation and Delivery considerations for use in LMICs:
The target age group, namely infants and young children under 5 for an ETEC vaccine in LMICs, has proven difficult to immunize effectively against enteric pathogens via the oral route.\textsuperscript{9} For this reason, other routes of administration and a variety of vaccine types are being evaluated. Oral vaccines avoid many of the delivery challenges associated with injectable vaccines in LMICs: they are relatively easy to administer, have the capacity to induce local mucosal immunity in the intestinal mucosa, and potentially can be produced at a relatively low cost.\textsuperscript{114} However, for policy recommendation, procurement and widespread use of oral vaccines in LMICs, it will be crucial to develop vaccine formulations and presentations that are efficacious and facilitate use in the target population.\textsuperscript{91, 115} Pre-clinical and human data suggests that intradermal delivery may be an alternative option for intra-muscular delivery, for inducing mucosal immunity.\textsuperscript{116, 117} Although this route may be a challenge in mass vaccination campaigns or in the EPI schedule, novel delivery devices may render this vaccination route more practical and attractive given its potential for improved immunogenicity and dose sparing.\textsuperscript{117}

It is imperative to consider the vaccine presentation requirements for programmatic delivery early in product development, so that a suitable presentation can be included in pivotal clinical trials that will support licensure. For oral formulations, considerations should be given to protection from gastric acidity to prevent antigen degradation in passage through the stomach.\textsuperscript{105, 118} In this regard, if small volumes of citrate buffer can protect antigens fed to infants, it may simplify vaccine administration compared to sodium bicarbonate buffer. Dose-volume optimization is also an important consideration for administration to infants and young children. While minimizing dose volumes reduces the storage footprint for the vaccine and facilitate delivery, it may impact on the osmolarity, which may have detrimental effects on vaccine stability and palatability of the final formulation.\textsuperscript{105}
Packaging technologies to improve product shelf life and allow for packaging of dry and liquid vaccine components in one container would help to address some of the delivery challenges.\textsuperscript{119, 120, 121} Several manufacturers are developing innovative designs for dry and liquid vaccine presentations.\textsuperscript{122, 123}

The number of doses, vaccination schedule and the possible need for booster doses also should be carefully considered based on the efficacy and capacity to induce immunological memory, safety and cost of the final vaccine presentation, as well as the fit within the immunization program.
### 7. PPCs for ETEC vaccines

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<th>Parameter</th>
<th>Preferred characteristic</th>
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| **Indication**  | Prevention of moderate-to-severe diarrhoea (MSD) due to ETEC infection                    | Primarily prevention of moderate-to-severe (MSD) diarrhoea due to ETEC is considered the optimal clinical end point to provide a measurable impact.  
  
  Note: Prevention of MSD diarrhoea does not imply induction of sterilizing immunity but prevention of severe and moderate infection. Prevention of mild disease is also considered important; however, this will not be possible to measure pre-licensure.  
  
  Other possible indications: Direct effects include reduction of stunting, prevention of malnutrition, risk reduction of ETEC associated infections, prevention of all cause diarrhoea. Indirect effects include decrease in AMR, induction of herd protection and financial risk protection. While these are important outcomes that will contribute to the value assessment for ETEC vaccines, they are challenging to assess as primary clinical endpoints pre-licensure. Where feasible, exploratory endpoints related to these indications should be collected during clinical studies. |
| **Target Population** | For initial licensure: Infants and children up to 24 months.  
  For introduction in LMICs: Children up to five years of age. | The goal is full protection of infants by the end of 9 months, to cover peak incidence and mortality through the first 24 months of life and the greatest burden in children in LMICs up to 5 years of age. Prevention of MSD in this group would significantly reduce death and morbidity due to both immediate and long-term sequelae, such as growth stunting associated with infection.  
  
  Some country and regional variation (+/- 6 months) in peak incidence is expected.  
  
  Other target populations that would likely benefit from a vaccine that is efficacious in infants are older children, adolescents, adults in LMICs and emerging markets, as well as travellers and military recruits to endemic areas. |
| **Dose Regimen & Schedule** | At least 2 doses are expected to be needed for primary immunization, between the ages of 6 to 12 months.  
  An additional booster/s dose may be required to maintain effective and long-lasting immunity through the first 5 years of age | The schedule should provide protection prior to the peak of infection to prevent the majority of ETEC infection and disease, and thus prevent the initiation of the EED pathogenic process.  
  
  Depending on the vaccine platform and formulation, two or three doses, 4-8 weeks apart might be needed for primary immunization, with the first dose ideally at 6 months and second dose to be given with measles containing vaccine (MCV) at 9 months.  
  
  This vaccine is expected to be delivered through the routine immunization schedule, although it may be
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<td><strong>implemented on a sub-regional or sub-national level in areas of heterogenous endemcity.</strong></td>
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<td>A booster dose, after the initial regimen may be needed in the second year of life and could be given with the second MCV at 15 months.</td>
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<td>The optimal delivery schedule will be determined by assessment of clinical efficacy and cost effectiveness. Consideration for coformulation with EPI vaccines or other pipeline vaccines that have a compatible route of administration, immunization schedule and delivery requirements would be advantageous.</td>
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<td>In some situations, such as outbreak, the ETEC vaccine may be delivered through campaigns. It could be also delivered pre-emptively with oral cholera vaccine and or typhoid vaccine.</td>
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<tr>
<td>Safety</td>
<td>A safety and reactogenicity profile at least as favourable as current WHO-recommended routine vaccines.</td>
<td>A favorable safety profile will need to be demonstrated in adults before progressing to younger ages and the target population.</td>
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<td>Live attenuated vaccines may have the greatest challenge for safety in infants, whereas non-living (both oral and subunit) approaches may be better tolerated.</td>
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<td></td>
<td>Contraindications should be restricted to known hypersensitivity to any of the vaccine components.</td>
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<tr>
<td>Endpoints</td>
<td>Reduction of MSD diarrhoea as defined by the case definition for MSD</td>
<td>Although there is alignment on the need to prevent MSD due to ETEC in the target population, there is no consensus on the case definition (severity score) for MSD in community settings. Alternatively, trials could assess vaccine impact on medically attended MSD using a passive surveillance study design.</td>
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<td>Secondary Clinical Endpoints may include initial and follow up HAZ to measure potential impact on growth stunting, with or without overt diarrhoea.</td>
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<td>Efficacy</td>
<td>Efficacy of 60% or more against moderate-to-severe ETEC diarrhoea.</td>
<td>Values proposed are based on observed lower performance of enteric vaccines in endemic pediatric settings.</td>
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<td>Moderate efficacy (approximately 50%) is considered clinically meaningful and would be comparable to rotavirus vaccine in some lower-middle-income countries.</td>
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<td>Vaccine Impact models should evaluate and guide the efficacy targets.</td>
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<td>Parameter</td>
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<tr>
<td>Duration</td>
<td>Duration of protection two years or longer, starting 14 days after the second dose.</td>
<td>Protective immunity should be present as early as 10 months of age and protect for a minimum of 2 years. Duration of protection up to 5 years is optimal, however a booster dose may be required.</td>
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<tr>
<td>Adjuvant requirement</td>
<td>Preference for the absence of an adjuvant.</td>
<td>Adjuvant could be included if proven enhancement of vaccine immunogenicity and efficacy observed with vaccines in primary target population in endemic settings.</td>
</tr>
<tr>
<td>Immuno-</td>
<td>Seek to establish correlate or surrogate of protection, based on a validated assay measuring immune effector levels and functionality that has been directly related to efficacy in the target population.</td>
<td>A correlate of protection would provide an immunological benchmark for the evaluation of ETEC vaccines and immunization regimen; however, these are not well defined. Field and CHIM studies suggest that ELISA based assays measuring the level of serum IgG or IgA against key colonization factor and LT toxoids in the vaccine may provide a practical correlate of protection (CoP) but further studies, including field efficacy studies, are needed to better establish and validate threshold levels that best correlate with protection. The longevity of the immune response should be characterized, and the relationship to the duration of protection should be investigated. Since ETEC infections are confined to the mucosal surfaces in the gut and immune protection is most likely provided by locally produced secretory IgA antibodies, it has been assumed that assessment of the relative immunogenicity of vaccine candidates should focus on antigen-specific antibody responses induced at the intestinal mucosa or on surrogate antibody measures of intestinally derived antibody responses, like the ASC-, ELISPOT or ALS responses.</td>
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<tr>
<td>Non-</td>
<td>Demonstration of favourable safety and immunologic non-interference upon co-administration with other vaccines recommended for use within the EPI schedule.</td>
<td>There should be no significant interference in relation to safety and immunogenicity with concurrently administered or co-formulated vaccines.</td>
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<tr>
<td>Route of administration</td>
<td>Oral or injectable (IM, ID or SC), using standard volumes for injection, as specified in programmatic suitability for prequalification (PQ), or needle-free delivery</td>
<td>Induction of local mucosal immunity can play an important role in protection against ETEC.</td>
</tr>
<tr>
<td>Product stability and storage</td>
<td>Two years at 2 to 8°C. For a powder formulation: Vaccine vial monitor (VVM) for 30 days at 40°C</td>
<td>If some components need to be kept separate from the vaccine until administration, i.e. buffer or diluent, it would be critical that these are not required to be stored in the cold chain.</td>
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For a liquid formulation: VVM for 14 days at 40°C

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<tr>
<th>Vaccine presentation</th>
<th>Low-dose vials or blow-fill-seal multi- mono-dose containers to reduce missed opportunities for vaccination and vaccine wastage.</th>
<th>Novel delivery technologies, such as dual chamber devices for an oral vaccine or microarray patches for a parental vaccine, may help to overcome the presentation and delivery challenges.</th>
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<tr>
<td>Registration, PQ, and programmatic suitability</td>
<td>The vaccine should be prequalified according to the process outlined.</td>
<td>WHO-defined criteria for programmatic suitability of vaccines should be met.</td>
</tr>
<tr>
<td>Access and affordability</td>
<td>The vaccine should be cost effective and price should not be a barrier to access, including in LMICs. Dosage, regimen and cost of goods amenable to affordable supply.</td>
<td>Total Cost per Fully Immunized Subject comparable to the incremental cost of cholera vaccine at the time of an ETEC vaccine launch, would be the analog for an EPI vaccine. It is imperative to capture the full burden of ETEC diarrhoea including morbidity burden in determining acceptable price. In addition to the direct and indirect effects of infection, herd immunity, assessment of the broader societal and economic benefits of vaccination are important to articulate the value of an ETEC vaccine from an LMIC prospective. The vaccine’s impact on health systems and other aspects of implementation science should be evaluated pre- or post-approval, as this will also contribute to assessment of vaccine value.</td>
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8. Main Research Gaps in ETEC vaccine Development and Implementation:

The following priority research gaps were identified in the development of this PPC. Improved data/evidence across these areas will enable refinement of the PPCs when available:

- Lack of consensus on how to define diarrhoeal diseases and ETEC diarrhoea severity in community-based studies in LMICs, which limits comparability between vaccine candidates and studies. Severity scoring as an important attribute given the ability to observe a biological effect, and a validated severity score that can be used in LMICs for grading/definition severity of diarrhoea among infants and young children is needed to demonstrate vaccine efficacy and impact. Many scores have been developed and suggested (see section 6.4), but there is a need to evaluate them in field settings and to reach consensus on which is optimal.

- Alignment on clinical endpoints of sufficient clinical and epidemiological importance to demonstrate prevention of MSD; correlates of protection and standardized assays and antigens assessing immunogenicity of ETEC vaccine candidates (see PPC table). Clinical trials for product down-selection decisions and to support licensure are ongoing in diverse settings and they offer both opportunities and challenges for endpoint determination.

- Refining and improving burden data, with sentinel surveillance leveraging the ongoing global expanded diarrhoea surveillance studies, using practical, rapid ETEC diagnostics for accurate assessment of the proportion of ETEC diarrhoea, among other pathogens. The most appropriate diagnostic tool for identifying microbiologically-proven ETEC episodes is quantitative PCR which is more sensitive for detecting infection with the target organisms, but it has limitations in its broad fieldability, particulate in remote areas, in characterizing the colonization factor antigens of ETEC sub/serotypes, and cannot be used to assess antibiotic susceptibility profiles. Therefore, when these methods are deployed in support of or to replace traditional ETEC diagnostics (e.g., GM1 ELISA, CFA dotblot) in ETEC clinical trials; it needs to be investigated and clear recommendations made so regulatory groups overseeing trials are more accepting of molecular-based methods. Additional studies comparing culture based and molecular based methods for determining microbiology endpoints in vaccine efficacy studies are needed. In addition, the full morbidity burden of ETEC infection and diarrhoea, and its long term sequalae need to be captured and quantified (see section 5.3).
• Reaching an alignment about optimal ETEC vaccine, antigen composition, dose regimen and schedule, which is important for vaccine implementation and delivery strategy. ETEC immunity is needed relatively early during infancy, as the fatality rate of MSD is significantly greater in the first 6 months of life favoring efforts for early administration. However, with the high rates of exclusive breast feeding in LMICs, ETEC antibodies can protect against oral vaccine take. In addition, it is difficult to stimulate local immune responses in very young infants for full protection against ETEC and improving coverage for ETEC strain producing only ST toxin remains a challenge. Further exploration of mucosal adjuvants, an ST toxoid and/or novel proteins that could help provide better coverage for ST only strains needs to be encouraged.
9. References:


20 Isidean SD, Riddle MS, Savarino SJ, Porter CK. A systematic review of ETEC epidemiology focusing on colonization factor and toxin expression. *Vaccine* 2011; **29**: 6167–78.


97 McKenzie R, Darsley M, Thomas N, *et al.* A double-blind, placebo-controlled trial to evaluate the efficacy of PTL-003, an attenuated enterotoxigenic E. coli (ETEC) vaccine strain, in protecting against challenge with virulent ETEC. *Vaccine* 2008; **26**: 4731–9.


