Status of vaccine research and development for enterotoxigenic Escherichia coli

A. Louis Bourgeois*, Thomas F. Wierzba, Richard I. Walker

PATH, Washington, DC, USA

ABSTRACT

Enterotoxigenic Escherichia coli (ETEC) is one of the most common bacterial causes of diarrhea-associated morbidity and mortality, particularly among infants and young children in developing countries. Still, the true impact on child and traveler health is likely underestimated. There are currently no licensed vaccines for ETEC, but studies indicate high public health impact, cost-effectiveness, and feasibility of immune protection through vaccination. ETEC vaccine development remains a World Health Organization priority. Traditionally, ETEC vaccine development efforts have focused on inducing antitoxin and anticolonization antigen immunity, as studies indicate that antibodies against both antigen types can contribute to protection and thus have potential for vaccines. Leading cellular vaccine candidates are ETVAX (a mixture of four inactivated strains) and ACE527 (a mixture of three live attenuated strains), both of which have been found to be safe and immunogenic in Phase 1/2 trials. ETVAX is the furthest along in development with descending-age studies already underway in Bangladesh. Other ETEC vaccine candidates based on protein subunits, toxins (both LT and ST), or novel, more broadly conserved ETEC antigens are also under development. Of these, a protein adhesin-based subunit approach is the most advanced. Impact and economic models suggest favorable vaccine cost-effectiveness, which may help expand market interest in ETEC vaccines. Combination vaccine formulations may help improve the economic case for development and use, and better point-of-care diagnostics will help to raise awareness of the true health burden of ETEC and highlight the potential public health benefit of ETEC vaccine introduction. Better diagnostics and vaccine demand forecasting will also improve vaccine development financing and support accelerated uptake once a licensed vaccine becomes available.

© 2016 World Health Organization; licensee Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/3.0/).

1. About the disease and pathogen

Enterotoxigenic Escherichia coli (ETEC) remains among the most common bacterial causes of diarrhea-associated morbidity and mortality [1–4]. 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) 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 seeking care for their illness at health centers in both regions [3]. Similarly, in a prospective community-based diarrhea study in South America, Africa, and Asia, the Interactions of Malnutrition and Enteric Infections: Consequences for Child Health and Development (MAL-ED) project found ETEC to be an important cause of diarrhea illness in the second year of life in these regions, where it was frequently associated with more severe acute illness and persistent diarrhea [4]. GEMS data also indicate that children with MSD are in a subgroup that is at higher risk of dying or being stunted, and that cases infected with heat-stable (ST) toxin-positive ETEC strains, which includes ETEC strains expressing ST toxin alone or in combination with heat-labile (LT) toxin, were more likely to have these poor health outcomes; although it remains to be determined if these relationships in GEMS
are causal [3]. In MAL-ED, diarrhea episodes associated with gut inflammatory markers or changes in permeability were associated with stunting. Illness associated with diarrheagenic E. coli, including ETEC, contributed to this linear growth faltering [5]. Although both studies highlight the continuing importance of ETEC as a significant cause of diarrhea-associated morbidity and mortality in developing countries, it is likely that these estimates, based on more traditional culture-based detection methods, may underestimate ETEC’s actual health impact. A recent reanalysis of GEMS samples using more molecular-based, quantitative polymerase chain reaction (qPCR) technology [6] suggests that this approach may give a better picture of the true burden of ETEC-associated MSD as well as other bacterial pathogen-related MSD and their relative roles in infant mortality and stunting.

In 2010, the Global Burden of Disease (GBD) study estimated annual mortality from illness due to ETEC at 157,000 deaths – 9% of all deaths attributed to diarrhea and approximately 1% of all deaths in children 28 days to 5 years of age [7]. The World Health Organization (WHO) Child Health Epidemiology Reference Group (CHERG), which uses a different methodology, estimated 42,000 (95% CI, 20,000–76,000) ETEC-associated deaths of children under five years of age in 2013 [8]. In older age groups, a meta-analysis of hospitalization and stool culture data projected that ETEC may contribute to an additional 89,000 deaths per year among age groups older than five years in Africa and South Asia [9]. In addition to mortality estimates, morbidity estimates in 2010 projected ETEC Disability-Adjusted Life Years (DALYs) at 8.5 million (10% of all diarrhoea DALYs) and Years Lived with Disability (YLDs) due to ETEC at 1 million (13% of all diarrhea YLDs) [10,11]. The meta-analysis also found that, in 2010, ETEC was significantly more common in age groups older than 5 years than cholera and typhoid combined, with 44 million cases of ETEC versus 9 million typhoid and cholera cases (6 and 3 million, respectively) [9]. Across all diseases, school-age children were at the highest risk for illness [9]. Moreover, another meta-analysis describing the etiology of diarrhea among individuals in older age groups suggests that ETEC may be associated with 10–14% of hospitalized cases and 6% of all diarrhea illnesses in outpatient and community settings.

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, 10–14% of travelers recovering from ETEC-associated travelers’ diarrhea may go on to develop irritable bowel syndrome [12], further highlighting the importance of ETEC prevention and the potential benefit of effective ETEC vaccines [2,12,13].

In the classical paradigm for ETEC pathogenesis, these bacteria first colonize the small intestine, where they employ plasmid-encoded fimbrial colonization factor (CF) or coli surface (CS) antigens to bind to enterocytes in the upper small intestine [1,2]. Here, they produce ST and/or 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]. These plasmid-encoded antigens are considered to be key virulence factors and have therefore been intensively studied over the last three decades. Recent data has supported an immune modulatory role for ST that may reduce the ability of infected hosts to mount effective innate and adaptive immune responses to the infecting ETEC organism [14]. Whether this proposed immunomodulatory role for ST toxin contributes to the poorer health outcomes associated with the ST-ETEC infections in GEMS and MAL-ED remains to be determined, but future investigation of ST toxin’s additional role(s) in ETEC pathogenesis warrants more in-depth study. In a similar vein, as indicated above, the LT toxin is a well-accepted virulence factor for those ETEC strains that produce it [1,2]. However, a less-appreciated role for this toxin in ETEC pathogenesis is its contribution as an accessory colonization factor and also as a potential co-factor in promoting intestinal colonization by other enteric pathogens, like Salmonella enterica [15]. Promotion of intestinal colonization by Salmonella has only been observed in pigs to date, but given the potential impact of this observation, it warrants further study in humans since it may contribute to some of the more long term poor health outcomes associated with ETEC infections in both the GEMS and MAL-ED studies, while highlighting the importance of including a toxoid component in future ETEC vaccine formulations [16,17]. Prior field studies of cholera and ETEC vaccines have suggested that formulations containing LT or the cross-reactive CTB toxoid not only can give protection against LT-producing ETEC strains but also intriguing short-term protection against Salmonella and possibly Campylobacter [18–20]. While more than 25 unique CF/CS types and putative factors have been characterized so far, one or more of these known CFs have been identified in only about 50% of ETEC clinical isolates. Therefore, as mentioned previously, 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 [6]. This has stimulated renewed interest in more sensitive and specific point-of-care diagnostics and in exploring more novel adhesins and other conserved ETEC proteins contributing to virulence that may go undetected using standard molecular biology and immunological approaches [2,19,21]. The prominent role played by ST–producing ETEC in GEMS and MAL-ED has also triggered renewed efforts to make a safe and immunogenic ST toxoid for inclusion in future ETEC vaccine formulations [21–23].

ETEC is transmitted via the fecal-oral route and is associated with the consumption of contaminated water or food. Like most enteric pathogens, multi-drug resistance is becoming more common [1,2]. Because ETEC-associated MSD is an enterotoxigenic, non-invasive disease of the small intestine, rehydration therapy can be beneficial. However, given growing concerns about the longer-term health effects of ETEC illness, prevention remains an important area to address. As indicated above, recent GBD data highlight ETEC as an important global contributor to diarrhea-associated mortality, DALYs, and YLDs [7,8,10,11] and, despite declining mortality, ETEC-associated morbidity has not changed significantly over the last 20 years. Moreover, based on the application of more sensitive detection methods mentioned above, there is likely a significant underestimation of its true impact on child and traveler health. Based on recent vaccine impact and cost-effectiveness modeling analyses done by the University of Florida, Johns Hopkins Bloomberg School of Public Health, and PATH [19,24,25,26], ETEC episodes are estimated to contribute to an additional 4 million children with moderate-to-severe stunting, which, in turn, would contribute to an additional 31,000 deaths annually from diarrhea and comorbidities like pneumonia, malaria, and measles.

These analyses found that the potential impact of an ETEC vaccine differed by region, with the greatest cost-effectiveness ratio (CER) and impact seen in Africa (US$32.00 for every DALY averted). In 90 low- and lower-middle-income countries, including those eligible for support from Gavi, the Vaccine Alliance, the analyses found a long-term impact and favorable CER of US$65.00/DALY averted for ETEC vaccines. The CERs are projected to be even more favorable when modeling efforts incorporate herd immunity effects into the analysis. Favorable cost-effectiveness estimates have also been obtained for the introduction of ETEC vaccine into the travel medicine community [13,19,25,27,28].
2. Overview of current efforts

2.1. Vaccine feasibility

There are currently no licensed vaccines for ETEC. However, field studies 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. In addition, in ETEC-endemic areas, age-specific attack rates for symptomatic ETEC infection decline after three years of age [1,2,19,29], and in human challenge studies, subjects who recovered from ETEC diarrhea were protected against disease when challenged a second time with the same strain [30–32]. Finally, active immunization with candidate vaccines has led to protective immunity in limited challenge studies and field efficacy trials [1,2,19,20,31,32].

Although vaccine development is clearly feasible, several challenges remain before success can be achieved. ETEC strains are highly diverse antigenically, expressing a multitude of colonization factor and coli surface antigens, toxins, and other virulence proteins. Consequently, vaccines would have to be multicomponent formulations to provide the strain coverage needed to be an effective public health tool [2,19,35]. They must also be formulated and delivered in such a way that their costs are reasonable and their tolerability and immunogenicity is assured in the two primary vaccine target populations: infants and young children in developing countries (0–5-year age range) and adult travelers to ETEC-endemic areas [19,28,35]. Effective immunization of young children and infants in developing countries has proven difficult because of underlying gut enteropathy and other health and nutritional issues, particularly when oral vaccines are being used [19,35,36]. Consequently, more novel routes of parenteral delivery need to be explored, as well as mucosal adjuvants that may help overcome the detrimental effects of gut enteropathy. In addition, vaccines should be formulated in such a way as to facilitate their use in future combination vaccine strategies that will likely serve to help ensure better uptake by both private- and public-sector markets [19,28,35,37–39] in the developed and developing world.

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

Traditionally, ETEC vaccine development efforts have focused on the induction of antitoxin and anticolonization immunity. Specifically, studies in animals and human subjects indicate that exposure to LTs, CFs, and CS antigens contribute to protection against ETEC and have potential for use in vaccines [1,2,19]. LT is structurally, functionally, and immunologically related to cholera toxin (CT); in particular, the B subunits of LT and CT are closely related and active immunization with vaccines formulated to contain LT or CTB have given some degree of protection in the field [1,2,19,20,34]. Whereas LT is strongly immunogenic on its own, ST is not immunogenic unless coupled to a carrier protein. To date, no safe ST toxoid is available for use in humans, but as mentioned above, encouraging progress is being made in this important area of vaccine development [1,2,19,22,23]. Renewed efforts to develop a safe and effective ST toxoid have been given further impetus by the recent GEMS and MAL-ED results, which indicate that an effective ETEC vaccine must be able to provide coverage for strains expressing ST alone or in combination with LT [3,4].

When considering CFs as vaccine components, it is important to remember that some CFs are more prevalent than others – e.g., CFA/I, CS3, CS5, and CS6 account for 50–80% of all CF-positive clinical ETEC isolates [36–38]. In addition, some CF/CS antigens are immunologically related to these more prevalent CFs (i.e., CFA/I and CS14) [2,19,21,37], so, depending on the vaccine formulation, cross-protection may be possible. Although CF/CSs may have potential as vaccine components, it has been suggested that an LT-toxoid may help augment CF/CS induced protection against LT/ETEC, at least in young, immunologically naive children. As alluded to above, inclusion of an LT toxoid in the vaccine may help to move potential vaccine strain coverage into the 70–80% range for both infants and young children in developing countries and travelers to endemic areas, since it may help provide coverage for LT-only strains that lack CF/CS antigens [19,20]. Active and passive immunization studies in human volunteers followed by experimental challenge with wild-type ETEC strains have also shown that CF and CS antigens alone or in combination with LT can provide protection [1,2,19,33,35,40]. In addition, as mentioned earlier, including and LT toxoid component in the vaccine may help broaden protection to potentially include other enteric pathogens in some field settings, like Salmonella or Campylobacter [15,16,18,19].

How these human challenge study results translate into ETEC vaccine efficacy in the field remains to be determined. However, prior challenge studies with inactivated whole-cell cholera vaccine (precursor of Dukoral®) were very predictive of field efficacy for this vaccine approach [41], and an LT-based prototype ETEC vaccine given by transcutaneous patch reduced the severity of ETEC illness in a human challenge study [42] and showed protective efficacy against ETEC strains making LT in the field [20]. Consequently, vaccines that show an impact on disease incidence and/or severity in rigorous human challenge models may at least warrant further development and field testing since they may have a positive impact on disease. The field trial results with the LT-patch were also significant because they showed for the first time that skin immunization could protect against an enteric infection like LT-ETEC in a field setting; thus highlighting the potential importance of skin immunization as a novel way to deliver new subunit enteric vaccines, like those being developed for ETEC and Shigella [17,19,35]. The LT-patch study also indicated the potential importance of including other ETEC antigens with LT in order to achieve broader levels of protective efficacy [17].

Efforts to improve vaccine immunogenicity and coverage remain ongoing on several fronts. One of the most intriguing recent developments is a new attenuated form of LT called double-mutant LT (dmLT), which possesses both antigen and adjuvant properties. Data from human subjects and animals indicate that oral and parenteral ETEC vaccine candidates may benefit from adding dmLT to these vaccine formulations to help improve and sustain strong intestinal immune responses [2,19,33,43]. Given dmLT’s unique antigen and adjuvant properties, its inclusion in ETEC vaccine formulations may potentially facilitate vaccine dose sparing and improved efficacy [2,19]. Data presented at the July 2015 8th International Conference on Vaccines for Enteric Diseases held in Edinburgh, Scotland provided the first evidence that inclusion of dmLT in an attenuated ETEC vaccine formulation can have a positive impact on its protective efficacy in a human challenge model [33]. An earlier trial of dmLT with the inactivated whole cell ETEC vaccine candidate ETVAX indicated that the addition of dmLT significantly improved the mucosal immune response to the CS6 component in this vaccine as well as the toxin-neutralizing antibody response to LT toxin [43,44]. Descending-age safety and immunogenicity studies of ETVAX, which are now underway at the icddr,b in Dhaka, Bangladesh, will evaluate the impact of dmLT on vaccine safety and may provide some insight regarding effects on immunogenicity and dose sparing in this ETEC-endemic area (R. Walker, PATH, personal communication).

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 [2,19,21]. 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 two essential steps in ETEC pathogenesis: intestinal colonization and effective LT toxin delivery [21,45].

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 ELISPOT or ALS responses. Cholera studies suggest that T-cell immunity may also be important in protection against Vibrio cholerae [46], which is also a luminal bacterial enteropathogen like ETEC. The extent to which T-cell immunity to the ETEC antigen may contribute to the quality of the intestinal antibody response to this pathogen remains to be determined. However, it is starting to become a greater focus of vaccine development efforts [47,48].

Finally, once an effective vaccine is developed, public health officials will need to consider vaccine schedules and delivery mechanisms. While infants, young children, and travelers are potential targets for ETEC vaccine use, the greatest public health benefit would likely be achieved by introducing an effective ETEC vaccine into the EPI schedule. With this approach, infants in developing countries would gain protection from a primary two- to three-dose immunization series before they move into the latter part of their first year of life, when age-specific attack rates for ETEC start to increase sharply [1,2,19,29]. To be an effective public health tool, it is projected that an ETEC vaccine would have to have 50% or greater efficacy against moderate-to-severe ETEC diarrhea in infants and young children.

3. Technical and regulatory assessment

The availability of appropriate ETEC challenge models will be critical to clinical assessment of how antibodies against CF/CS, LT and/or ST toxoids, and the novel antigens mentioned above may contribute to protection [2,19,30,32]. The ETEC challenge model was first established in the early 1970s, and its evolution and contributions to our understanding of ETEC pathogenesis and immunology, as well as to the evaluation of new treatment and preventive interventions for ETEC, are the focus of a recent in-depth systematic review [32]. In more recent work, extension of the fasting time in volunteers prior to challenge appears to facilitate lowering the ETEC dose needed to see illness in the majority of subjects, thus potentially moving the model closer to doses that may actually be encountered in natural field exposure [30]. Three ETEC strains (H10407, E24377A, and B7A) have been given to a significant number of subjects under US-FDA IND, and in rechallenge studies, infection with H10407 and B7A results in strong immunity against rechallenge with the homologous strain [29,30]. In addition, antitoxin and CF/CS antibody responses in this model appear to mirror those seen among individuals infected with ETEC in the field.

It remains to be determined, however, whether these three ETEC challenge strains can meet all the needs of ongoing ETEC vaccine development efforts. It is anticipated that additional strains may need to be evaluated that are representative of other ETEC pathotypes. For example, all three of the strains outlined above produce both LT and ST, so there may be a need to further identify and develop challenge strains that express only ST or more newly identified colonization factors.

The apparent success in lowering the challenge dose for the H10407 strain of ETEC suggest that this change in fasting time may enable the ETEC challenge model to become more analogous to the cholera model, where doses in the 10^6 cfu range are generally used [41]. This modification also potentially addresses longstanding concerns regarding the ethical issues of exposing volunteers to higher doses of ETEC than necessary to achieve reasonable disease attack rates and that higher doses of ETEC might overwhelm any potential vaccine effects, leading to the premature abandonment of vaccine candidates that may have public health potential [32]. Presently, it is difficult to judge accurately how significant this model change may be for the field. The observations made with the H10407 strain need to be extended to other ETEC challenge strains, and more vaccine immunization and challenge studies need to be done with this new model before its value to the vaccine development field can be fully appreciated. However, initial studies with two vaccine concepts, the live attenuated ACE527 vaccine and the adhesin-based subunit vaccine prototype, have yielded data suggesting its usefulness in the early-stage assessment of vaccine efficacy [33,49].

Another issue is that, despite the clear evidence of protection against diarrheal illness in subjects following experimental infection and to some extent in the field [16,24], clear and consistent immune correlates of protection or functional assays predicting immunity against ETEC infection have yet to be established [1,2,19]. The association between the observed antigen-specific antibody 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 currently are not being measured may also contribute to protection. As mentioned above, the synergistic interaction of anti-LT toxin and anti-EtpA to more effectively block intestinal colonization and toxin delivery in preclinical mouse studies illustrate the potential value of functional read-outs as indicators of antibody activity, as well as tools in looking at the interaction of antigen-specific antibody responses in helping to mediate more complete protection. Therefore, the development of better functional assays for assessing ETEC immunity is an important technology gap that needs to be addressed. For example, high throughput assays are 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, bactericidal assays, much like the ones 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 immune profiling, which may be better predictors of broad protection. These new technologies represent important new tools that could help further accelerate ETEC vaccines toward licensure.

4. Status of vaccine R&D activities

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 (a mixture of four inactivated strains) and ACE527 (a mixture of three live attenuated strains)
Table 1
Development status of current ETEC vaccine candidates (POC = proof-of-concept trial).

<table>
<thead>
<tr>
<th>Candidate name/identifier</th>
<th>Developer</th>
<th>Stage of development</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivated tetravalent whole cell supplemented with LTB–CTB hybrid toxoid; may include dmLT adjuvant (ETVAX)</td>
<td>PATH; SBH</td>
<td>X</td>
<td>[1,2,19,43,44,48,50]</td>
</tr>
<tr>
<td>aroC, omp F, and Omp C-based live attenuated; may include dmLT adjuvant (ACE527)</td>
<td>PATH*</td>
<td>X</td>
<td>[1,2,19,33]</td>
</tr>
<tr>
<td>ZH9 attenuated typhoid vaccine expressing LT-ST toxoid (Typheteor)</td>
<td>Prokarium</td>
<td>X</td>
<td>[19,43]</td>
</tr>
<tr>
<td>Second-generation 120RS attenuated Shigella vaccine expressing CF/CS antigens and LT toxoid</td>
<td>CVD</td>
<td>X</td>
<td>[19,43]</td>
</tr>
<tr>
<td>Anti-adhesin based subunit vaccine</td>
<td>NMRC; PATH</td>
<td>X</td>
<td>[2,19,43,47,49]</td>
</tr>
<tr>
<td>Anti-adhesin-toxoid (MEFA)</td>
<td>KSU; JHSPH</td>
<td>X</td>
<td>[2,19]</td>
</tr>
<tr>
<td>dmLT</td>
<td>PATH</td>
<td>X</td>
<td>[2,19,23]</td>
</tr>
<tr>
<td>LT-ST fusion/LTB–ST conjugate</td>
<td>EntVac consortium; GLOBVAC; STOPENTERICS; PATHb</td>
<td>X</td>
<td>[2,19,23]</td>
</tr>
<tr>
<td>Flagellin; EtpA; EarA; EaeH; Yghj</td>
<td>Variousb</td>
<td>X</td>
<td>[2,19,21,35,45]</td>
</tr>
</tbody>
</table>

a PATH is seeking developing-country manufacturing partners that could help further develop this vaccine concept.
b Novel toxoids and antigens are being explored by a number of investigators from: Washington University in St. Louis; University of Maryland; University of Virginia; University of Bergen; South Dakota State University; Kansas State University; the Sanger Institute; Johns Hopkins Bloomberg School of Public Health; and Antigen Discovery, Inc.

[1,2,19]. Both have been aided in their development by PATH [1,2,19] and were 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 these two, ETVAX is the most advanced – it is a fully formulated, complete vaccine ready for field testing. PATH is working in a manufacturing partnership with Scandinavian BioPharma (SBH) for further development and testing of ETVAX. This vaccine approach builds on and extends the prior work of Ann-Mari Svenerholm and Jan Holmgren from the University of Gothenburg who, along with SBH Vaccines, developed and tested a first-generation inactivated whole cell vaccine through efficacy trials in travelers and Egyptian infants and young children. The Phase 3 studies were conducted in collaboration with Johns Hopkins University, the U.S. Department of Defense, the U.S. National Institute of Child Health and Human Development, the U.S. National Institutes of Health, and WHO [1].

Despite showing significant protection against the more severe forms of travelers’ diarrhea [1,2] and reasonably good immunogenicity in children in both Egypt and Bangladesh, the vaccine was not protective in the Egyptian Phase 3 pediatric trial [1,2,19]. After completion of the Phase 3 trial in Egypt, WHO reviewed the data from the Egyptian studies in the context of an international meeting held in Montreux, Switzerland in 2003 to review progress in ETEC vaccine development and to make recommendations for future directions in research and development [50]. WHO recommended further development and study of the inactivated whole cell vaccine approach for ETEC, with the following directives aimed at improving vaccine efficacy in infants and young children: (1) increase the amount of CF antigens delivered in each dose of vaccine; (2) include CS6 in the vaccine formulation to improve coverage in the field; (3) evaluate the potential value of adding a mucosal adjuvant to the formulation; and (4) in future Phase 3 efficacy trials, the primary endpoint should be protection against severe disease and/or hospitalization associated with vaccine-preventable cases. Aside from the point on efficacy trial design, SBH has successfully addressed the other three WHO recommendations by increasing the CF/CS antigen content per dose, adding CS6, and adding an adjuvant (dmLT) to the formulation [1,2,19,48]. Consequently, there is optimism among many investigators in the ETEC vaccine field that this second-generation vaccine, ETVAX, will show even better immunogenicity, and ultimately protective efficacy, among infant and young children in ETEC-endemic areas.

The ACE527/dmLT candidate demonstrated significant protective efficacy (PE) in a Phase 2b challenge study (PE of 58.5% against ETEC diarrhea of any severity) [33]. At the concentrations of cells in the vaccine, it was necessary to have the dmLT in the vaccine to demonstrate significant efficacy. This vaccine 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 ETEC-specific CF/CS antigens and LTB (constructed in GuaBA Shigella mutants) [1,2]. In a similar vein, the biotechnology company Prokarium has a combined ETEC-typhoid vaccine candidate in preclinical development (Typheteor®) that utilizes an attenuated typhoid vaccine strain to vector an LT-ST fusion protein plus a broad array of colonization factor epitopes [43].

Other ETEC vaccine candidates based on subunits, toxins, or novel antigens are also under development. An innovative, new subunit ETEC candidate using fimbrial tip proteins and administered intradermally with an LT-based adjuvant (mLT) is the most advanced subunit approach. In a recent Phase 1 study, a CfaE (CFA/I tip adhesin) prototype induced strong immune responses at systemic and mucosal sites [2,19,43,47]. In follow-up, the U.S. Naval Medical Research Center (NMRC) also recently completed a Phase 2b immunization and challenge study with a CfaE-dmLT
prototype vaccine, which also yielded encouraging results supporting the concept that, under appropriate conditions, a parenterally administered subunit vaccine may prevent ETEC diarrhea. Based on these promising Phase 1/2b results, NMRC is working with PATH and other partners to accelerate the further development of this concept as a complete vaccine and to optimize the formulation, dose, adjuvant(s), and route of delivery to further improve and broaden vaccine-induced protection [49].

Using a more classic approach, Kansas State University and the Johns Hopkins Bloomberg School of Public Health have developed a multicomponent fusion protein that will deliver the most common CFs as well as an LT–ST hybrid toxoid [2,19,23]. This innovative concept is currently still in the preclinical stage of development. As mentioned earlier, 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 improve strain coverage and efficacy. This work is being carried out by the EntVac Consortium, which is comprised 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 the 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 and for facilitating combined vaccine approaches [2,19,21,35]. However, these approaches have also yet to move beyond preclinical animal studies.

5. Likelihood for financing

In 2011, PATH and BIO Ventures for Global Health (BVGH) published an investment case for ETEC vaccine development, which found that recent increases in donor investment in ETEC vaccine research and development as well as encouraging technological developments and promising field data on the protective efficacy of ETEC vaccine candidates in travelers may serve to help reduce the perceived risk associated with investment in ETEC vaccines [27]. The analysis demonstrated that ETEC vaccines represent a modest investment opportunity for industry with an estimated annual revenue potential of more than US$600 million ten years after global launch, with the public and private sectors in emerging middle-income economies representing approximately 38% of the anticipated revenues. This suggests a significant potential return that may help draw the interest of more vaccine manufacturers in emerging economies, such as India, Brazil, and China. The potential market would also include military and low-income country components. The growing body of evidence about longer-term, post-infection health conditions resulting from travelers’ diarrhea as well as recent data from GEMS strengthening the association between a high ETEC disease burden, poor physical and cognitive development of children in endemic areas, and family-related health costs are also likely to help bolster these market estimates.

Gavi, the Vaccine Alliance has indicated an interest in enteric vaccines. While Gavi has shown interest in a vaccine for ETEC alone, their strongest preference would be for a combined vaccine that includes Shigella or another pathogen. From the market assessment mentioned above, it is clear that the travel medicine industry would also be more enthusiastic about use of a combined ETEC-Shigella vaccine than a standalone product. In addition, recent PATH efforts to collaborate with the LiST modeling group at the Johns Hopkins Bloomberg School of Public Health [19,24–26,35] and investigators at the University of Florida on vaccine impact and cost-effectiveness estimates have started to build an even stronger public health case for the development of an ETEC vaccine that could eventually be used together with vaccines against Shigella or other enteric pathogens in a combination vaccine formulation.

Most ETEC vaccine development efforts to date have been conducted by governmental agencies, academia, and the military. However, in recent years, nonprofit organizations have begun to play a more important role in moving the field forward, which has helped to justify further investment.

In general, financing options for ETEC vaccines are limited but may be further enhanced if ETEC vaccines were part of a combined vaccine approach [35] or if awareness of ETEC disease burden and its impact on more long-term health and family- and national-level economics were better appreciated [51]. Higher disease burden estimates forthcoming from the recent GEMS re-analysis as well as the development of better point-of-care diagnostics could help local, regional, and global public health stakeholders gain a better awareness of the impact of ETEC-associated illness on child health and development.

Acknowledgments

The authors are grateful to the WHO Product Development for Vaccines Advisory Committee for the invitation to prepare the ETEC vaccine landscape assessment. The authors also wish to thank Dr. Deborah Atherly, Laura Edison, and Allison Clifford of PATH for their very helpful review and editing of the manuscript. This work was done with the support of the Bill & Melinda Gates Foundation and the United Kingdom’s Department for International Development. Conflict of interest: None declared.

References

2886

A.L. Bourgeois et al. / Vaccine 34 (2016) 2880–2886


