


The Case for Investment in **Enterotoxigenic *Escherichia coli* Vaccines**

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BIO Ventures for Global Health is a non-profit organization whose mission is to save lives by accelerating the development of novel biotechnology-based drugs, vaccines, and diagnostics to address the unmet medical needs of the developing world. The organization spurs biotech industry involvement in global health product development by increasing biotech and global health partnerships, designing and advocating for compelling market-based incentives, and synthesizing and disseminating critical information and quantitative analysis.

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List of Acronyms

BVGH	BIO Ventures for Global Health	FTA	fimbrial tip adhesins
CF	colonization factor	LT	heat-labile toxin
CFA	colonization factor antigens	NATO	North Atlantic Treaty Organization
CFU	colony forming unit	ORS	oral rehydration solution
DALY	disability-adjusted life year	ORT	oral rehydration therapy
dmLT	double mutant heat-labile toxin	R&D	research and development
EPI	Expanded Programme on Immunization	ST	heat-stable toxin
ETEC	enterotoxigenic *Escherichia coli*	WHO	World Health Organization

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Executive Summary

Diarrhea is the second-leading cause of death among children under the age of five worldwide, killing an estimated 1.5 million and hospitalizing millions more, mostly in developing countries.[1] Beyond its potentially devastating and immediate impacts on health, diarrheal disease can also have long-term implications, including malnutrition and adverse consequences on physical and cognitive development. Infants and young children in the developing world bear the brunt of death and illness from diarrheal disease,[2] but older children and adults in these settings also suffer from its symptoms and the resulting severe, life-threatening dehydration.[3] In addition, travelers and military personnel visiting the developing world are at high risk of suffering from diarrhea. However, these individuals usually have access to immediate and sufficient health care to alleviate illness and prevent death—an option that many children in the developing world simply do not have. Despite this better access to care and treatment of diarrhea, there is growing evidence that acute illness experienced by these visitors to developing countries can lead to more long-term health conditions, ranging from functional gastrointestinal disorder, like irritable bowel syndrome, to reactive arthritis in approximately 10 percent of individuals recovering from an episode of travelers' diarrhea.[4]

One of the leading bacterial causes of diarrhea is enterotoxigenic *Escherichia coli* (ETEC). Bacterial pathogens are spread more easily in areas with poor sanitation and limited access to clean water, a frequent concern in the developing world. As a result, ETEC may be the first enteric illness encountered by many infants and is responsible for an estimated 300,000 to 500,000 deaths each year, mostly among children.[5] ETEC is also the leading cause of diarrhea among travelers to the developing world, as well as one of the top infectious-disease threats to military personnel deployed in ETEC-endemic countries. Recent studies suggest that ETEC incidence among all of these populations—children in the developing world, travelers, and the military—may be even higher than current estimates.[6]

Recent studies suggest that ETEC incidence among all of these populations—children in the developing world, travelers, and the military—may be even higher than current estimates.

An effective ETEC vaccine could have a significant impact on global health, saving the lives of hundreds of thousands of children each year and preventing considerable physical suffering and malnutrition due to repeated bouts of illness. Beyond this, an ETEC vaccine could also benefit visitors to endemic countries, saving millions of dollars in lost productivity and acute and chronic medical costs. In addition, a vaccine may benefit local economies in these endemic areas by providing an effective safeguard against the risk of travelers' diarrhea, which could encourage tourism. Many prevention and treatment options to address diarrheal illness from ETEC exist and are important parts of the solution. Global access to improved sanitation and clean water is an important long-term goal for addressing all diarrheal diseases. However, interventions like new ETEC vaccines could play a critical and complementary role in many parts of the world

where appropriate medical treatment for severe diarrhea and dehydration is limited and access to sanitation and safe water is currently inadequate.

In the last few years, momentum has been building in both the public and private sectors around research and development (R&D) efforts to develop new diarrheal disease interventions, including an ETEC vaccine. A recent report found that although the global share of R&D investment captured by diarrheal diseases remained approximately the same from 2007 (4.4 percent) to 2008 (4.5 percent), the overall number of organizations contributing to these investments increased. Also during this timeframe, public funding for diarrheal diseases from high-income country governments and multilaterals increased substantially, from US$43.8 million in 2007 to 60.4 million in 2008 (up from 38.5 percent to 45.7 percent of global funding), with innovative developing-country governments contributing a further $5.2 million (3.9 percent of the global total).[7] In addition, at least three pharmaceutical/biotechnology companies—Intercell,[8] Sanofi Pasteur,[9] and Novartis AG[10]—have recently shown an interest in ETEC vaccine development. Two of these companies have already made direct investments in specific products, but one appears not to be pursuing further development. Major philanthropic players, including the Bill & Melinda Gates Foundation,[11] the United Kingdom's Department for International Development,[12] and the Research Council of Norway,[13] have also announced new investments in ETEC vaccines over the last several years. In addition, global agencies have made greater commitments to understanding diarrheal disease burden and the impact of specific pathogens. Finally, there have been increased opportunities to leverage private markets for the public good through implementation of tiered pricing schemes, which allow companies to achieve a return on investment in profitable markets, such as the traveler and military segments, while providing those products at substantially lower cost in the developing world.

> The purpose of this report is to provide a rationale for the development of ETEC vaccines.

In 2006, BIO Ventures for Global Health (BVGH) developed a model to evaluate the business case for investment in ETEC vaccines. The model and resulting analysis were presented to representatives at several industry and global health meetings and conferences. In 2008, technological advances since the first analysis and growing interest in the ETEC vaccine field led BVGH to team with PATH to review and update the analysis, develop a revised product profile, and generate revised estimates of global market demand based on this new information.

The purpose of this report is to provide a rationale for the development of ETEC vaccines. Specifically, we aim to increase the awareness of biotechnology and pharmaceutical companies in Europe and the United States, as well as companies in emerging markets like China and India, about the opportunities and potential markets that exist for low-cost and effective ETEC vaccines. In addition, we hope to provide donors and commercial investors with a better understanding of the potential risks, rewards, and gaps in knowledge relative to these opportunities as they consider their own investment strategies.

The report first provides relevant background information for our updated assessment, including an overview of ETEC illness, disease burden, current treatment and prevention methods, and the scientific feasibility and current status of ETEC vaccine development. We then present the market assessment itself, detailing the key inputs used in the analysis and resulting estimates for potential pricing, market penetration, and revenue for each of the markets we analyzed. Given the myriad investment scenarios that can arise in this market, this report focuses on the primary inputs to financial-return scenarios. This allows companies

and their investors to run their own scenarios to estimate net present value and internal rate of return, based on their individual circumstances.

Through this assessment, we found that the potential developing-country, traveler, and military markets for ETEC vaccines continued to expand since the earlier market analysis results that BVGH presented in 2006.[14] In addition, the recent increase in investments in ETEC vaccine R&D, as well as encouraging technological developments and promising field data on the protective efficacy of ETEC vaccine candidates in travelers, may help to reduce the perceived risk associated with investment in this technology. Our analysis demonstrates that ETEC vaccines may represent a moderate opportunity for industry investment with an estimated annual revenue potential of more than $600 million, 10 years after global launch. This opportunity is driven primarily by travelers and middle-income markets (both public and private), but military and low-income markets are also represented. The growing body of evidence about longer term, post-infection health conditions (or sequelae) from travelers' diarrhea also bolsters the potential market. However, it should be noted that it may be challenging to meet the target product profile used in this assessment within the next decade, and there are some key uncertainties that affect the results of this estimate, which are further detailed in the report. These uncertainties are typical for a market assessment conducted years in advance of an actual product and for products that rely on the limited epidemiological data available from developing countries.

This market assessment estimate represents a significant increase over the 2006 estimate, which can be attributed to updates to the major drivers of revenue, specifically: anticipated travel market penetration, the estimated number of annual travelers to endemic countries, and the prices per course in the public low- and middle-income markets. This report presents the opportunities and inherent risks in this vaccine R&D effort, and elucidates the potential global markets that exist for an effective, low-cost ETEC vaccine.

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The Global Need for ETEC Vaccines

An effective and affordable ETEC vaccine could save the lives of hundreds of thousands of children each year[15] and prevent considerable physical suffering and malnutrition due to repeated bouts of illness. It may also be a potentially lifesaving intervention among older children and adults[16] in ETEC-endemic areas, as well as in natural disaster scenarios when large-scale outbreaks of enteric illness, frequently associated with cholera and ETEC, can occur.[17] Beyond these important benefits, an ETEC vaccine could also reduce the most common infectious disease among visitors to endemic countries, saving millions of dollars in lost productivity, acute and chronic illness medical costs, and tourism revenues in countries travelers frequent. Many prevention and treatment options to address diarrheal illness from ETEC exist and are an important part of the solution. Global access to improved sanitation and clean water is an important long-term goal for addressing all diarrheal diseases. However, interventions like new ETEC vaccines could play a critical and complementary role in many parts of the world where appropriate medical treatment for severe diarrhea and dehydration is limited and access to sanitation and safe water is currently inadequate. In addition, growing evidence[18-20] indicates that ETEC disease episodes may lead to a number of functional gastrointestinal disorders in returning travelers or military members, which may serve to push the balance more in favor of prevention, thus giving the development of an safe and effective ETEC vaccine a higher priority.

In 1991, the World Health Organization (WHO) identified the most important causative agents of diarrhea, dysentery, and enteric fever as ETEC, *Salmonella enterica*, serovar Typhi (typhoid); *Shigella dysenteriae* type 1; *Vibrio cholerae* (cholera); and rotavirus. Since then, several candidate cholera vaccines have advanced to clinical trials and two have been licensed, two typhoid vaccines have been licensed, and two vaccines for rotavirus have been approved. In addition, WHO's June 2009 global recommendation that rotavirus vaccination be included in all national immunization programs,[21] as well as a commitment by the GAVI Alliance (an organization that subsidizes immunization costs in developing countries) to fund rotavirus vaccine introduction,[22] indicate that these key organizations will at least consider support for enteric vaccine uptake.

To date, no vaccines have been approved to specifically target ETEC. However, the inactivated whole-cell cholera vaccine marketed under the name of Dukoral® has shown short-term protection against ETEC in both travelers and endemic populations. While the vaccine was licensed with both a cholera and ETEC indication in 29 countries, its lack of significant coverage and limits in duration of effectiveness mean that it does not meet current requirements for an ETEC vaccine to have significant impact on public health, particularly in the developing world. There have been some limited successes beyond Dukoral®, but none of the ETEC vaccine candidates reaching advanced development to date has demonstrated sufficiently broad or prolonged protection to meet acceptable efficacy thresholds for the endemic, commercial traveler, and military markets (see Appendix A).

An ETEC vaccine may be beneficial to endemic countries in more ways than just reducing illness. A number of recent health-related economic studies propose a strong link between

population health and economic growth in developing-country settings. One study indicated that vaccination can play a significant role not only in improving overall population health, but also economic development and national wealth.[23-24] Concerning diarrheal diseases, a recent study estimated that successful uptake of rotavirus vaccine could prevent as many as 2.4 million deaths and more than 82 million disability-adjusted life years (DALYs) in low-resource countries introducing the vaccine between 2007 and 2025. Introduction of rotavirus vaccine was also found to be highly cost-effective in these countries when the gross domestic product per DALY averted was used as the threshold.[25] These results suggest that the benefits of rotavirus vaccination may be much greater than previously estimated, and it is likely that an ETEC vaccine would have similar benefits.

A recent study using a model evaluated what donors and developing-country governments would do if they were asked to choose between implementing a cholera vaccine or improving water quality. The study concluded that, in the long-term, improved water quality would more likely yield an attractive cost-benefit outcome at the community level than vaccination. However, the study also found that in more short-term scenarios, donors and governments viewed vaccination as a more equitable intervention particularly when budgets are constrained because more people may receive the benefit for every dollar invested.[26] In addition, studies have found that a herd protection effect was observed upon the introduction of new cholera,[27] rotavirus,[28] and typhoid vaccines[29] when their use has increased in endemic areas. Such a benefit has additional cost-effectiveness implications for an ETEC vaccine. These studies may provide insights into the decision to implement other enteric vaccines by donors and developing-country governments.

Beyond populations in endemic countries, travelers and the military have also shown an interest in ETEC vaccines. A number of studies have already shown the cost-effectiveness of vaccination for preventing travelers' diarrhea, regardless of trip duration or continent visited, using Dukoral® as a model ETEC vaccine.[30-32] (At least two of these studies were conducted independent of the company producing the vaccine.) Also, among travelers, candidate ETEC vaccines have in some instances been shown to induce at least short-term immunity against other enteric pathogens.[33-36] This benefit is likely to be amplified as ETEC vaccine candidates with broader strain coverage and greater efficacy become available. Recent data also suggest that active vaccination against major causes of travelers' diarrhea, like ETEC, may be further justified because of the growing recognition that acute episodes can often lead to chronic functional gastrointestinal disorders. From the US military perspective, a vaccine against ETEC would likely be cost-neutral if compared to acute-care costs and loss of duty time.[37] However, if one considered the reduction in attendant health care costs and veteran benefits associated with post-deployment chronic sequelae (e.g., irritable bowel syndrome), such a vaccine would likely be cost-saving among troops deploying to high-risk destinations.[38]

There is a clear need for additional studies to assess the societal impact of ETEC diarrhea, including cost-effectiveness studies in all at-risk populations. However, current estimates among American and European travelers project nearly $300 million in medical costs and more than $650 million in lost productivity costs per year for the US and €200 million in medical costs and €450 million for lost productivity in the European Union due to travelers' diarrhea,[39] of which ETEC is by far the most common cause.[40]

The sections that follow provide an overview of ETEC illness, disease burden, currently available treatment and prevention methods, and the scientific feasibility and current status of vaccine development, all of which aim to serve as background information to support our assessment of the ETEC vaccine market.

Disease Description

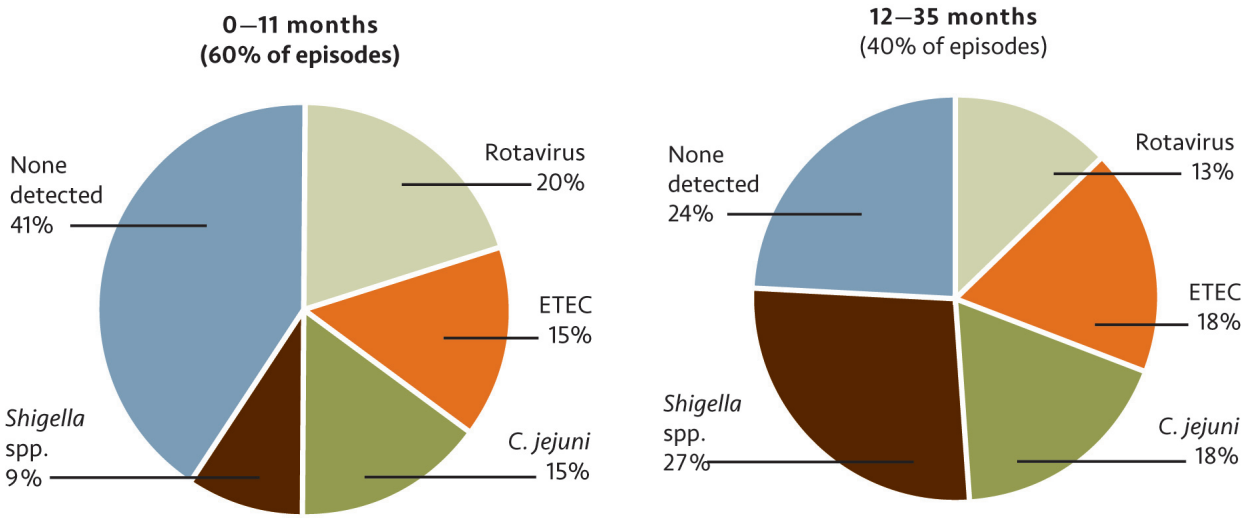
Diarrhea is defined as the passage of frequent, watery, loose bowel movements. In some instances, stools may also contain blood. These symptoms may also be accompanied by cramping, nausea, and fever. The condition has four main causes: bacterial, viral, parasitic, and non-infectious. Different organisms cause similar symptoms through somewhat different processes. Most diarrheal illnesses are transmitted through the fecal-oral route and are spread through contaminated food and drinking water or from person to person as a result of overcrowding or poor hygiene and sanitation. Diarrhea can be life-threatening because it increases the volume of liquid and electrolytes in the small intestine, leading to fluid loss that can cause severe dehydration. If visible blood is present in the stool, the episode will more generally be classified as dysentery.

ETEC are diarrhea-causing bacteria that colonize the mucosal surface of the small intestine and produce toxins that cause intestinal epithelial cells to secrete excess fluid. ETEC produces two toxins: heat-labile toxin (LT) and heat-stable toxin (ST). Some ETEC strains produce one toxin and not the other, while some produce both toxins simultaneously. Many ETEC strains also produce surface proteins called colonization factors (CF) that help them stay in the human intestine and in close contact with mucosal epithelial cells lining the gut surface, particularly in the small intestine. Most of these CFs are fimbrial structures that appear as hair-like projections on the surface of the bacteria. All of these elements represent potential target antigens for inclusion in an ETEC vaccine. Once colonization occurs, the process of ETEC bacteria adhering to mucosal epithelial cells facilitates the transfer of enterotoxins and ultimately drives liquid from the cells lining the intestinal walls, causing the large quantities of watery stool and the dangerous dehydration associated with diarrhea caused by these bacteria.

Disease Burden

According to the WHO, diarrheal disease is the most common illness in the world today.[41] While there are a number of causes of diarrhea, ETEC is responsible for an estimated 300,000 to

FIGURE 1. **Etiology of acute diarrhea among hospitalized children: A multi-center study in five developing countries**[53]



Data compiled from China, India, Mexico, Myanmar, and Pakistan.

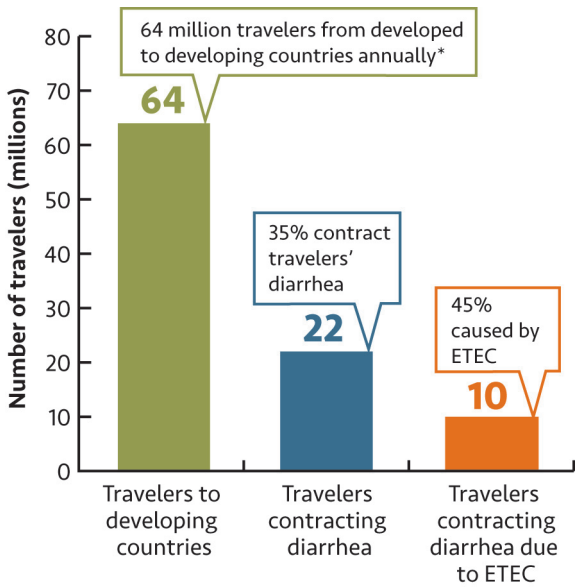
500,000 deaths per year—second only to rotavirus.[42] Because the bacteria is difficult to culture and the symptoms of infection are similar to other types of diarrhea, many experts believe the incidence of ETEC-associated morbidity and mortality is actually significantly underestimated.[43]

Mortality from ETEC is primarily among young children, and studies in Bangladesh and Egypt found that most cases occur in children younger than two years of age. In community and clinic settings, the prevalence of ETEC-associated disease among children less than three years old has consistently ranged between 15 and 18 percent, according to the most recent data available (see Figure 1). In many instances, ETEC may be the first enteric infection encountered by infants in the developing world and essentially all children in endemic countries will have at least one symptomatic ETEC diarrhea episode by their first birthday. In general, multiple episodes during the first few years of life are common until natural immunity begins to develop around 18 to 36 months of age.[44–45] Children older than five years of age, adolescents, and adults are also affected by ETEC, but at lower rates, with older age groups being the most vulnerable.[46–50] In fact, recent studies in endemic areas suggest that ETEC and cholera may contribute to approximately half of the hospitalizations due to diarrhea occurring among these age groups, as well as a significant percentage of the 1.15 million diarrhea-associated deaths estimated to occur in individuals older than five in Africa and Southeast Asia.[51–52]

Mortality is not the only way to measure the burden imposed by diarrhea. Repeated ETEC infection, like other instances of diarrheal disease, is associated with malnutrition, growth stunting, and cognitive deficits in children.[54–60] In the developing world, small children may suffer bouts of severe diarrhea 10 or more times in a year, with several potentially associated with ETEC. The resulting dehydration and malnutrition can delay and diminish mental development, which in turn causes a loss of 15 to 20 percent of productivity in adult life.[61–62] A 2002 study in Brazil found that when children were given a variety of intelligence and function tests, those who suffered repeated bouts of diarrhea in their first two years of life—four to seven years earlier—scored significantly lower than those who did not endure frequent occurrences of the disease.[63] An earlier study also showed that growth shortfalls were significantly associated with early childhood diarrhea.[64]

ETEC is also the leading cause of diarrhea among travelers from industrialized to developing countries, estimated to be responsible for 30 to 45 percent of all cases (see Figure 2).[65] Indeed, nearly one out of every six travelers to endemic regions contract ETEC.[66] Furthermore, recent studies using more sensitive molecular detection techniques suggest that ETEC incidence among travelers may actually be considerably higher with incidence projected in the 45 to 50 percent range.[67] ETEC is responsible for a much higher proportion of diarrheal illness in travelers compared to children living in endemic areas because the range of pathogens generally associated with travelers' diarrhea is much more limited than what infants and young children are exposed to, and the epidemiology associated with exposure may be significantly different between the two groups.

FIGURE 2. **Estimated cases of travelers' diarrhea due to ETEC[68-71]**

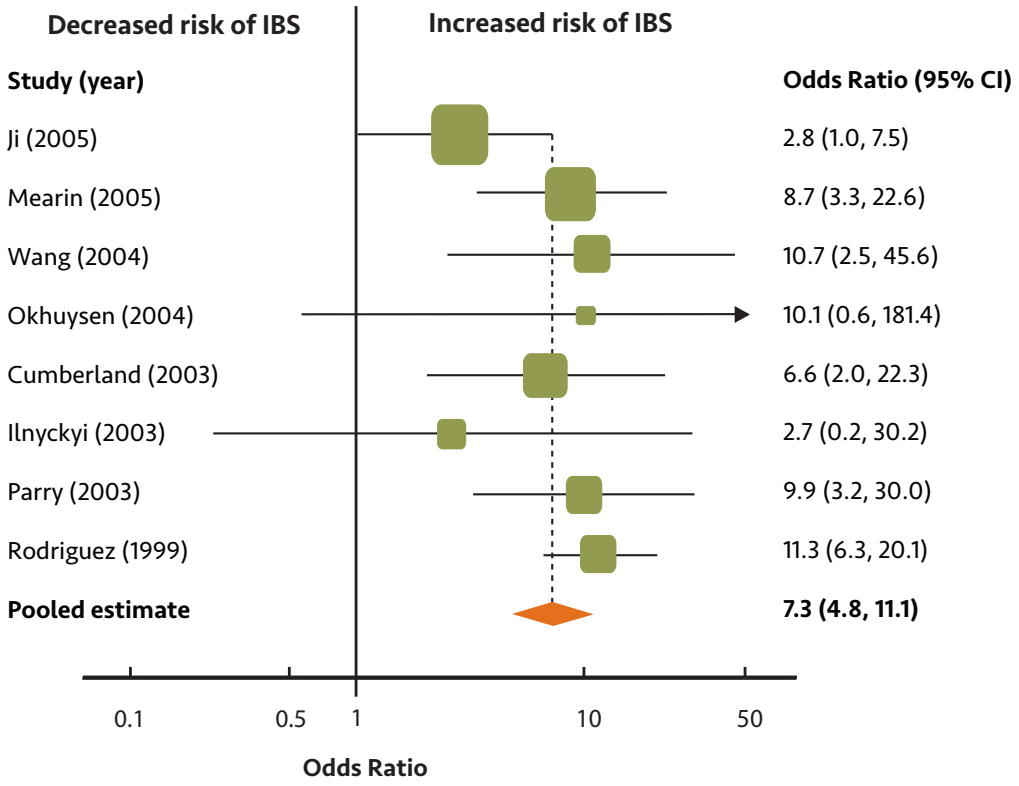


* Adjusted for 15% repeat travelers for one year

Mortality is not an issue for travelers, because ETEC strains respond within a day or two to powerful antibiotics like ciprofloxacin or azithromycin, and oral rehydration therapy can help all patients. Still, travelers' diarrhea is severe enough that 40 percent of affected travelers have to alter planned activities, business meetings, or their itinerary, while 20 percent have to stay in bed for at least one day.[72-73] Using standard criteria from the US Food and Drug Administration for classifying the severity of diarrheal disease episodes, the majority of ETEC-induced travelers' diarrhea cases would fall into the moderate-to-severe category, which is associated with either a change in or prevention of normal daily activity.[74] While it is standard travel medicine practice to provide travelers with antibiotics for use in self-treatment during travel, there is currently a lack of understanding about the frequency of possession and use of symptomatic and antibacterial therapy among travelers to high-risk regions, the clinical response to self-treatment, and the frequency of treatment failures.[75] Furthermore, while chemoprophylaxis is considered appropriate management in some high-risk groups, it is currently not recommended for use in a broad travelers' population setting.[76]

Beyond the acute effects, travelers' diarrhea often results in post-infectious sequelae ranging from functional gastrointestinal disorder to reactive arthritis and even severe and debilitating inflammatory bowel disease (see Figure 3 for a summary of data from the latest studies showing an increased risk of irritable bowel syndrome after acute gastroenteritis).[77-81] Consideration of these sequelae and their attendant medical costs and disability would likely find that the acute disease costs are at least matched, if not exceeded, by the burden associated with the functional chronic sequelae.[82] While a number of studies among travelers have described the risk of these

FIGURE 3. **Summary of independent epidemiological studies evaluating the increased risk of irritable bowel syndrome (IBS) after acute gastroenteritis**[83]



sequelae after an episode of travelers' diarrhea, more research is needed to further elucidate the ETEC-specific risk of these sequelae, as well as the pathophysiological mechanisms of disease.

While travelers' diarrhea may represent an inconvenience for recreational and business travelers, the problem is heightened for military personnel. The US Department of Defense describes diarrheal disease as one of the top infectious-disease threats to deployed American forces. Military studies cite that diarrheal illnesses during deployments have been associated with considerable impact in duty days lost, decreased performance, and operational impacts.[84-85] ETEC episodes among military personnel are similar to that of travelers and have even exceeded 70 percent during deployments to high-risk areas. For example, according to a personal communication from Dr. Mark Riddle, Deputy Head of the Enteric Diseases Department at the US Naval Medical Research Center, in July 2010, among the nearly 2.3 million individual US deployments between 2001 and 2007 to operations in Iraq, Afghanistan, and the Middle East region, it was estimated that there were more than 3.8 million cases of diarrhea, 11.5 million troop days with diarrhea, and more than 850,000 visits to medical services, resulting in more than 1.1 million duty days lost and direct medical costs climbing higher than $124 million.[86] Furthermore, while not directly related to deaths or injury among combat troops, acute diarrhea and its associated symptoms can serve as a significant distraction and compromise an individual's or unit's combat capabilities.[87]

Despite ETEC's considerable disease burden, it is seldom recognized as a significant cause of severe illness and death. A coordinated advocacy effort at country and global levels to increase awareness about the impact of ETEC on global health will be critical for ensuring vaccine uptake and will likely require the development of improved field diagnostics and the generation of more precise epidemiological and disease impact data at a country level.

Current Methods of Prevention and Treatment

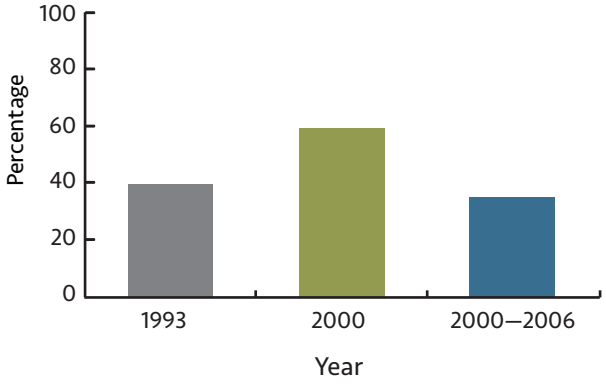
Proven, lifesaving interventions to prevent and treat diarrheal disease, including illness caused by ETEC, already exist. They include prevention methods, such as improved sanitation and hygiene, access to safe drinking water, exclusive breastfeeding, optimal complementary feeding and micronutrient programs, and vaccines against other pathogens (e.g., rotavirus and measles). In addition, treatment options such as oral rehydration solution (ORS)/oral rehydration therapy (ORT) and zinc treatment can speed recovery time. However, while both strategies have been used successfully over the past decades (particularly during the 1980s and 1990s), there are notable limitations and issues with coverage and sustainability. Over the last decade, momentum to support the implementation and scale-up of these interventions in the developing world has slowed, despite the fact that diarrheal disease remains a top killer of children.[88]

In addition, some of the most effective prevention interventions, such as improving water quality and sanitation, tend to be expensive, difficult, and time-consuming to implement and sustain. Breastfeeding provides a wide array of proven benefits to infants and young children; however, it can also serve to delay the risk of infections[89] and is not always practical or acceptable in all cultures. In addition, while multi-pathogen, low-tech alternatives such as cloth filtration of water and hand-washing may be more cost-effective and easier to implement, vaccines are much less dependent upon behavioral change, which can be difficult to sustain over the long term.

Treatment of diarrhea, including ETEC, is complicated by three main factors: uncertainty in recognizing when the disease is not self-limiting, difficulty getting to treatment centers, and delays in receiving therapy—particularly in epidemic situations when supplies and staff are limited and antibiotic therapy is not recommended.[90] ORT, the process of replacing fluids and electrolytes lost

through diarrhea, is a key treatment intervention used in the developing world. While the use of ORT has reduced mortality due to diarrhea by almost 50 percent, there are constraints on its impact, such as its suboptimal usage in developing countries, with reported rates as low as 18 percent in some countries.[91-93] In addition, as another intervention dependent on behavioral change, it is not always long lasting. Implementation of widespread ORS use has been successful with historical utilization rates approaching 70 to 80 percent, but use of ORS has not been consistently maintained over time (see Figure 4, which shows ORS use in developing countries peaking in 2000 and then declining through 2006). And, while zinc treatment for diarrhea is an important way to speed recovery,[94] like ORS, there can be challenges related to geography, access, and cost, particularly if supplies are not locally made.

FIGURE 4. **Percentage of all cases of diarrhea in children under five treated with oral rehydration solution or recommended home fluids in developing countries**[95–96]



Although treatment interventions are lifesaving, primary prevention of diarrheal illness is key to minimizing the associated mortality, morbidity, disability, delays in physical and mental development, and economic consequences. A comprehensive strategy that encompasses all of these prevention and treatment interventions, of which vaccines are a critical element, can be a highly effective way to reduce the incidence of diarrheal disease in the developing world.

Scientific Feasibility of an ETEC Vaccine

Natural exposure to ETEC strains in both endemic settings and among travelers, as well as experimental infection of human volunteers in clinical trials, results in the development of protective immunity.[97-102] For this reason, vaccination is considered a feasible option for disease prevention and control among all risk groups. However, most ETEC vaccine research is still in the early stages, and to date, clinical studies have primarily been conducted with adult travelers with only limited evaluation of candidates in endemic populations and children.[103-105]

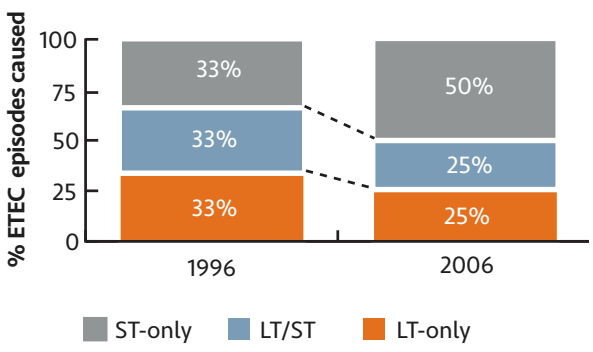
Technological advances in genomics and proteomics have given the research field better tools to examine pathogens in depth, revealing many possible targets for inclusion in an ETEC vaccine. ETEC bacteria bear certain proteins on their surface that appear to help them colonize the small intestine. Because of the key role these proteins play in causing illness and the fact that they are discrete proteins, these CFs are potentially valuable components of a vaccine. Human challenge studies and passive protection trials have shown that CF antigens (CFAs) can induce protective immune responses and that antibodies against these proteins can protect volunteers in passive immunization studies.[106] Therefore, the optimal combination of toxin and CFAs for a specific target population may not always be obvious. In ETEC, there are more than 20 CFAs expressed in different combinations, and some strains of the bacteria apparently produce none that are identifiable.[107] Researchers studying ETEC isolates and the CFAs they produce by geographic region conclude that a candidate ETEC vaccine should at least provide coverage for the following CFAs: CFA/I, CS3, and CS6.[108] However, a candidate vaccine formulated to cover these CFAs would only provide coverage for approximately 50 to 60 percent of the ETEC strains associated with diarrhea in travelers or endemic pediatric populations. Consequently, an additional antigen (or antigens) would need to be included to meet even minimal vaccine coverage thresholds. To

this end, early proteomic research has already identified the fimbrial tip proteins of ETEC CFAs as a candidate-conserved vaccine antigen that could significantly broaden vaccine strain coverage to above threshold levels,[109] and antibodies raised against a model tip protein, CfaE, have been shown to passively protect volunteers against oral challenge with a wild-type ETEC strain sharing this tip protein, thus providing very strong evidence that these tip proteins can indeed be protective antigens.[110]

In addition to CFAs, vaccine candidates could also target the two toxins produced by ETEC bacteria: LT and ST. Based on animal studies, both anti-LT toxin and anti-CFA immunity can sufficiently provide protection at least in the short term, but the strongest level of immunity is achieved when both anti-LT toxin and anti-CFA immunity are induced.[111–112] One challenge is that, as with the CFAs, LT and ST occur in varying proportion, dispersed across ETEC strains, geographic regions, and population types. A second challenge is related to developing a vaccine construct that would offer protection against ST. ST is reportedly present in about 75 percent of ETEC strains; this prevalence, as well as the toxin's established correlation to virulence, is appealing to vaccine developers.[113–114] However, ST may be too small to effectively provoke much of an immune response. In addition, according to a personal communication from Dr. John D. Clements of the Department of Microbiology & Immunology at Tulane University's Health Sciences Center, tests of sera in patients recovering from ETEC diarrhea do not reveal the presence of neutralizing antibodies against ST. Animal model studies evaluating ST toxoid conjugated to carrier proteins to improve their immunogenicity have shown that anti-toxin neutralizing antibody can be induced, but these conjugates have tended to exhibit poor immunogenicity or unacceptable levels of reactogenicity.[115–116] More recently, improved immunogenicity has been achieved in animals when the ST protein has been fused to a genetically detoxified form of the heat-labile enterotoxin of *E. coli* (LTR192G),[117] so there may be a promising new way forward for this vaccine approach.

An LT-only vaccine should face fewer technical hurdles, but is unlikely to provide broad enough protection to meet market needs. Field studies suggest that anti-LT-based immunity may be short lived[118–119] and may only protect against 50 percent of diagnosed ETEC cases because LT and LT/ST strains each cause 25 to 35 percent of the total number of cases (see Figure 5).[120–121] These proportions appear to remain relatively stable over time; however, they do vary by geography, the population studied, and the methodology used for detection.[122–124] In addition, in some endemic settings LT-only strains appear to be less virulent.[125–126] Consequently, an LT-only vaccine may not be able to provide the desired strain coverage and may only provide short-term protection against milder cases. Thus, additional protective antigens such as CFAs or other conserved antigens may need to be added to increase the potential strain coverage, particularly for the toxin and CFA phenotypes associated with more severe illness. However, despite these concerns, several field studies evaluating vaccines based on LT or cholera toxin B subunit have shown levels of protective efficacy above expectations, suggesting that there are either potentially preventive effects being conferred to non-ETEC pathogens and/or there is ETEC disease being prevented that is not being diagnosed.[127–129] Animal model studies suggest that the most effective ETEC vaccine candidate may be one that would be able to induce both anti-toxin and anti-CF immunity.[130–132]

FIGURE 5. **Etiology of ETEC episodes by toxin type**[133–134]



One of the most promising new areas in ETEC vaccine research has evolved from genomic- and proteomic-based antigen discovery efforts which have identified several conserved protein antigens among ETEC strains.[135-136] Of these new protein antigens, fimbrial tip adhesins (FTAs) are the most well-characterized, and they have the potential to substantially broaden strain coverage with fewer protein components because they are conserved across different ETEC CFA types.[137-139] In addition, antibodies raised against a model tip protein, CfaE, have been shown to passively protect volunteers against oral challenge with a wild-type ETEC strain sharing this tip protein and human clinical studies of the anti-CfaE antibody induced in cows and fed to volunteers in milk preparations protected subjects against ETEC challenge,[140] thus providing very strong evidence that these tip proteins can indeed be protective antigens.[141]

Current Research and Development

The current pipeline of ETEC vaccine candidates is somewhat limited, although it represents significantly more activity than this field has seen over the last two decades (see Figure 6 for a review of the current ETEC vaccine landscape by stage of development). The pipeline contains one candidate in Phase 2 studies, five candidates in early-stage Phase 1 clinical evaluation, and four candidates in preclinical development. See Appendix A for a brief summary of the current status of each vaccine candidate in development.

Research on environmental enteropathy

Historically, oral enteric vaccines have experienced poor underperformance (reduced immunogenicity and protective efficacy) among pediatric populations in developing countries.[142-144]

FIGURE 6. **Current ETEC vaccine landscape by stage of development**

Preclinical	Phase 1	Phase 2	Phase 3	Regulatory
LT/ST-based anti-toxin vaccine candidates	LT-based anti-toxin vaccine/adjuvant candidate (double-mutant LT)	Attenuated ETEC vaccine candidate		Inactivated whole cell cholera vaccine*
Fimbrial tip adhesin vaccine candidate	Inactivated ETEC vaccine candidate			
EtpA glycoprotein vaccine candidate	Vectored ETEC vaccine candidate (attenuated *Salmonella Typhi*)			
Siderophore receptor and porin vaccine candidates	Vectored ETEC vaccine candidate (attenuated *Shigella*)			
	Transgenic plant vaccine candidates			

*Licensed for ETEC indication in 29 countries

This phenomenon, often broadly referred to as tropical or environmental enteropathy, includes a wide range of factors that may contribute to this issue, such as the influence of breastfeeding and maternal antibodies, poor nutrition, heavy worm infestation, aberrant microbiota, and host genetic factors. This issue may be one of the most significant challenges to developing effective ETEC vaccines for use among pediatric populations in endemic countries.[145] Complementary R&D efforts are currently under way that may impact the effectiveness of ETEC vaccines when they move into studies designed to evaluate their immunogenicity and protective efficacy among these children. For instance, the development and testing of new mucosal adjuvants, like the LT(R192G/L211A) mutant toxoid from ETEC, may help to improve the mucosal immunogenicity of new ETEC vaccine candidates. In addition, more basic research is being conducted to gain a better understanding of the biological basis for this phenomenon. Finally, research is also under way to examine how alternative delivery methods, such as topical routes like sublingual or intradermal, may improve vaccine effectiveness and circumvent the problem.[146–147]

PATH/Satvir Malhotra

An Assessment of the ETEC Vaccine Market

Study Rationale, Objectives, and Process

In 2006, BIO Ventures for Global Health (BVGH) developed a model to evaluate the business case for investment in ETEC vaccines. A team consisting of BVGH staff, several industry advisors, and the Boston Consulting Group completed the model and the initial analysis and pressure-tested the findings with a broad range of advisors from industry, academia, foundations, global-health providers, and government representatives. The results were then presented to representatives at several industry and global-health meetings and conferences.

Technological advances and growing interest in the ETEC vaccine field led PATH and BVGH to partner in 2008 to review and update the underlying data and assumptions used in the original analysis, develop a new target product profile, and generate revised estimates of global market demand, again in consultation with a wide range of key opinion leaders in the enteric vaccine-development arena (see Appendix B for a member list of the ETEC market assessment team). Through this effort, PATH and BVGH aimed to provide better insight into the risks, opportunities, and needs relative to ETEC vaccine investment for donors, private investors, and companies. The information is also intended to provide key inputs for investors to conduct their own financial return analyses, including calculations of net present value and internal rate of return. By bridging this knowledge gap we hope to help bring additional vaccine developers into this important global public-health area.

To date, limited information has been available on the global market opportunity for ETEC vaccines, and companies have had little incentive to pursue this information on their own. The lack of substantial R&D investment in this area seems in contrast to the growing international recognition that an effective vaccine against this important bacterial pathogen is an unmet global public-health need. We hope this assessment can help fill this knowledge gap. The primary objectives of our analysis are:

- To assess the potential costs and revenues associated with the development and marketing of an ETEC vaccine in order to help inform short- and long-term investment strategies.
- To determine the key drivers of an ETEC vaccine market.
- To define the major uncertainties in determining the market for an ETEC vaccine.
- To provide key inputs for investors in ETEC vaccines to calculate individualized potential financial returns.

Using the model developed in 2006, we prepared market scenarios built around a sample target product profile and estimates of price, country and market sector willingness to pay, and time to adoption. These variables can and will change as epidemiological data improves, ETEC vaccines progress in their development, and international policy evolves. Our model can readily adjust to these different scenarios, yielding quantitative information about potential market opportunities.

Our analysis is based both on primary and secondary research initially conducted in 2006 and updated in 2009 to 2010. We conducted more than 70 interviews to inform our assumptions regarding thresholds for efficacy and price, country decision-making processes for determining whether and when to adopt, and other key factors influencing vaccine supply and demand (see Appendix C for list of interviewees). We also prepared a comprehensive review of the

scientific literature since 1984 on the etiology and epidemiology of diarrheal disease among children in developing countries, international travelers, and deploying military units. This helped us to develop more current estimates of regional- and country-level ETEC disease burden and to better assess the impact that improving diagnostics may have on the precision of these disease-burden projections. Analysis of existing global-health data, precedents, and trends, as well as new information on the potential value of ETEC vaccine prototypes in providing broader than anticipated protection against travelers' diarrhea, further helped to shape our hypotheses. For example, we used health system status and vaccine adoption history to predict country behavior, while demographic and macroeconomic information drove our analysis on country willingness to pay for a vaccine.

The resulting assessment, described in detail below, projects potential costs and revenues from an ETEC vaccine. We have presented a base-case revenue scenario alongside a range of sensitivity analyses to address the inherent uncertainty of market projections.

Key Inputs

To evaluate the global market for ETEC vaccines, it is necessary to understand the key factors that drive demand in different markets. However, ETEC vaccines are still at an early stage of development, and the attributes of the final vaccines are yet to be determined. In order to develop a realistic estimate of market demand, we consulted with a wide range of industry and public-health experts, as well as health officials from a variety of endemic countries. These consultations allowed us to build a realistic target product profile and generate assumptions about development costs, probability of success, development timing, price, and country-level adoption.

Market segments

Given that demand will vary across different markets, we analyzed four different market segments:

- Public markets in low- and middle-income countries.
- Private markets in low- and middle-income countries.
- Travel markets in the United States, Europe, and Asia.
- Military markets in the United States, North Atlantic Treaty Organization (NATO) countries, and non-NATO European Union countries.

We split endemic populations in the developing world into public and private sectors, as each has different needs and expectations. With the public market, governments and/or global-health organizations may pay in part or in full to protect at-risk populations, such as young children, refugees, or victims of natural disasters like flooding. These countries also have private markets used by individuals who can afford to pay out-of-pocket for a vaccine.

Travel and military markets represent important segments as well. Travelers to endemic countries may opt to get vaccinated to avoid ETEC infection. Militaries that deploy troops in endemic regions also have a strong interest in ETEC vaccines.

We conducted primary research exploring the unique characteristics and major drivers of demand for each of these market segments:

- The **low- and middle-income country public market** is primarily driven by the local government's ability to pay (and the likelihood of donor support).
- The **low- and middle-income country private market** is driven by an individual's own ability and willingness to pay.
- The **travel market** is driven largely by the desire not to be inconvenienced by illness while traveling.
- The **military market** is driven primarily by the desire to minimize lost duty days and medical utilization within a theater of operations and reduce post-infection sequelae in returning veterans.

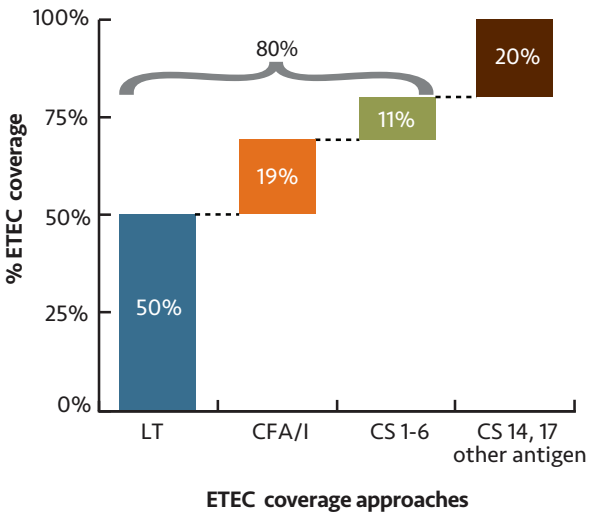
Target product profile

An ETEC vaccine faces a number of hurdles to be accepted in the markets where it is needed most, including:

- Multivalent approaches are likely required to achieve sufficient efficacy.
- Uptake is highly sensitive to the vaccine's impact against all causes of diarrhea, not just ETEC.
- Pricing in target markets needs to be competitive and affordable.
- Achieving sufficient immunogenicity among target populations in low- and middle-income countries may be difficult due to environmental enteropathy.
- The side-effect profile needs to be minimal.
- Implementation in field conditions must be easy.

Because of ETEC's diversity, creating a high-efficacy vaccine requires a multivalent approach, which is something that few vaccine candidates to date have accomplished. As discussed earlier, an LT-only vaccine may only cover up to 50 percent of the ETEC burden and epidemiological data suggest that this may not include the most virulent forms of the disease.[148–150] However, a vaccine with LT and CFA coverage could improve overall efficacy against ETEC. For example, a vaccine targeting a combination of LT with CFA/I and CS 1 to 6, could bring coverage to 80 percent. For a vaccine with 70 percent efficacy, the modeled vaccine will yield a 56 percent adjusted effectiveness against all ETEC (see Figure 7). We selected a 70 percent efficacy level for this assessment as a conservative target given what has historically been the performance of enteric vaccines in endemic pediatric settings compared to adult travelers from industrialized countries.[151–152] We also assumed that, based on past enteric vaccine performance (e.g., Dukoral® and current rotavirus vaccines), protective efficacy is likely to be better against severe life-threatening diarrhea than it is against mild disease.[153–155]

FIGURE 7. **Reaching high efficacy requires multivalent approaches because of ETEC diversity**[156–158]



Market-based efficacy thresholds

Based on feedback from our interviews, we learned that uptake may be sensitive to the vaccine's impact against all causes of diarrhea (which includes both infectious and non-infectious causes, but is primarily presumed to be due to bacteria, parasites, and viruses), not just ETEC. For instance, health officials in endemic countries suggested that an ETEC vaccine that provides protection against at least 10 percent of all forms of diarrhea would be only marginally acceptable.

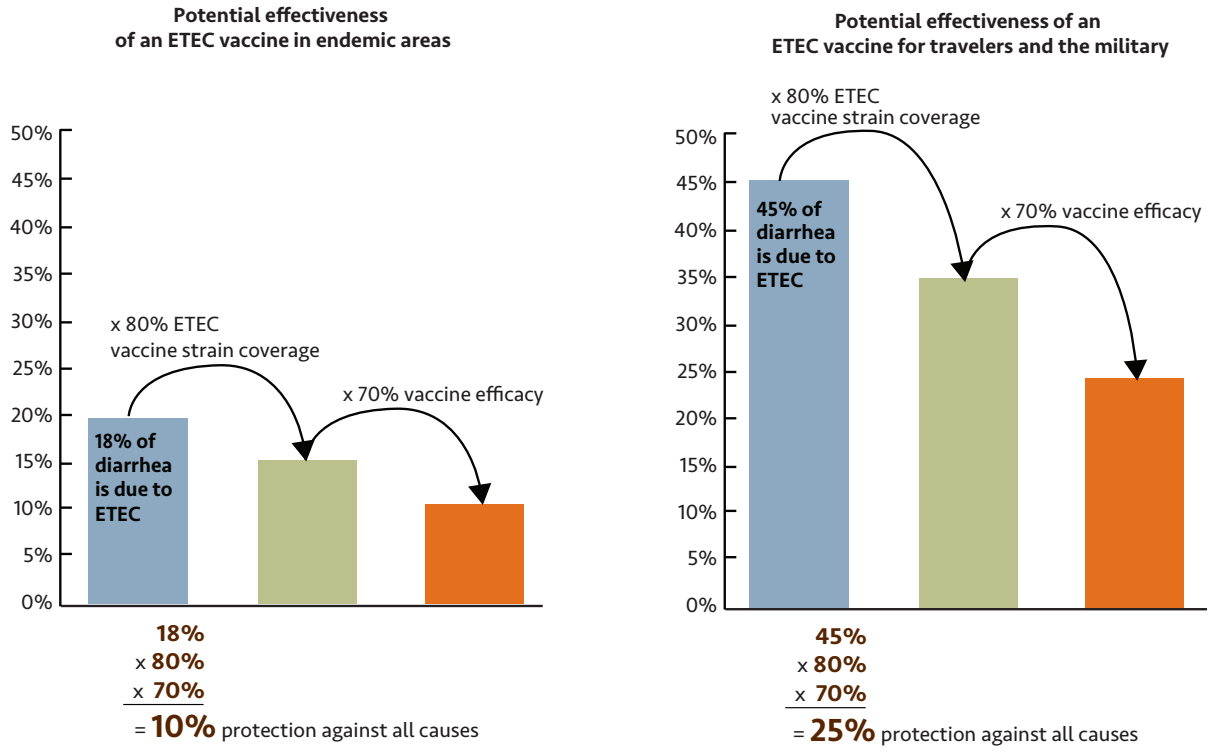
Information on the travel market was less certain. While traveler adoption is likely to be highly sensitive to the level of efficacy against all causes of diarrhea, we heard differing views from travelers' physicians and their patients. Acceptance of an ETEC vaccine will depend upon how well a product meets consumers' and physicians' expectations for protection against diarrhea. Travel health experts we interviewed did not perceive a vaccine that reduces a traveler's risk of diarrhea by 30 percent as highly desirable because antibiotics like ciprofloxacin or azithromycin can relieve symptoms in 12 hours or rifaximin prophylaxis can prevent 70 to 80 percent of episodes in travelers.[159] (Current practice guidelines do not recommend general use

of antimicrobial chemoprophylaxis.[160] However, rifaximin, which is licensed for the treatment of diarrhea by the US Food and Drug Administration, has been found to effectively prevent travelers' diarrhea among short-term travelers in three studies. This has led to its off-label use.[161–163]) Travel health experts also emphasized the importance of combination vaccines that could result in greater overall reductions in the risk of travelers' diarrhea. This is an important area that warrants further market research. Furthermore, with new data documenting the association between travelers' diarrhea and long-term sequelae, a more complete understanding of the potential preventable burden of disease is emerging and could result in higher uptake of an ETEC vaccine. Finally, as mentioned earlier, the protective efficacy of some ETEC vaccines against all causes of travelers' diarrhea has been higher than anticipated, which could make vaccination an option that is potentially more cost-effective and more competitive with antibiotic treatment or prophylaxis.[164–170]

The US military has defined criteria for the various target product profile aspects. They have established a threshold efficacy of 80 percent against all severe ETEC disease to support vaccine acquisition. The efficacy against moderate-to-severe ETEC in our target profile is 70 percent, so the efficacy against severe disease alone would likely be higher (80 percent or more) based on the profiles of other enteric vaccines such as rotavirus[171] and cholera.[172]

Clearly, the percentage of strains covered will be a key factor in determining a vaccine's success. Overall efficacy against all causes of diarrhea is dependent upon the range of enteric pathogens covered in the formulation, particularly the percentage of all ETEC toxin and CFA phenotypes covered by a vaccine, the vaccine's efficacy against those strains, and the prevalence of ETEC in the population (see Figure 8).

FIGURE 8. **Potential effectiveness of an ETEC vaccine against all causes of diarrhea**



- In endemic countries, the proportion of diarrhea due to ETEC is estimated to be 18 percent, so if a vaccine meeting the target product profile could cover 80 percent of ETEC strains and is 70 percent effective against moderate to severe ETEC disease, such a vaccine could achieve 10 percent protection against all causes of diarrhea.
- For travelers and the military, the proportion of diarrhea due to ETEC is estimated to be 45 percent, so if a vaccine meeting the target product profile could cover 80 percent of ETEC strains and is 70 percent effective against moderate to severe ETEC disease, such a vaccine could achieve 25 percent protection against all causes of diarrhea. Based on epidemiological data, it is assumed that the proposed vaccine construct would cover more severe ETEC-associated disease, meeting the US Department of Defense's target product profile for efficacy.

We evaluated a range of scenarios in our effort to define a target product profile. For the purposes of this analysis, we focused on a vaccine that has the potential to meet the needs of all markets and is achievable given the current state of vaccine development. Table 1 outlines the minimally acceptable characteristics of this modeled vaccine: two- or three-dose regimen administered to infants on the Expanded Programme on Immunization (EPI) schedule that provides 80 percent coverage of ETEC strains and 70 percent vaccine efficacy. The vaccine would provide protection for at least two years. While developers may pursue multivalent vaccine candidates with different combinations of toxins and/or CFAs, we believe the vaccines must provide, at a minimum, the levels of protection against ETEC and all causes of diarrhea specified in the target product profile.

TABLE 1. **ETEC vaccine target product profile**

Parameter	Minimum Requirements
Strain coverage	LT and CFAs (CFA/I and CS1 to 6) theoretically yield 80% strain coverage
Efficacy	70% minimum—all severity (require higher efficacy [80% or more] in more severe ETEC-associated diarrhea and adult travelers)
Route of administration	Oral or alternate route that induces intestinal mucosal immunity (e.g., transcutaneous, sublingual, or intradermal may be options)
Regimen	Endemic: 2 to 3 doses compatible with EPI schedule Travelers/military: 2 doses with short interval (e.g., days 0 and 14 to 21)
Duration	Confers protection for at least 2 years, with boosting possible to extend protection
Formulation and stability	Powder, solid, or liquid stable at 2–8°C (may include adjuvant)
Protection threshold	Endemic and travel markets: 10% against all causes of diarrhea Military market: 80% efficacy against *severe* ETEC disease

Development and production costs

The costs of developing any new vaccine are high, and accurate numbers for past development costs are elusive at best. Rather than relying solely on existing benchmarks for these costs, we also conducted a number of interviews with industry and global-health experts to arrive at a range of development costs for an ETEC vaccine with our target product profile. Both private and public funding often contribute to these development costs, and historically, a large proportion of R&D costs for new vaccines against infectious diseases have been covered by the public sector. However, the proportion covered by each will vary by project.

Our analysis, outlined in Figure 9, resulted in an attrition-adjusted range of $339 to $624 million (without preclinical costs and before addressing the investor's required return or interest costs). Although they are generally not considered "typical" development costs and do not impact the attrition-adjusted totals, we also included estimates for regulatory and post-marketing costs in this figure because they remain important elements in making a new vaccine available to the relevant markets. The estimates for post-marketing costs used here include activities such as effectiveness studies, demonstration projects, and surveillance work, within each of the markets discussed in this report.

Based on our interviews, we also estimated an initial manufacturing cost of 75 cents to $1.50 per dose for manufacturers in developing countries. Initial manufacturing costs of $1.50 to $3.00 per dose were assumed for developed-country manufacturers. We assumed startup costs for a 60 million dose-per-year production plant of $45 and $85 million, for developing and developed countries respectively, three years' time construction and commissioning, and a nominal 10-year equipment life.

Timing

There are currently six ETEC vaccine candidates with potentially broad applications, including endemic country markets, in Phase 1 and 2 trials. Since our analysis aims to estimate the business case for a product that would meet broader market needs, entry could occur within eight to ten years, assuming development challenges can be met.

FIGURE 9. **Costs of ETEC vaccine development**

Non-attrition-adjusted costs (millions)

	Research & Discovery	Phase 1	Phase 2	Phase 3	Process Development	Regulatory	Post-Marketing Activities	TOTAL
LT-only vaccine	$14–24	$10–12	$19–36	$159–190	$12	$2–5	$20–75	$236–354
Multivalent vaccine	$26–36	$16–22	$32–45	$184–214	$12	$2–5	$20–75	$292–409
Probability of success	10–30%	72–75%	79–80%	71–85%				
Estimated duration	3 years	4 years	4 years	4 years		1–2 years		

Attrition-adjusted totals range from $339 million to $624 million

Note: Does not include preclinical development

Pricing and Market Penetration

As with other vaccines, pricing of an ETEC vaccine will need to be far lower in the developing world than in the developed world if its use is to become widespread. Indeed, our analysis assumes differential pricing within each of six identified market segments (see Table 2).

TABLE 2. **Differential pricing by market segment**

Market Segment	Price
Low-income, endemic-country public market	$2 per regimen
Middle-income, endemic-country public market	$6 per regimen
Low-income, endemic-country private market	$20 per regimen
Middle-income, endemic-country private market	$60 per regimen
Travel market	$100 per regimen
Military market	$75 per regimen

To simplify and focus the analysis on the overall potential revenue for ETEC vaccines, we assume one vaccine manufacturer will receive approval and enjoy 100 percent of the market for the duration of the modeling period. We have not modeled for earlier entrants or the effect on total market demand and resulting revenue changes, nor have we adjusted pricing in the event of earlier entrants. If the innovator is a developed-country manufacturer, we acknowledge that technology transfer to an emerging-market supplier may increase the likelihood of meeting pricing thresholds in the developing-world markets. Our model assumes fixed pricing in each market segment and has not made adjustments for inflation or other price increases.

Public markets in low- and middle-income endemic countries

Based on our interviews and research, we expect an ETEC vaccine with the right profile and price to have strong uptake in public markets. However, adoption will be highly sensitive to price. Many of the endemic-country officials we interviewed suggested that they would pay, on average, no more than $2 per regimen for an ETEC vaccine. Other studies suggest a similar upper bound.[173] In contrast, however, current pricing of rotavirus vaccine in Latin America suggests that this number may be an underestimate. In recent years, the GAVI Alliance has also paid higher prices for newer vaccines (e.g., $10.80 per regimen for a combination vaccine against diphtheria, tetanus, whooping cough, hepatitis B, and *Haemophilus influenzae* type b [DTP-hep B-Hib] and $16.00 per regimen for a rotavirus vaccine). However, these prices are highly subsidized to the countries with average country co-payments of $0.30 to $0.60 per regimen. For the purposes of this analysis, we assumed prices for the public markets set at $2.00 per regimen in low-income countries and $6.00 per regimen in middle-income countries.

ETEC vaccine developers could see their products reach a significant number of people if WHO decides to include it in the EPI schedule. The EPI protocol presently calls for routinely vaccinating children against diphtheria, *Haemophilus influenzae* type b, hepatitis B, measles, polio, tetanus, tuberculosis, and whooping cough, and more recently rotavirus and pneumococcal have been added. To project the likelihood and timing of an ETEC vaccine being included in EPI, we considered:

- A country's prior history of adopting other new vaccines, using timing of hepatitis B vaccine adoption as a proxy.
- Current capacity of a country's vaccination program, using coverage levels for DTP vaccine as a proxy.
- A country's gross national income per capita and access to donor support, particularly the GAVI Alliance.

Private markets in low- and middle-income endemic countries

Private-market uptake of an ETEC vaccine in endemic countries will depend on individuals' ability and willingness to pay out-of-pocket for protection against diarrhea caused by this pathogen. Markets have demonstrated that a large fraction of the population is also willing to pay for access to new vaccines before they are available through public markets.

We assume that some individuals in low- and middle-income countries will be willing to pay one-half to one full day's wage for vaccines on the private market. A study of the Brazilian market supports this assumption. The researchers also found that 50 percent of individuals able to pay are willing to pay a day's wages for EPI vaccines, even when the vaccine is available at no cost through public markets.[174] In this analysis, however, we assume that once a product has been introduced in the public market, the portion of the population willing to pay for access will drop by one-third.

Our analysis also assumes that private-market prices in ETEC-endemic countries would be 10 times greater than public prices. This estimate is based on an average of prices charged to private payers in Brazil for vaccines against *Haemophilus influenzae* type b and rotavirus.[175]

Travel market

Travelers to endemic countries could represent a major source of revenue for ETEC vaccine producers. About 64 million people travel from developed to developing countries annually, when adjusted for 15 percent repeat travelers.[176] Of these, about 30 to 50 percent, or approximately 26 million people, experience diarrheal illness, of which 30 to 45 percent is caused by ETEC. The travel market has been primarily defined by visitors from the United States, Canada, Europe, and Japan to ETEC-endemic areas for business or holiday travel. However, it is important to note that emerging economies, like China, may represent a new, rapidly evolving travel market as their role in African economic development expands, which could increase the size of this market in future analyses.

Travelers who are motivated enough to go to travel clinics before venturing to developing nations are the likeliest purchasers of an ETEC vaccine. Yet few travelers actually do get vaccinated against any pathogen. For example, just three percent of all travelers get vaccinated against hepatitis A, and only two percent are vaccinated against typhoid.[177]

The experiences with hepatitis A and typhoid vaccines present available baseline proxies for estimating potential market penetration and the likely speed of uptake. However, ETEC-associated travelers' diarrhea is more common than hepatitis A by a factor of 100[178] and more common than typhoid by a factor of 2,000.[179] Even considering the lower relative severity in a minority of cases for ETEC compared with these other diseases, the high incidence of disease, combined with increased understanding of how these vaccines might prevent chronic gastrointestinal sequelae, may lead to stronger support among travel medicine physicians for the use of this vaccine and higher levels of comparable vaccine uptake. In addition, an ETEC vaccine could be more beneficial, as well as more cost-effective, for a larger proportion of travelers.[180]

Military market

Military purchasing could also have a substantial impact on the commercial potential of ETEC vaccines. Based on interviews with US Department of Defense officials, we estimate that the US military would procure enough vaccines to immunize all troops deploying to high-risk regions for travelers' diarrhea, as well as civilian beneficiaries who travel to these areas.

The US military values vaccines, as indicated by its present purchasing contracts for 13 separate vaccines. According to a personal communication

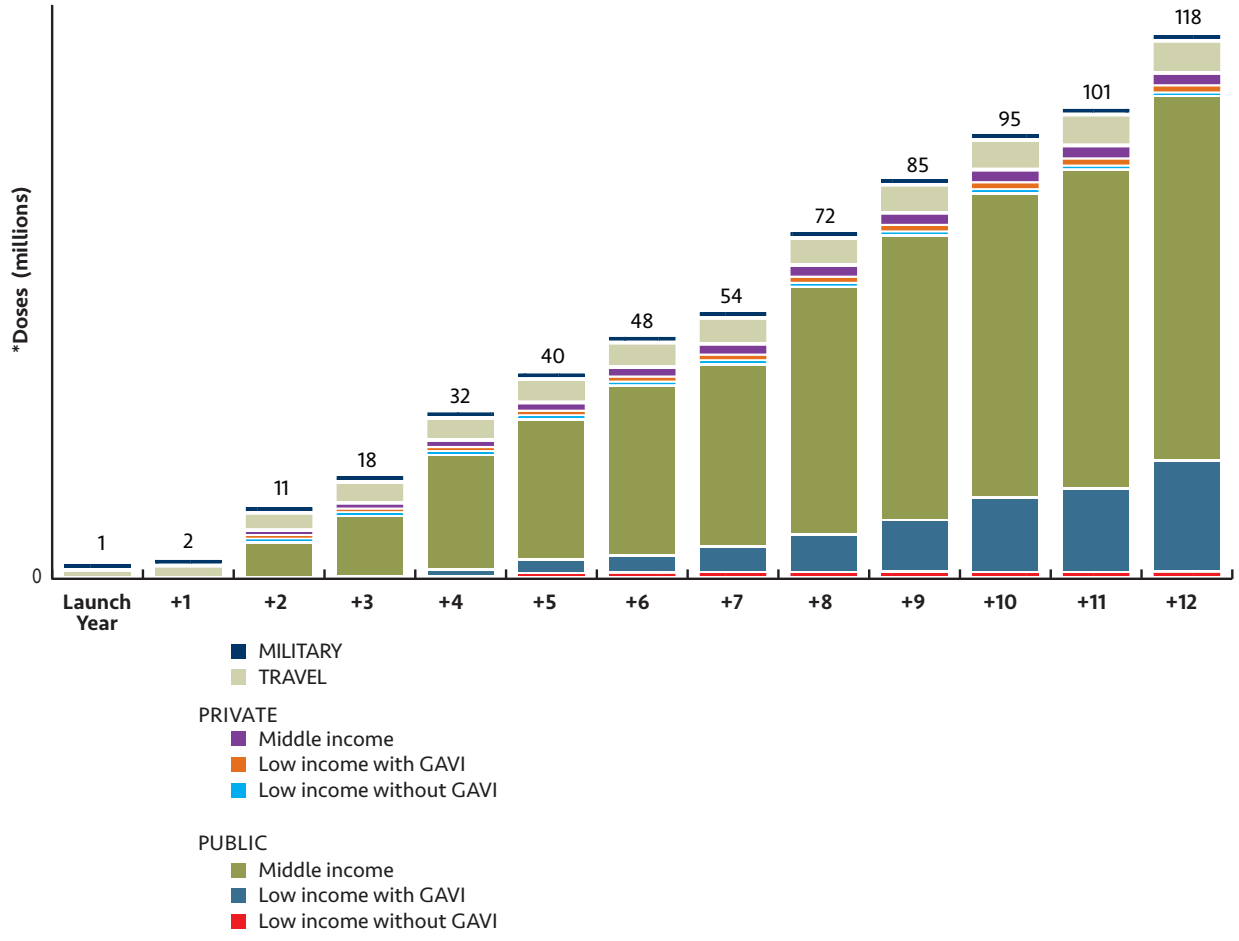
from Dr. Mark Riddle in July 2010, developers would be dealing with a single purchaser known for demanding volume discounts that are substantially lower than the travel-market price. In the case of an ETEC vaccine, we have estimated that the military would pay $75 per regimen, versus $100 per regimen in the travel market.

Adoption would be immediate rather than gradual, and dosing would then be expected to hold steady over many years. Because NATO militaries typically follow US medical intervention policies, we have extended our assumptions on military uptake to all NATO military personnel. However, adoption of any vaccine would require a demonstration of cost-effectiveness prior to acquisition.

Distribution Channels

We expect ETEC vaccines to rely on existing distribution channels within the various markets, making distribution more a question of procurement than logistics. Children within most endemic populations, where the greatest impact will be felt in terms of alleviating pain, suffering, and mortality, currently receive several immunizations through EPI. Our current base-case product profile is compatible with the EPI schedule, and uptake of the vaccine by EPI would provide access to a significant portion of both the public and private markets in endemic countries. Based on current delivery channels for pediatric vaccines in most developing-world settings, candidate vaccines that are not compatible with the EPI

FIGURE 10A. **ETEC vaccine doses by year and market**



*Based on 2 doses per course presentation

schedule would be at a serious disadvantage for uptake in these markets. Consequently an R&D effort to develop a final ETEC vaccine formulation that could be used effectively within the current EPI program is considered an important developmental cost for the vaccine at this time. However, an ideal ETEC vaccine would be one that would be compatible for distribution within both the travel medicine and endemic markets.

Given the sophistication of the health-care delivery systems in the developed world, access to vaccines is not a significant barrier. But vaccine developers do need strategies to reach them and encourage demand from travelers. Convincing travel clinics to stock and recommend the product and inform patients of its existence are critical steps.

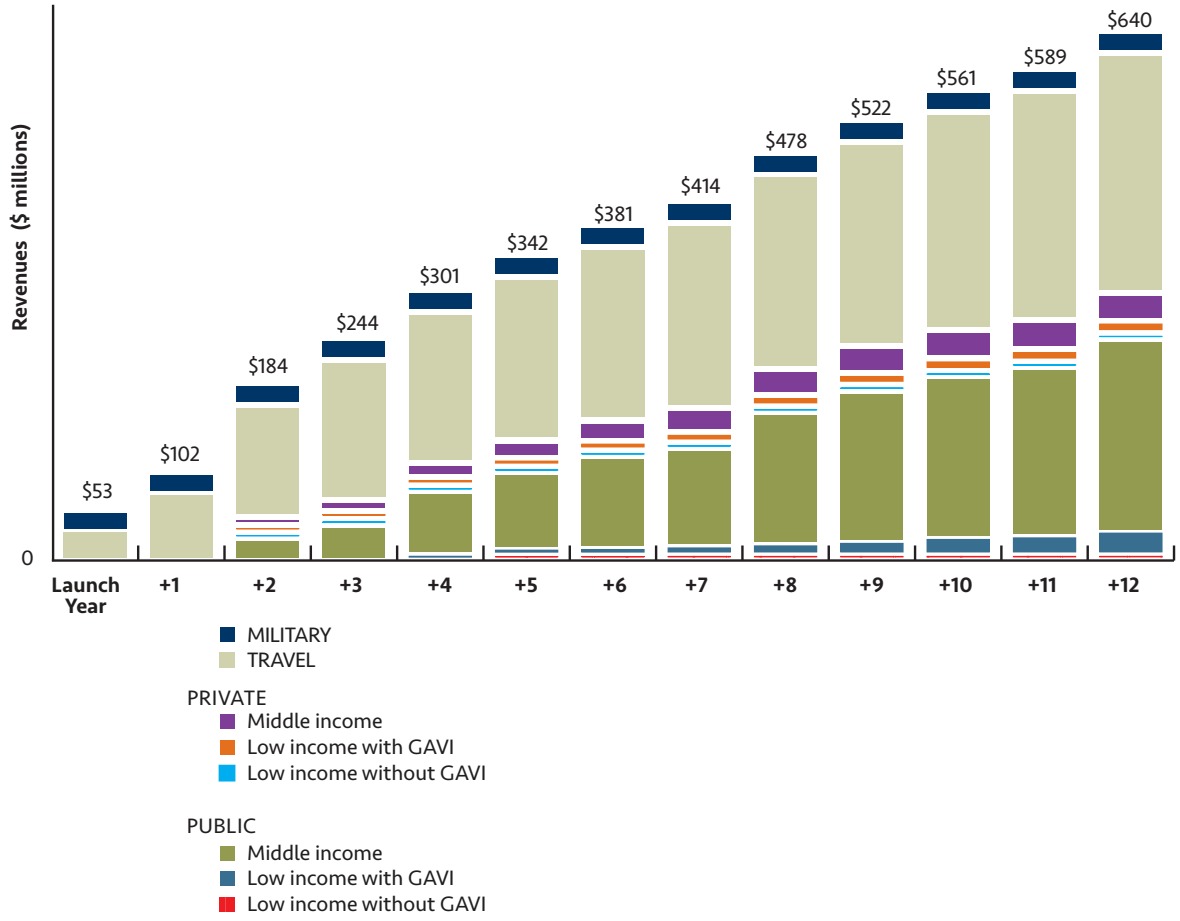
The US and NATO armed forces have a robust distribution system as well. Therefore, we anticipate that uptake would be rapid once the adoption decision and procurement process were completed.

Results of the Base Case

Market demand: Estimated doses and revenue per year, by market

Applying our assumptions, as described in the sections above, we derived global market demand estimates for each market segment (see Figures 10A and B). Global demand is estimated to reach 118 million doses per year, corresponding to more than $600 million in revenues, 12 years post-launch.

FIGURE 10B. **ETEC vaccine revenues by year and market**



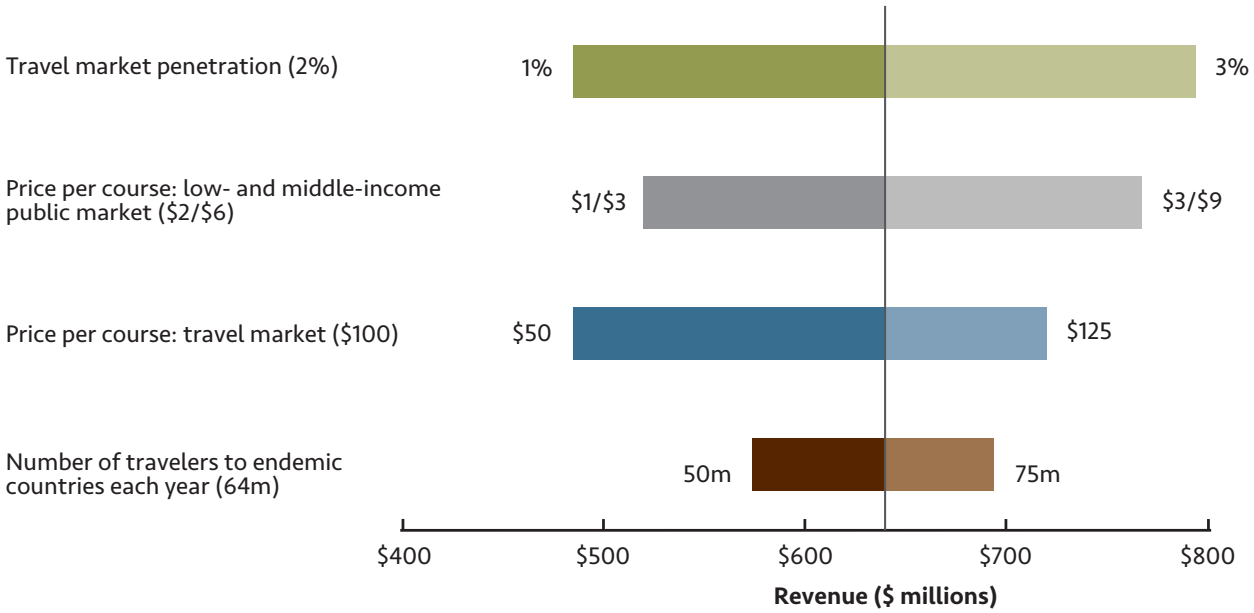
LOW- AND MIDDLE-INCOME MARKETS: Not surprisingly, public- and private-market revenues would be dominated by middle-income countries, rising steadily over time. Within 12 years of launch, demand for vaccine is estimated to be just over 100 million doses per year with public-market revenues peaking at around $274 million per year. While private-market doses represent only a fraction of the public-market doses, peaking at just over 2.5 million per year, we expect faster uptake in private markets, with a decline after public-market availability of the vaccine. Private-market revenues reach $35 million per year.

TRAVEL AND MILITARY MARKETS: Uptake among travelers is projected to increase by the rate of growth in travel to endemic countries—about five percent per year. Under base-case assumptions, the demand for an ETEC vaccine would be similar to typhoid given comparable pricing, or two percent of travelers. We expect military uptake to be stable following almost immediate ramp-up, and under our base-case scenario of low deployment of US and NATO forces, we estimate the demand for an ETEC vaccine to be 500,000 doses per year. The travel and military markets are also important sources of revenue. For travelers, if uptake follows the curve of typhoid vaccines, we expect peak revenues of $309 million with approximately 6 million doses sold. For the military, we estimate a peak market of $21 million based on sales of 0.5 million doses.

Sensitivity analyses

Figure 11 illustrates the key influences on revenue estimates within the market model. A small change of one percent in travel market penetration shows a change of over $100 million in revenue. Changes in the price per course in the public markets of endemic countries and the travel market also have significant effects on revenue as illustrated below. The number of travelers to endemic countries also drives changes in revenue.

FIGURE 11. **Major drivers of revenue estimates**



Revenue = Total annual revenue 10 years post-global launch of vaccine, all markets

( ) = base case value

Figure 12 displays three different uptake scenarios in the travel market—base case, high, and low—and the expected revenues associated with each one. The high-uptake scenario reflects a three percent uptake rate for the vaccine, similar to hepatitis A. The low-uptake scenario represents a one percent uptake rate. On this basis, peak year revenues range from approximately $155 million to $464 million.

Public-market demand and revenues are highly influenced by uptake in middle-income countries. Two scenarios are shown in Figure 13—the base case that includes vaccine adoption in 50 middle-income countries and a more conservative situation reflecting adoption in 25 middle-income countries.

FIGURE 12. **Potential travel market revenues based on three uptake scenarios**

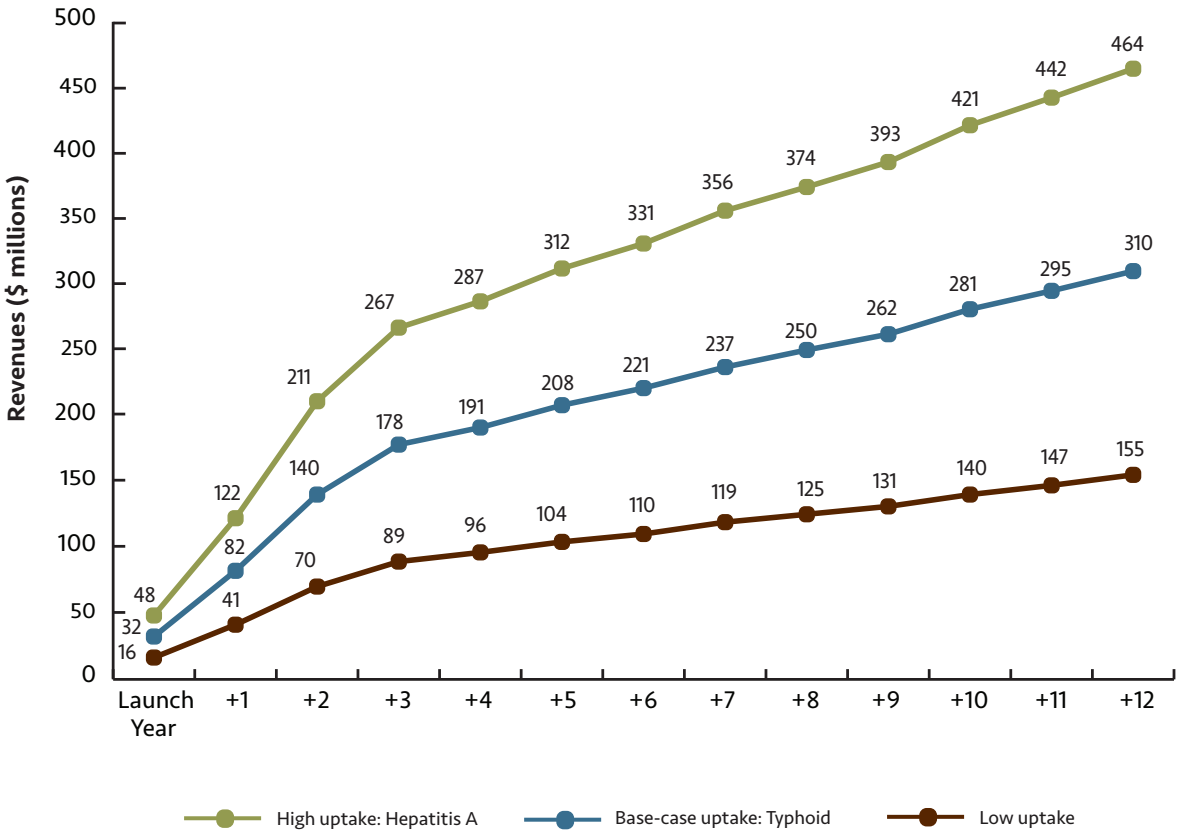
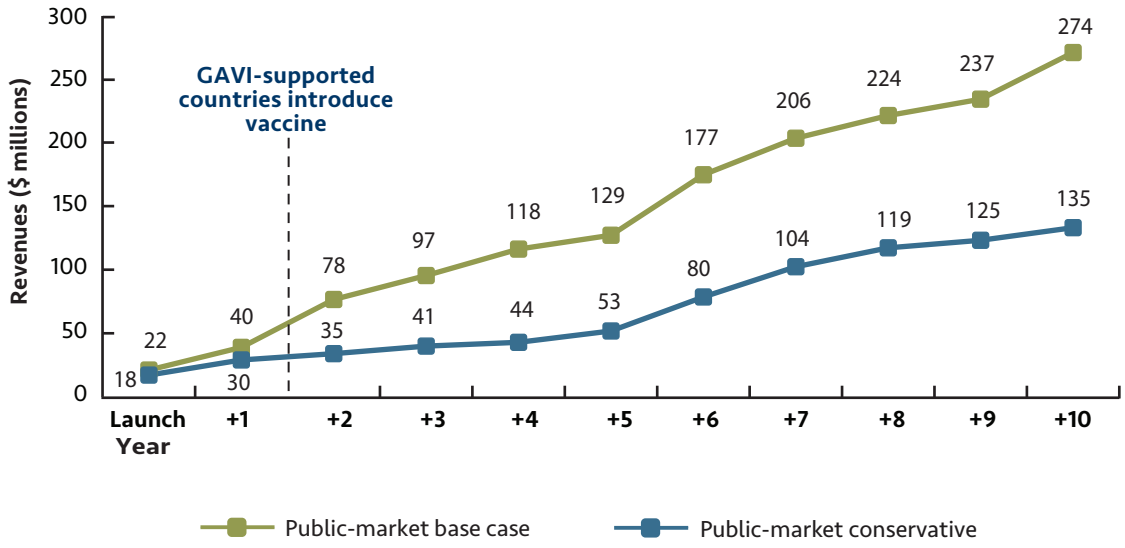


FIGURE 13. **Public market revenues for an ETEC vaccine**



For military market demand, baseline adoption assumes vaccination of newly deployed US and NATO troops to ETEC-endemic regions. A conservative scenario assumes only US demand for an ETEC vaccine, excluding NATO, resulting in estimated revenues of $16 million, while an aggressive scenario assumes war-time troop deployment, with associated revenues of $44 million per year.

Limitations

We recognize that there are some limitations to this analysis. Our results assume that one product will meet most market needs, although alternative scenarios (e.g., one product profile for travel and military markets and one for children in endemic countries) are also a possibility. However, our estimated revenues by market, as well as our estimates of development and manufacturing costs, should remain pertinent to any developers considering investment in ETEC vaccines. As vaccine development evolves, future market assessments can reflect current and projected development and financing scenarios.

In addition, the results of this analysis may not accurately reflect the future vaccine market for ETEC due to the significant challenges in projecting demand for a new vaccine. Whether endemic, travel, or military markets, there is a great deal of uncertainty, and many factors may influence the decision to use a vaccine and the extent to which it is adopted. Some of these key uncertainties that could affect the results include: burden of disease, vaccine strain coverage and efficacy, effectiveness thresholds, travel-market uptake, and endemic country-market uptake. However, we attempted to address these uncertainties by setting broad ranges for vaccine uptake in each market and running sensitivity analyses to measure the impact of the variations on revenues.

Challenges and Opportunities

Challenges

While conducting this assessment, we identified several significant challenges to our understanding of ETEC disease and demand for a vaccine.

LIMITED DISEASE-BURDEN DATA. Any illness with multiple causes represents a challenge to medical science and diarrhea is no exception. Our efforts to forecast demand for an ETEC vaccine are no better than the available disease-burden data. Country-specific etiological studies based on the most advanced and sensitive diagnostic techniques are limited in number, yet certainly possible, as has been demonstrated with the improved availability of rotavirus disease-burden data collected in recent years. We believe that such studies are critical to defining the burden and potential reduction of the overall morbidity and mortality associated with ETEC infection, regardless of the solutions being considered.

Beyond the acute effects, there are also gaps in our knowledge base related to linking the causes of post-infectious sequelae with particular pathogens. Furthermore, the likely impact of ETEC on individual health, cognitive function, and productivity in endemic countries would also benefit from further study.

Endemic-country awareness of the true impact that a disease has or may have on a country's population is fundamental to making sound health-policy decisions. Policymakers in some endemic nations are unaware of the significance of ETEC and the burden of diarrheal illness. Unless the level of awareness improves over time, the likely impact of an ETEC vaccine may be limited.

TECHNICAL HURDLES. ETEC vaccine development faces stiff technical hurdles. A global ETEC vaccine needs to be efficacious at minimum levels, as stated above, and have an acceptable side-effect profile among travelers, the military, and children in endemic populations. To date, trials of multivalent strains have had mixed results, with success in travelers but failure in children in endemic areas. Creating a vaccine that has broad enough coverage and elicits sufficient protective response in endemic populations is a key challenge to overcome. An ETEC vaccine also must elicit lasting mucosal immunity. Strategies to improve vaccine immunogenicity through improved antigen production, antigen exposure, delivery routes, and/or adjuvants may be necessary.

Overcoming this issue of environmental enteropathy may be one of the most significant challenges to developing an effective ETEC vaccine for endemic pediatric populations. More basic research is under way to help gain a better understanding of this issue, but continued research will be necessary to fully understand the root causes and ways to overcome this challenge.

CLINICAL TRIAL CHALLENGES. Clinical development of an ETEC vaccine also presents some challenges. ETEC vaccines will require multiple clinical trials that span specific populations, large cohorts, and across time. For example, developers must address the challenges of measuring efficacy across disparate populations and ages; identifying interactions with other vaccines or diseases, such as HIV; observing the effects of imperfect coverage due to serotype diversity and imperfect causality due to colonization; and measuring appropriate outcomes, especially related to long-term sequelae. Clinical development is further complicated by the lack of an accepted immune correlate for anti-ETEC immunity. Current indicators are viewed more as surrogate measures of protection and not direct correlates of protection at the individual level. Other approaches being considered may hold promise as possible immune correlates for protection, but these options have not yet been studied sufficiently.

CROWDED VACCINE LANDSCAPE. An ETEC vaccine may be rolled out in a time frame when vaccines for other diseases, such as malaria and tuberculosis, are also emerging. These vaccines, in addition to a number of the more recently approved vaccines (e.g., human papillomavirus, pneumococcal, and rotavirus), raise the hurdle for ETEC. Competition could cause an ETEC vaccine to be de-prioritized, further increasing the need for a strong target product profile, improved disease-burden data, greater disease awareness, and an affordable vaccine.

POOR DIAGNOSTIC TOOLS. Disease prevention tools like vaccines require specific data about the causative agent in order to prevent unnecessary immunizations, inform the patient and caregivers of the likely prognosis, and assist health policymakers in making choices about how to address the illness. ETEC has likely been underestimated as the cause of diarrhea in many studies due to the fact that most laboratories do not have readily available methods in place for ETEC diagnosis. This deficiency is confounded by recent studies that suggest that our reliance on phenotypic techniques may result in an inaccurate measurement of ETEC incidence in both pediatric and adult-traveler populations. Given the rapid adoption of molecular techniques in a broad number of geographic settings, it should be feasible to introduce simple and reliable methods for diagnosis of ETEC in any laboratory that is involved in identifying microbes associated with diarrhea. However, this needs to be done with caution and careful consideration of clinical disease-specific data, comparison with traditional laboratory techniques, the population being studied, and the characteristics of the test being used. Until diagnostic techniques can be standardized and improved, it will be difficult to ensure comparability in global ETEC disease epidemiology.

Opportunities

Despite these challenges, our analysis shows that there are several key pathways and opportunities for accelerating ETEC vaccine development.

INCREASING COMMERCIAL INTEREST AND DONOR COMMITMENT. As outlined in the Executive Summary, momentum has been building over the last few years in both the public and private sectors around R&D efforts to develop new diarrheal-disease interventions, including an ETEC vaccine. There has been increased private-investor interest in ETEC vaccines and treatments for all markets, and some major philanthropic groups have increased their support of ETEC vaccine research.

While there are a number of disease-focused organizations that receive substantial funding to support vaccine development in their respective areas, there is currently no such organization dedicated solely to the development of ETEC vaccines. However, there are signals that funding for ETEC vaccine development is growing, especially in the area of product development partnerships (PDPs). In 2007, PATH received a five-year grant to collaborate with public- and private-sector partners to advance the development of safe, effective, and affordable vaccines against *Shigella* and ETEC. In 2010, a European government donor expanded this program at PATH with an additional five-year grant. Other private- and public-sector donors are also showing a potential interest in funding the development of vaccines against diarrheal diseases, including ETEC, and the PDP model appears to be the most attractive to donors.

While it seems that the market opportunity for ETEC vaccines may be sufficient to attract investment, to date, vaccine developers have shied away from developing ETEC vaccines that address endemic-country needs, given the additional cost and risk involved. However, our analysis shows that the potential market that can be reached in middle-income countries through vaccines targeting endemic-country needs may broaden the appeal of a vaccine that reaches more than just travelers and the military. Donors have a significant opportunity to leverage this potential commercial interest and direct resources to encourage industry to pursue vaccines that address endemic-country vaccines as well as other markets. Targeted "push funding" for research and development of a vaccine can also improve the commercial return on investment, thereby improving the market opportunity.

LOW FIELDABILITY HURDLES. Because ETEC vaccines will only address one cause of diarrheal disease and will face stiff competition for scarce resources from other new or existing vaccines, they must incorporate "fieldability" features

into their design from the outset to make them more attractive. There are several ways this can be addressed for most vaccine candidates in the early stages of development. For instance, aligning vaccine administration to the EPI schedules in developing countries can lower administration costs. In addition, low-technology, emerging-market production can help keep costs low, provided the requisite quality standards can be met. Developing-country manufacturers are most likely to produce a vaccine within the range of government affordability but must meet challenges of scale as well as quality. In addition, these manufacturers can have lower overall costs of production and may possess expertise in fermentation technologies for bacterial growth.

ANTICIPATED DISEASE-BURDEN DATA. As described above, disease-burden data for ETEC has been severely lacking. However, there are currently two ongoing projects that will likely improve this situation soon, specifically in endemic countries:

- The Global Enterics Multi-Center Study (GEMS) project, currently being conducted by the Center for Vaccine Development at the University of Maryland, Baltimore, will quantify the burden and identify the microbiologic etiology of severe diarrheal disease among children aged 0 to 59 months living in developing countries in sub-Saharan Africa and South Asia. Data from this study will significantly advance our ability to assess vaccine demand and generate sound country-level estimates for the developing world.
- The Interactions of Malnutrition & Enteric Infections: Consequences for Child Health and Development (MAL-ED) project is a multinational study of enteric infections and their relation to malnutrition, growth, development, and vaccine response. Data from this study will broaden and complement the results of the GEMS project.

In addition, efforts are under way to further explore the association between travelers' diarrhea and post-infectious sequelae, specifically looking at ETEC-associated diarrheal illness. If future findings bear out such an association, it is envisioned that a higher priority within the commercial traveler and military markets will be given to preventive interventions, like vaccination.

Conclusions

This market assessment demonstrates that ETEC vaccines represent a potentially viable opportunity for industry investment with an estimated peak-year annual revenue stream of more than $600 million 10 years after global launch. This opportunity is driven primarily by travelers and middle-income markets (both public and private), but military and low-income markets are also represented. Although a number of uncertainties and challenges remain, PATH and BVGH believe that this outcome highlights the potential opportunities that exist for a low-cost and effective ETEC vaccine in these global markets. We have recommended several next steps, described below, that can help to move the development process forward and make affordable, safe, effective, and accessible ETEC vaccines a reality for each of these markets.

- Initial public-sector investment should address key risks to enable the private sector to carry forward with later-stage development and commercialization. This includes designing vaccines that confer broad strain coverage to meet efficacy thresholds, exploring the development of combination vaccines, demonstrating developing-world needs with improved epidemiological data in ETEC-endemic areas using standard methodologies and identifying disease impact beyond mortality, and showing the potential of a vaccine to prevent long-term sequelae from ETEC illness.
- Donors should provide funding and incentives to encourage developers to pursue vaccine approaches that meet at least the endemic markets, and which could potentially address all market needs. Donors need to develop a reasonable or realistic estimate of how much of an investment on their part may be required to help move the field forward. For later-stage products, donors also need market information to better understand the financing required to support the uptake of vaccines in developing countries and to prepare countries for vaccine adoption.
- Financial return scenario-planning is dependent on a number of key assumptions that can vary widely, such as time and cost of development, uptake, pricing in various market segments, number of approved products, duration of exclusivity in various commercial markets, and discount rates used. The investment-return calculation based on these assumptions varies significantly, and needs to be conducted by each investor to reflect individual conditions. Initial estimates based on our model suggest that in order to attract private investment, public-sector funding may well be essential to facilitate development of ETEC vaccines in order to offset the high front-end costs and technical risks of development.
- Although challenging, vaccine developers would be best served to design ETEC vaccines that would meet the needs of all global markets, taking advantage of the established travel and military markets as well as the growing opportunities in the endemic market. This may involve pursuing low-technology, fieldable vaccines, and seeking partnership opportunities that could help lower development costs (e.g., manufacturing agreements with developing-country suppliers). At the same time, developers should keep an eye toward robust technologies that would be most suitable for the other markets.

- Improved ETEC disease monitoring, supported by improved diagnostics, should be established to better demonstrate burden of disease and define morbidity, mortality, sequelae, and other societal impacts at the country level and among at-risk groups like travelers and the military.
- Global- and country-level advocacy efforts should be increased to help lay the groundwork for uptake in high-risk, ETEC-endemic areas. It is particularly important to reach the broader public-health community in developing countries to increase their knowledge of the array of prevention and treatment interventions for diarrheal diseases, including vaccines in development, as they will be relied upon to eventually adopt these new tools. It may also be useful to combine these efforts with those already occurring with other enteric vaccines that may be further along in development or adoption (e.g., rotavirus vaccines).
- PATH and BVGH suggest that the further development of this ETEC market-assessment model be pursued to account for estimated societal returns on investment (e.g., lost work time, quality of life, cost of long-term sequelae) from global- and country-level perspectives and including travel and military markets. This refinement of the model should be pursued to help policymakers and other decision-makers understand the additional benefits that an ETEC vaccine could provide as a public-health tool.

PATH

Appendix A: Current Research and Development

The current pipeline of ETEC vaccine candidates is somewhat limited, although it represents significantly more activity than this field has seen over the last two decades. The pipeline contains one candidate in Phase 2 studies, five candidates in early-stage Phase 1 clinical evaluation, and four candidates in preclinical development. The following figure and associated text provide a brief summary of the current status of each vaccine candidate.

Current ETEC vaccine landscape by stage of development

Preclinical	Phase 1	Phase 2	Phase 3	Regulatory
LT/ST-based anti-toxin vaccine candidates	LT-based anti-toxin vaccine/adjuvant candidate (double-mutant LT)	Attenuated ETEC vaccine candidate		Inactivated whole cell cholera vaccine*
Fimbrial tip adhesin vaccine candidate	Inactivated ETEC vaccine candidate			
EtpA glycoprotein vaccine candidate	Vectored ETEC vaccine candidate (attenuated *Salmonella Typhi*)			
Siderophore receptor and porin vaccine candidates	Vectored ETEC vaccine candidate (attenuated *Shigella*)			
	Transgenic plant vaccine candidates			

*Licensed for ETEC indication in 29 countries

Inactivated, whole-cell cholera vaccine

Only one licensed vaccine currently on the market has shown efficacy against ETEC—the inactivated, whole-cell cholera vaccine (Dukoral®) manufactured by SBL Vaccines AB of Sweden. Dukoral® has been administered to more than 200,000 people for the prevention of cholera and, in limited field studies, it has been shown to provide 50 to 70 percent short-term protection against ETEC, particularly against those strains producing LT toxin alone or in combination with ST.[181] Dukoral's® ability to induce protection against ETEC is based on the observation that the B subunit of the cholera enterotoxin present in this vaccine is able to induce antibodies that will also inactivate or neutralize the heat-labile LT toxin of ETEC. In post-marketing surveillance studies, Dukoral® has also been shown to provide higher than expected efficacy (30 to 60 percent) against all causes of travelers' diarrhea,[182-183] which has motivated researchers to increase their efforts to develop a more robust anti-toxin vaccine for ETEC.

LT-based anti-toxin vaccine candidates

Intercell AG of Austria extensively evaluated an LT-based anti-toxin vaccine candidate in US and European adults for safety, immunogenicity, and protective efficacy. In a Phase 2 field study, immunization with the LT-patch ameliorated the severity of ETEC illness and dramatically reduced the risk of moderate to severe travelers' diarrhea in adult travelers to Guatemala and Mexico (protective efficacy of 76 percent; p=0.007).[184] Study results also suggested that the candidate may provide at least short-term protection against both LT- and ST-producing ETEC, as well as possibly other enteric pathogens.[185] Intercell launched a pivotal Phase 3 efficacy study in October 2009 to evaluate the protective efficacy of the vaccine, delivered via transcutaneous immunization (TCI) using a skin patch, in US and European travelers visiting Guatemala, Mexico, and India. Because of the enterotoxicity inherent in the native LT protein, delivery of this type of vaccine is restricted to topical routes like TCI.

In December 2010, Intercell announced the results of that study, indicating that it had failed to meet primary efficacy endpoints to protect against all strains of ETEC and other enteric pathogens. As a result, they decided not to pursue further development of this vaccine candidate. However, the trial results did confirm previous observations from the Phase 2 study in that vaccination was associated with a statically significant reduction in the duration of all causes of travelers' diarrhea episodes and in the total number of unformed stools passed during illness. In addition, the results strongly suggested that vaccination did induce some degree of protection against LT-positive ETEC strains. However, the overall number of cases associated with LT-positive ETEC in the trial were too low to achieve statistical significance for this indication. Based on these encouraging observations, Intercell determined that continued investigation of the patch technology as a suitable route of immunization for future vaccine candidates, including those targeting mucosal pathogens, seems warranted. However, it is currently unclear whether any further development of the LT-patch as an ETEC vaccine candidate will take place. In addition, this delivery approach still needs to be evaluated in developing-world populations and the cost of goods involved, including the vaccine containing patch, its packaging, and the skin preparatory device, may serve to limit its use in these settings.

In addition, investigators at Tulane University recently detoxified the LT protein using targeted amino acid substitution. These efforts yielded an attenuated form that has been designated double-mutant LT (dmLT), which appears to retain both the antigenic and adjuvant properties of native LT and could be safely delivered using other routes.[186] The US National Institutes of Health and PATH began a Phase 1 clinical trial of the dmLT vaccine/adjuvant in 2010, which is projected to be completed in the second quarter of 2011.

Attenuated ETEC vaccine candidate

TD Vaccines A/S (formerly ACE Biosciences) of Denmark, in partnership with PATH, is currently developing ACE527, a live, attenuated ETEC vaccine candidate comprised of three strains that all express the B subunit of LT (LTB) individually and together express CFA/I and CS1 to 3, 5, and 6. Expected coverage is 70 to 80 percent of ETEC strains that affect both pediatric populations in developing countries and travelers to ETEC-endemic areas. In early Phase 1 studies, a monovalent prototype of the vaccine was found safe and immunogenic in adult volunteers, inducing serum antibody levels to CFAs at titers associated with reduced risk of enteric illness[187] and fecal IgA levels to these antigens comparable to that induced by wild-type ETEC infection.[188] A new Phase 1 trial of the complete three-strain vaccine was completed in 2009, using doses of 10^{10} colony-forming units (cfu) (approximately $3x10^9$ of each strain) and 10^{11} cfu (approximately $3x10^{10}$ of each strain) in an ascending-dose trial design. Participants receiving the $3x10^{10}$ dose of each strain responded with excellent mucosal responses to the CF and LT toxin antigens (LTB) present in the vaccine. The protective efficacy of the ACE527 (optimal dosing level) was evaluated in a Phase 2b human challenge study at The Johns Hopkins Bloomberg School of Public Health, with complete results expected to be available in 2011. Although the data analysis is not yet complete, the initial findings are encouraging because of the vaccine's positive impact on reducing disease incidence and severity, as well as colonization by the ETEC challenge organism, though the primary endpoint for the study was not met.

Inactivated ETEC vaccine candidate

A first-generation, inactivated, whole cell ETEC vaccine candidate was found to be safe and protected adult travelers against the more severe forms of ETEC-induced travelers' diarrhea,[189-190] but it was not sufficiently immunogenic in children. The candidate was an rCTB-ETEC vaccine, containing five strains expressing CFA/I and CS1 to 5 and recombinant cholera toxin B-subunit. The apparent lack of efficacy may have been due in part to the fact that it did not induce levels of anti-CF antibodies associated with a reduced risk of developing ETEC diarrhea in the field or in challenge trials. To this end, the University of Gothenburg and Crucell-SBL Vaccines in Sweden have been working in partnership with PATH to improve the level of CFA expression in *E. coli* strains projected for inclusion in an improved second-generation candidate. The first prototype strain, called SBL 109, expresses CFA/I at levels 8 to 16 times above that expressed by its counterpart strain in the original first-generation vaccine. The SBL 109 strain was recently evaluated in a Phase 1 study to determine whether it is significantly more immunogenic than the strain used in the first-generation vaccine. This Phase 1 trial was completed in late 2010 and the results, which are expected to be available in 2011, will determine steps for further development of the complete second-generation candidate. The new second-generation vaccine candidate is also projected to provide broader coverage than the earlier version since it will include a strain over-expressing CS6, a major colonization antigen found in common virulent strains, and an improved LTB-CTB toxoid component that should induce better toxin neutralizing antibodies against both LT and cholera enterotoxin.

Vectored ETEC vaccine candidates

Vectoring of ETEC vaccine antigens by other attenuated bacterial vaccines represents an innovative attempt to construct combination vaccines that may have higher commercial-market potential. Three early efforts in this area made it into Phase 1 trials, with two already undergoing improvements to design and stability. US-based Emergent Technologies, Inc. (formerly MicroScience) successfully used an attenuated *Salmonella* Typhi vaccine strain (ZH9) expressing LTB to induce both anti-LTB and *Salmonella*-specific immune responses in human volunteers.[191] However, this combined ETEC-typhoid vaccine candidate is currently undergoing a redesign

in the hopes that it might be able to immunize against both the LT and ST toxins of ETEC as well as typhoid. More recently, the Center for Vaccine Development at the University of Maryland, Baltimore, used an attenuated *Shigella* vaccine strain to deliver CFA/I and the LTA2B fragment of the LT toxin. This combined ETEC-*Shigella* vaccine candidate was well-tolerated in Phase 1 studies, but *in vivo* expression of the vectored ETEC antigens was found to be unstable in the human intestine.[192] Consequently, efforts are currently underway to evaluate improved plasmid stabilization systems or expression of ETEC antigens from chromosomal sites which may help to achieve more stable expression following vaccination. The third candidate, developed by Matrivax R&D Corp., is being evaluated by the US National Institute of Allergy and Infectious Diseases and uses the Peru-15 live attenuated cholera vaccine as a vector to deliver and over-express the B subunit of cholera toxin which induces antibodies that cross-react with and neutralize the LT toxin from ETEC. This vaccine is currently in an ongoing Phase 1 trial.

Transgenic plant vaccine candidates

Transgenic plant-derived antigens offer an innovative new strategy for the development of potentially safe, stable, and inexpensive vaccines. The vaccine antigen can be delivered by ingesting the edible part of the plant or by producing the antigen to commercial scale in plants and purifying it for delivery using more traditional vaccine formulations, either alone or with an adjuvant.[193] In Phase 1 clinical studies of prototype antigens in potato- or corn-based vaccine candidates, these preparations have been found safe and immunogenic without the need for a buffer or delivery vehicles other than plant cells. LTB has been one of the lead vaccine antigens to be evaluated using this technology in both preclinical studies and Phase 1 trials, which has primarily been supported by the US-based ProdiGene, Inc. Similarly, CTB or a detoxified form of the cholera holotoxin (dmCT) have also been delivered to animals using this approach.[194–196] In both cases, results have been encouraging. However, the true promise of this vaccine approach will be better addressed once the regulatory hurdles associated with the use of genetically modified plants can be overcome.

Fimbrial tip adhesin vaccine candidate

Investigators at the US Naval Medical Research Center (NMRC) have shown that the distal tips of ETEC fimbrial CFs consist of relatively conserved, highly immunogenic proteins with the potential to provide a more functional target to block colonization and broader protection than traditional cellular vaccine candidates. These novel proteins, known as fimbrial tip adhesins (FTAs), have been shown to be protective in non-human primates and other animal models as well as in passive protection studies in humans. Animals immunized with a prototype trivalent FTA vaccine-developed antibodies that inhibited adherence of a broader range of CFA types than other ETEC vaccines currently under development. Researchers at NMRC and the University of Colorado have also recently shown that FTA–LTB chimera proteins are able to induce both anti-FTA and anti-LT immunity.[197] The FTA approach has not yet been evaluated in humans, but clinical studies are projected to begin in 2011.

LT/ST-based anti-toxin vaccine candidates

As discussed earlier, a safe and immunogenic LT/ST-based vaccine candidate could provide coverage against all ETEC strains, but the small size of ST and its apparent lack of immunogenicity have been the primary roadblock to this approach. Limited studies indicate that ST can be made immunogenic when conjugated to a larger protein, particularly the B subunit of LT (LTB). However, the ST molecule must be fully detoxified. Currently, the EntVac Consortium, a collaboration of researchers supported by the Research Council of Norway, is working to develop an immunogenic and well-tolerated ST-based vaccine candidate. The consortium will also determine whether ST can be made more immunogenic by coupling it to a

protein carrier such as LTB. Immunogenic LT-ST conjugates have been made previously, but delivery and safety have remained a concern. These concerns must be addressed in preclinical animal studies before human trials can be considered. Recently a prototype LT ST fusion protein was shown to be well tolerated in animal models and to induce both LT and ST toxin neutralizing antibody,[198] thus encouraging progress on this vaccine concept is being made.

EtpA glycoprotein vaccine candidate

Recent use of advanced genomic and proteomic techniques by investigators at the University of Tennessee have led to the identification of a novel virulence factor for ETEC, designated EtpA.[199] EtpA is a 170 kD glycoprotein adhesin that is secreted by a number of ETEC strains. The EtpA protein appears to be conserved across a number of different ETEC toxin and CFA pathotypes, and consequently may have potential as a candidate ETEC vaccine either alone or in combination with other ETEC-conserved proteins. Vaccine development on this protein is still in a very early preclinical stage, but work to date has shown in mice that the EtpA protein is required for optimal colonization of the intestine and immunization with either a non-glycosylated, truncated 110 kD form of the protein or the full-length glycosylated protein induced protection against colonization with human ETEC strains. More preclinical studies, as well as manufacturing process-development work, are needed before clinical studies can determine if this conserved protein has real potential as a vaccine, particularly from the standpoint of its ability to induce broad cross-strain protection and whether protective forms of the protein can be produced on a commercial scale.

Siderophore receptor and porin vaccine candidates

Siderophore receptor and porin (SRP) vaccines, currently used in agriculture, contain multiple surface proteins derived from bacterial cells following growth in iron-restricted media. A high level of sequence conservation exists between the SRP proteins of many species, particularly ETEC and *Shigella*, which may improve the prospect that a single vaccine could provide cross-protection among different strains and species of bacteria. SRP vaccines have been used successfully to protect millions of agricultural animals against a variety of gram-negative bacteria (e.g., *Salmonella*) for more than a decade. SRP vaccines are highly efficient and use relatively inexpensive manufacturing methods. US-based Syntiron is conducting preclinical studies to screen ETEC and *Shigella* isolates for expression of the largest variety of conserved SRP. They plan to prepare an SRP vaccine candidate from a single selected strain of each pathogen for use in subsequent immunogenicity and cross-protection studies in mice, in the hopes of paving the way for future Phase 1 trials.

Appendix B: ETEC Market Assessment Team

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Karen Chang, 2009 Summer Intern, PATH

David Cook, (former) Head of Business Advocacy, BIO Ventures for Global Health

Don Joseph, Chief Operating Officer, BIO Ventures for Global Health

Priya Mehta, Director, Global Health Markets, BIO Ventures for Global Health

Melinda Moree, Chief Executive Officer, BIO Ventures for Global Health

Mark Riddle, Deputy Head, Enteric Diseases Department, Naval Medical Research Center

Cindy Roberts, Project Administrator, PATH

Duncan Steele, Senior Technical Advisor, PATH

Wendy Taylor, (former) Vice President of Strategy and Operations, BIO Ventures for Global Health

Appendix C: Stakeholders Interviewed

Note: Interviewees listed below are arranged by their area of expertise. Affiliations for interviewees were effective at time of interview and may no longer be current. Some interviews were conducted as part of the original 2006 assessment. The content of this report and the views expressed herein are solely those of PATH and BVGH and do not necessarily reflect the views of these individuals.

Vaccine Developers

Dr. Ray Barlow, Emergent Bioscience

Dr. Eileen Barry, Center for Vaccine Development, University of Maryland

Dr. Steve Chatfield, Emergent Bioscience

Dr. Tim Cooke, Avant Immunotherapeutics

Dr. Stan Cryz, independent consultant

Dr. Mike Darsley, TD Vaccines

Dr. Stanley Erck, IOMAI Corporation

Dr. Jorge Flores, PATH

Dr. Greg Glenn, IOMAI Corporation

Dr. Patricia Guerry, US Naval Medical Research Center

Xie Guilin, China National Biotec Group's Lanzhou Institute of Biological Products

Dr. Björn Gustafsson, Scandinavian BioPharma

Dr. Thomas Hale, Walter Reed Army Institute of Research

Dr. Dennis Kopecko, US Food and Drug Administration

Li Qing Lang, Zhejiang Tianyuan Bio-Pharmaceutical Co., Ltd.

Dr. Stephen Savarino, US Naval Medical Research Center

Gong Su, Walvax Biotech Co. Ltd.

Dr. Ann-Mari Svennerholm, University of Gothenburg, Sweden

Dr. Georges Thiry, PATH

Dr. Malabi Venkatesan, Walter Reed Army Institute of Research

Dr. Richard Walker, US Food and Drug Administration

Dr. Xiaoming Yang, China National Biotec Group's Wuhan Institute of Biological Products

Weidong Yin, Sinovac Biotech Ltd.

Global Demand

Dr. Jan Agosti, Bill & Melinda Gates Foundation

Amie Batson, World Bank

Debbie Burgess, Bill & Melinda Gates Foundation

Tracey Goodman, World Health Organization

Evan Simpson, PATH

Nand Whadwani, The Rehydration Project

Dr. Lara Wolfson, World Health Organization

In-country Demand

Viktor Ankran, United Nations Children's Fund (Ghana)

Dr. Nana Antwi-Agyei, Directorate of Public Health (Ghana)

Dr. Robert Davis, United Nations Children's Fund (Kenya)

Dr. Paul Francis, World Health Organization-Regional Office for South-East Asia (India)

Dr. John Grundy, PATH (Cambodia)

Dr. Kalilani, Ministry of Health (Botswana)

Dr. Karen Keddy, National Institute for Communicable Diseases (South Africa)

Dr. Kittipong, private practice (Thailand)

Dr. Harish Kumar, World Health Organization-Regional Office for South-East Asia (India)

Dr. Lisa Lee, World Health Organization (China)

Dr. Chariya Lekyananda, private practice (Thailand)

John Mboya, Ministry of Health (Botswana)

Dr. Jeffrey Mphahlele, Medical University of Southern Africa (South Africa)

Dorothy Ochola-Odongo, Ministry of Health (Botswana)

Isabella Segoe-Moses, Ministry of Health (Ghana)

Dr. Julitasari Sundoro, Ministry of Health (Indonesia)

Dr. Janet Tuli, Ministry of Health (Botswana)

Dr. John Tumbo, Medical University of Southern Africa (South Africa)

Dr. Johann Van der Heever, Ministry of Health (South Africa)

Travel Market

Dr. Virasakdi Chongsuvivatwong, Songkla University, Thailand

Dr. Charles Ericsson, University of Texas

Dr. David Hamer, Boston University Medical Center

Dr. Edward Ryan, Massachusetts General Hospital

Dr. Robert Steffen, World Health Organization

Knowledge/opinion: supply

Dr. John Clemens, International Vaccine Institute

Dr. Jerry Keusch, Boston University

Dr. Marian Neutra, Harvard Medical School

Dr. Firdausi Qadri, International Center for Diarrheal Disease Research, Bangladesh

Dr. David Sack, International Center for Diarrheal Disease Research, Bangladesh

Dr. Potjanee Srimanote, Mahidol University, Thailand

Knowledge/opinion: epidemiology

Dr. John Brooks, US Centers for Disease Control and Prevention

Dr. Jobayer Chisti, International Center for Diarrheal Disease Research, Bangladesh

Dr. Fatima Chowdhury, International Center for Diarrheal Disease Research, Bangladesh

Dr. A.S.G. Faruque, International Center for Diarrheal Disease Research, Bangladesh

Dr. Richard Guerrant, University of Virginia

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