



## Innovation in enteric vaccine development

### Addressing bacterial causes of diarrhea

Since 2007, PATH has been pursuing a wide range of innovative approaches to controlling diarrheal disease through the development of new vaccines against bacterial causes of diarrhea. Each year, nearly 600,000 children younger than five years of age die from severe, dehydrating diarrhea and dysentery worldwide, and millions more are hospitalized, mostly in low-resource countries. In addition, many more children suffer from diarrheal disease-associated malnutrition and its adverse consequences on physical and cognitive development, which perpetuates the cycle of poverty.

Two of the leading causes of diarrhea are the bacteria enterotoxigenic *Escherichia coli* (ETEC) and *Shigella*, and together they account for at least one billion cases of diarrhea annually. Insufficient data exist, but conservative estimates suggest that ETEC and *Shigella* are responsible for almost one-third of child deaths from diarrhea, as well as many deaths in older age groups. Results from the Global Enterics Multicenter Study confirmed that both ETEC and *Shigella* remain among the top four pathogens causing moderate-to-severe diarrhea among children in Africa and South Asia.

Access to appropriate medical care for severe diarrhea and dehydration is limited in low-resource areas, and *Shigella*

is becoming increasingly more resistant to the antibiotics most commonly used to manage febrile diarrhea and dysentery. In addition, bacteria that cause diarrhea are spread more easily in areas with poor sanitation and limited access to clean water, which are frequent concerns in the developing world. For these reasons, prevention through vaccination is a critical part of the strategy to reduce the impact of diarrheal disease. Currently, there are no licensed vaccines against either pathogen.

PATH collaborates with private- and public-sector partners to accelerate the development of safe, effective, and affordable vaccines against ETEC and *Shigella* for children in endemic areas, with the goal of identifying



at least one vaccine candidate for each pathogen to prioritize for late-stage development. A scientific advisory board provides us with strategic guidance, with experts in the enteric vaccine field playing a key role in shaping our portfolio and providing strategic recommendations. We are also assessing manufacturing partners, mostly in emerging countries, to participate in the late development and eventual manufacture and distribution of these new vaccines.

In 2011, PATH helped conduct an assessment of the market opportunity for ETEC vaccines to provide product developers and donors with a business case for investment. The resulting analysis demonstrated that ETEC vaccines may represent a moderate opportunity for industry investment, with an estimated annual revenue potential of more than US\$600 million ten years after global launch. We also identified a clear need for additional studies to assess the societal impact of diarrhea, such as disease burden, lost work time, and quality of life. We are now preparing a similar investment case for *Shigella* vaccines and an updated assessment of ETEC vaccines, with a heavier focus on these societal impacts, as well as an assessment of the feasibility of and markets for a combined ETEC/*Shigella* vaccine.

## PATH'S ENTERIC VACCINE PORTFOLIO

**Live attenuated strains** have shown promise with ETEC and *Shigella* vaccines, as they mimic natural infection and may induce potentially more protective immune responses. However, some live vaccine candidates have shown unacceptable levels of reactogenicity in clinical trials or reduced immunogenicity in developing countries, particularly in infants and young children.

- ACE527 is an oral, whole-cell ETEC vaccine candidate comprised of three attenuated strains. PATH previously supported early clinical research on ACE527, including a Phase 1/2b challenge trial of the vaccine given in combination with a novel mucosal adjuvant

called double-mutant heat-labile toxin (dmLT). Results indicated that the ACE527/dmLT combination provided significant protection against diarrhea of any severity and was highly efficacious against severe ETEC diarrhea. PATH is currently engaging with emerging-country vaccine manufacturers to further the development of ACE527.

- WRSS1 is an oral, single-strain *Shigella* vaccine component developed by the Walter Reed Army Institute of Research (WRAIR). PATH is currently supporting a descending-age study of WRSS1 in Bangladesh with the long-term goal of developing a multivalent vaccine designed to prevent illness from the most common disease-causing *Shigella* strains.

**Killed whole-cell vaccines** offer a superior safety profile as well as a relatively simple and cost-effective manufacturing process, although it remains to be seen whether these vaccines will demonstrate sufficient immunogenicity and protective efficacy among infants and young children living in endemic countries.

- PATH is working with the University of Gothenburg and Scandinavian BioPharma in Sweden to evaluate a tetravalent, inactivated whole-cell ETEC vaccine candidate called ETVAX. After successful proof-of-concept studies to test the vaccine's immunogenicity compared to an earlier version of the candidate, we assessed it in combination with the dmLT adjuvant. Results showed that ETVAX was safe, well tolerated, and more immunogenic than expected. We are now conducting additional studies to evaluate the ETVAX/dmLT combination among children in Bangladesh, particularly the extent to which the dmLT adjuvant may facilitate vaccine dose sparing.
- In collaboration with WRAIR, PATH conducted a current good manufacturing practice (cGMP) manufacture and preclinical assessment of a formalin-inactivated, trivalent whole-cell *Shigella* vaccine candidate. It was previously thought that live attenuated bacteria were the most promising approach to eliciting a strong immune response, but a prototype of this vaccine elicited a robust immune response during an initial clinical trial. We are currently planning additional clinical studies to evaluate the safety, immunogenicity, and protection of this new multi-strain vaccine given in combination with the dmLT adjuvant.

**Subunit vaccines** offer excellent safety profiles and potential broad coverage, but it has been difficult to effectively achieve mucosal immunization with them because they do not survive oral delivery. To address this challenge, PATH is involved in innovative research on intradermal and sublingual delivery routes to improve intestinal mucosal response.



PATH/Eric Becker

- A vaccine that targets the conserved fimbrial tip adhesin proteins of ETEC is being developed by the US Naval Medical Research Center (NMRC) with technical support from PATH. This candidate completed early clinical testing using the intradermal and transcutaneous routes. Intradermal immunization resulted in higher immunogenicity results, and additional testing is being planned.
- Heat-stable enterotoxin (ST) is associated with the most common and serious ETEC infections. PATH is partnering with the International Enteric Vaccine Consortium, a group of universities anchored by the University of Maryland School of Medicine, to conduct preclinical research on an ST toxoid vaccine to determine if ST can be rendered non-toxic while retaining its ability to induce toxin-neutralizing antibody responses.
- The invasion plasmid antigens (Ipa proteins) of *Shigella* may be able to serve as broadly protective antigens against diverse serotypes and species. PATH worked with researchers at Oklahoma State University (now located at University of Kansas) to develop a *Shigella* vaccine based on IpaB and IpaD that, when combined genetically into a single fusion protein (DB Fusion), successfully protected mice against infection with several different *Shigella* serotypes. Activities are now underway to produce a cGMP lot of DB Fusion suitable for clinical testing as an intradermally delivered vaccine. This groundbreaking product could be the first serotype-independent vaccine against *Shigella*.
- PATH and WRAIR are working in partnership to conduct a preclinical evaluation of different formulations of Invaplex, a vaccine candidate consisting of *Shigella* lipopolysaccharide (LPS) in complex with IpaB, IpaC, and IpaD proteins. Using detoxified forms of the LPS component, the candidate is being evaluated in combination with the original Ipa proteins.

## INNOVATIVE RESEARCH TO SUPPORT VACCINE DEVELOPMENT

**Adjuvants:** Adjuvants—ingredients that may enhance the effectiveness of some vaccines—could be a potential game-changer for the field of enteric vaccines. PATH in-licensed from Tulane University the highly promising dmLT adjuvant, an ETEC antigen that may protect against both diarrhea and intestinal infection. LT is also one of the most effective mucosal adjuvants known. Due to its improved attenuation, dmLT could provide a breakthrough in mucosal adjuvants. In addition to testing the dmLT adjuvant in conjunction with several of our vaccine candidates, we are working with the US National Institutes of Health’s Division of Microbiology and Infectious Diseases on early clinical studies of dmLT being administered orally, sublingually, and intradermally.

**Formulation and delivery:** In recognition that new vaccines must be practical for use with infants and children in low-resource countries, PATH is studying several innovative approaches to enteric vaccine formulation and delivery:

- To address the challenge of ensuring the survival of multiple strains in attenuated vaccines, we have developed a holding buffer system that may allow live cells to be stored until all components are available to be mixed and lyophilized rather than completing this step for each strain individually.
- We are also conducting research on novel vaccine-formulation options, such as a fast-dissolving tablet technology platform. Because infants may not be able to tolerate the large volumes of buffer used with oral vaccines, we are developing a low-volume buffer that can still effectively neutralize stomach acids.
- We are supporting preclinical studies in several animal models to evaluate intradermal vaccination as a means to elicit intestinal immunity. This delivery route has been gaining interest due to its potential



PATH/Gabe Blenczycki

for dose sparing and recent technological advances that enhance its safety and simplicity.

- Finally, work supported by PATH has shown that intradermal coadministration of an enterotoxin-based adjuvant such as dMLT with an antigen induces a robust immune response in the intestine. This novel paradigm for inducing intestinal immune responses may facilitate the development of new vaccines.

**Systems biology approaches:** Recently, PATH began exploring innovative, iterative approaches to vaccine development based on systems biology. One such approach involves applying novel immune analysis technologies such as protein microarrays to samples from our clinical trials in order to gain a better understanding of the immunological mechanisms involved in protection. These technologies can also provide insights into novel antigens that could better guide the composition of future vaccines and help us discern subtle responses induced by adjuvant and formulation changes in a vaccine. This information will ultimately make the vaccine development process more robust and efficient.

**Microbiome research:** Another new PATH effort is to develop a better understanding of the intestinal microbiome—or the collection of microorganisms that live in the human gut—with a particular focus on the pediatric intestinal microbiome. These microorganisms may play a role in determining children’s susceptibility and/or resistance to infection and vaccine uptake. Gaining insight into the pediatric intestinal microbiome will help better guide the development of vaccines geared towards children in ETEC- and *Shigella*-endemic countries.

**Controlled human infection models:** PATH is supporting the development of controlled human infection models for enteric diseases, including the refinement of ETEC human challenge models to improve the sensitivity of vaccine testing. PATH aims to continue refining human challenge models for *Shigella* as well as ETEC.

**Vaccine combination and integration:** PATH is tackling difficult questions around the feasibility of developing combined ETEC/*Shigella* vaccines as well as how new stand-alone ETEC and *Shigella* vaccines can be integrated into the existing childhood immunization regimen. We want to know how this integration could impact diarrhea-associated morbidity and mortality in younger age groups.

**Consensus building:** Finally, PATH supports research and other efforts that aim to benefit the broader enteric vaccine community and maintain the pipeline of future vaccines. We have organized several expert scientific meetings to address cutting-edge problems, such as the potential autoimmune consequences of some vaccines or the cause of poor intestinal responses to oral enteric vaccines.

## LASTING PROTECTION FOR THOSE WHO NEED IT MOST

Diarrhea has plagued humans for thousands of years. Current interventions to prevent and treat diarrhea have reduced disease levels considerably—especially in the developed world. However, additional tools are still needed to address the significant disease burden that remains, particularly among children in low-resource settings. PATH’s innovative and pioneering efforts to develop new vaccines against ETEC and *Shigella* are helping to move us closer to making diarrheal disease a thing of the past.



PATH/Cabe Blenczycki

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