Conference report

Title: Recommendations regarding the development of combined enterotoxigenic *Escherichia coli* and *Shigella* vaccines for infants

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Abstract: PATH hosted a workshop on October 14 and 15, 2013 in Washington, DC to solicit expert opinions on the potential merits and challenges of developing combined enterotoxigenic *Escherichia coli* (ETEC) and *Shigella* vaccine products to benefit children in developing countries. This article summarizes the key issues raised during the workshop and provides an analysis of the recommendations regarding the strategic, clinical and regulatory, and manufacturing considerations for the development of a combined enteric vaccine, which aim to guide future vaccine development efforts and donor investment strategies in this area. Notwithstanding the potential technical, legal, financial, and other constraints that would be faced in developing a combined ETEC/*Shigella* vaccine, it is clear that this is the preferred approach over standalone products. There are many advantages to a combined vaccine, such as the potential cost-effectiveness and easier logistics of introducing a combined vaccine instead of two standalone vaccines in low-resource, endemic countries.

- 24 **Keywords:** enterotoxigenic *Escherichia coli*, *Shigella*, diarrhea, combination vaccine, developing
25 world, investment strategy

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27 **1. Introduction**

28 Each year, nearly 600,000 children younger than five years of age die from severe, dehydrating
29 diarrhea and dysentery worldwide, and millions more are hospitalized, mostly in low-resource
30 countries [1]. In addition, many more children suffer from diarrheal disease-associated
31 malnutrition and its adverse consequences on physical and cognitive development, which
32 perpetuates the cycle of poverty [2,3]. Together, enterotoxigenic *Escherichia coli* (ETEC) and
33 *Shigella* account for almost one-third of child deaths from diarrhea each year, as well as many
34 deaths in older age groups [4]. Recent results from the Global Enterics Multicenter Study
35 (GEMS) confirmed that both ETEC and *Shigella* remain within the top four pathogens causing
36 moderate-to-severe diarrhea among children in Africa and South Asia [5]. Currently, there are no
37 licensed vaccines against either pathogen.

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39 There has been increasing interest in enteric vaccines over the last several years, and it is an ideal
40 time to determine the best strategy for moving the field forward as efficiently as possible. Given
41 that the main target populations for these vaccines are in resource-poor settings, it may be a
42 better investment strategy for the major donors contributing to this field to take on the
43 development of a combined vaccine that addresses both ETEC and *Shigella* rather than pursuing
44 the separate development of individual vaccines. In addition, due to an already-crowded vaccine
45 landscape, some countries may not be interested in introducing single-agent vaccines, and the
46 GAVI Alliance, which subsidizes the cost of vaccines for the world's poorest countries, may not
47 be willing to purchase single-agent vaccines for bacterial causes of diarrhea. From a commercial
48 standpoint, there is some interest in a standalone ETEC vaccine for use with traveler and military

populations [6], but there has been less demonstrated commercial interest in developing a standalone *Shigella* vaccine for these groups.

PATH hosted a workshop on October 14 and 15, 2013 in Washington, DC to solicit expert opinions on the potential merits and challenges of developing combined ETEC and *Shigella* vaccines to benefit children in developing countries. The more than 30 workshop participants represented both the public and private sectors and a wide range of institutions and specialties from across the globe. Through intensive discussion, they reviewed the current landscape of enteric vaccines and the technical hurdles involved in the development of a combined enteric vaccine. Targeted sessions focused on analyzing the strategic, clinical and regulatory, and manufacturing considerations and devising recommendations for the realization of combined vaccines including ETEC and *Shigella* components. This article summarizes the key issues raised during the workshop and provides an analysis of the recommendations regarding the potential for developing a combined enteric vaccine. These recommendations aim to guide future vaccine development efforts and investment strategies in this area.

2. Rationale for a combined ETEC/*Shigella* vaccine

To date, most vaccine development efforts to address ETEC and *Shigella* have been conducted in the pursuit of single-agent vaccines. As these vaccines reach proof-of-concept, there can be real advantages in their further development as combined vaccines. A combined ETEC/*Shigella* vaccine would be appealing to all markets in lower-resource countries. Despite the commercial success of the rotavirus vaccine as a single-agent diarrhea vaccine, this was considered by many in the enteric vaccine field to be a “low-hanging fruit” because of its universality of disease

burden. The incidence of other diarrhea pathogens may be too small and localized for many developing-country governments to manage introducing single-agent vaccines. In addition, in these areas where incidence remains high, there is a lack of awareness that diarrhea may be associated with ETEC and *Shigella*.

In addition to protecting against multiple pathogens, a combined vaccine would make things easier for GAVI-eligible countries with regard to cold chain and logistics issues, as well as significantly reduce the total number of vaccine administrations in an already-crowded Expanded Program on Immunization (EPI) schedule. In non-GAVI eligible lower- and middle-income countries, where cost is an even more important factor for considering the introduction of a new vaccine, these issues may be even more acute.

From the standpoint of disease burden, GEMS found that four main pathogens (including ETEC and *Shigella*) cause the great majority of diarrheal disease in Africa and South Asia [5]. GEMS used a statistical approach to determine an “attributable fraction” for the disease burden of each pathogen, in order to take into account the prevalence of multiple pathogens frequently found in the stools of infants and young children living in endemic areas, as well as the lack of symptoms seen in some children [7]. Vaccines against one of the primary GEMS pathogens (rotavirus) already exist and are having a major positive effect on disease burden and mortality from diarrhea where they have been introduced [8]. It can be assumed that vaccines against the other three top enteric pathogens would make a major difference in the overall health of children in these areas, including a reduction in mortality and stunting. The weight-adjusted impact for these agents in the GEMS data calculations for pathogen-specific attributable fractions tend to be very

conservative estimates of disease burden and impact [5]; therefore, the health benefits to be realized through the introduction of a vaccine are likely to be much greater. In addition, in places where rotavirus vaccine has already been introduced, there has been a reduction of mortality simply because the number of diarrhea cases is lower in general. However, it is not expected that additional enteric vaccines will result in eliminating *all* cases of diarrhea in the developing world.

Ideally, an aspirational goal would be a single “enteric/diarrheal disease vaccine.” If children are exposed to multiple pathogens at once, it is not efficient to go after just one at a time with vaccines. In addition to ETEC and *Shigella*, this pan-enteric/diarrhea vaccine could include components to vaccinate against moderate-to-severe diarrhea, dysentery, and fever caused by rotavirus, cholera, typhoid, paratyphoid, non-typhoidal *Salmonella*, *Cryptosporidium parvum*, other types of diarrheagenic *E. coli*, and many other pathogens. However, the potential for this type of vaccine becoming a reality in the near term is extremely unlikely. Given the current state of the science, it is impossible to predict how the intestinal mucosa would respond to a combination of so many antigens at once, and the technical hurdles would be immense. In addition, the concept of a combined ETEC/*Shigella* vaccine already has a lengthy timeline, so adding more pathogens would only elongate it.

Putting aside potential technical, legal, financial, and other constraints, it is clear that a combined ETEC/*Shigella* vaccine is the preferred approach over developing standalone products. However, in recognition that there are some single-agent products that are more advanced than others, it is important to note that if a good standalone product is ready first, it should be used until a

combined vaccine may be able to replace it in the future. Although a combined vaccine is optimal, holding out for the ideal may result in many more children suffering from morbidity, disability, and mortality due to ETEC and *Shigella* in the interim. As a potential model to follow, the typhoid and enteric fever vaccine field recently made a decision to move ahead with the existing typhoid conjugate vaccine to support its roll-out, while a bivalent typhoid-paratyphoid conjugate vaccine is still in development. The rationale for this is that if the technology is available, it should be used now. The same reasoning should hold true for a combined ETEC/*Shigella* vaccine. In addition, there are few, if any, known instances in which a combined vaccine was formulated *de novo* and launched into clinical development without prior evidence that the individual components had protective efficacy in the field or triggered immune responses that could be accepted as true correlates of protection in Phase 1/2 or 2b immunization and challenge studies.

3. Landscape of near-term product possibilities

Due in part to increased interest in recent years, there is a fairly broad landscape of ETEC and *Shigella* vaccine candidates currently under development. Generally, the candidates fall into two major categories: cellular and subunit. Cellular vaccines include those based on live attenuated strains, vectored strains, and inactivated whole cells; these are typically delivered orally (via the mouth). Subunit vaccines are based on specific proteins or O-antigens derived from the bacteria; these are typically delivered parenterally (via intradermal or intramuscular injection). A detailed overview of the ETEC and *Shigella* vaccine candidates currently under development is provided in another article to be published separately.

Generally, combinations across cellular and subunit categories of vaccines are not considered viable. However, Dukoral® [9] (a licensed cholera vaccine that includes an ETEC indication in 29 countries) and ETVAX [10] (an inactivated ETEC whole-cell candidate) are cellular vaccines with a recombinant bacterial toxin subunit component incorporated; therefore, the suitability of antigens for combination with a cellular vaccine is highly dependent on the gastric stability and immunogenic presentation of each. It should also be noted that the pathogenic properties of ETEC and *Shigella* are very different. *Shigella*, an invasive organism, is more like *Salmonella*; ETEC, a toxigenic organism, is more like cholera. Given this, a combined vaccine would need to recruit many potentially diverse/divergent arms of the innate and adaptive immune system in order to successfully address both bacteria.

Another way to consider combinations is by route of administration. A parenterally administered subunit vaccine that induces a mucosal immune and memory response may avoid the environmental enteropathy prevalent among children in developing countries [11,12], making it an ideal candidate. However, there are already so many injected vaccines being given to infants, with more on the horizon (e.g., RTS,S vaccine against malaria), it may be difficult to make the case for adding another to be administered during the same period. Furthermore, parenterally administered vaccines have limited success in eliciting the strong immune responses needed on mucosal surfaces to protect against localized intestinal pathogens such as ETEC and *Shigella* [13]. New adjuvants and regimens may be able to overcome this in the future.

Oral vaccines have a great history of success as well, especially for eliciting mucosal immunity (e.g., oral polio, rotavirus, and cholera vaccines). A major concern with this route is the volume

of the vaccine and buffer required to induce an immune response and neutralize gastric acidity, respectively. These factors will have to be taken into consideration for formulating an oral vaccine for infants. In addition, subunit vaccines are often not successful when given orally, so that may limit the options for their inclusion in a combined oral vaccine. Finally, the need for cold chain storage is a concern for both parenteral and oral vaccines.

Other novel administration routes are currently under review, such as sublingual (under the tongue), which could help avoid environmental enteropathy and the volume and buffer issues associated with oral delivery [14]. Both cellular and subunit formulations could be delivered this way, however it is still too early to know how practical and affordable this delivery route may be for infants in the developing world. Another option under review is the use of a skin-gut nexus to induce both systemic and mucosal immunity to enteric pathogens [15], though the feasibility and success of this approach has not yet been fully assessed.

Given the current landscape, it is clear that there is a number of vaccine candidates emerging that could be used to make combined vaccines. Some of these are fairly advanced, while others are still in preclinical development. There are certainly benefits and disadvantages to the various options for considering a combined ETEC/*Shigella* vaccine, in terms of vaccine category and delivery route. There is no clear choice for the optimal vaccine type or route of administration to pursue at this time. Instead, the best option should be determined by examining each potential combination on a case-by-case basis as the field advances.

4. Strategic recommendations

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187 *4.1 Intellectual property considerations*

188 Pragmatic concerns arise immediately when considering the possibility of developing a
189 combined ETEC/*Shigella* vaccine. For instance, the potential for a combined vaccine will rely
190 heavily on who owns the different components that need to be brought together. Neither entity
191 will want the development of a combined vaccine to interfere with the development of their
192 single-agent vaccines. In addition, each product owner must make sure that their single-agent
193 vaccine has a large enough safety database to make clear that the effect of any safety issues
194 observed in a combined product are a result of the combination and not an effect of any
195 individual component. It is critical for vaccine developers and potential investors to not
196 underestimate the amount of time and complexity that may be involved in bringing the
197 components together if they are owned by different entities.

198

199 *4.2 Dosing schedules*

200 The EPI vaccine schedule for infants is already crowded in many countries, and it will become
201 more so with the increasing roll-out of newer vaccines (e.g., rotavirus, pneumococcus). In
202 addition, there are some changing perspectives emerging on whether developing new vaccines to
203 target the EPI schedule is still the right approach. Reasons for this include the feasibility of
204 giving so many injected vaccines to infants at the same time, potential interference between the
205 different vaccines, the immaturity of the immune system during infancy, and the role of maternal
206 antibodies. However, it is also important to avoid necessitating wholly new interactions with the
207 healthcare system to administer a combined vaccine, as it would likely be too expensive and
208 logistically difficult in endemic countries. It is possible that there will be revisions to the EPI

schedule in the near future, which may benefit the potential dosing schedule for a combined ETEC/*Shigella* vaccine.

Shigella is more of a concern for older children while ETEC largely affects younger infants. If a combined ETEC/*Shigella* vaccine were not given to an infant within the first six months, it would not optimally address the high burden of ETEC morbidity and mortality among these young children that generally starts to rise significantly during the second six months of life. In addition, after the six-month mark, many children are no longer breastfed, so they are no longer receiving maternal antibodies to protect them from infection. Environmental enteropathy is also a major issue of concern after weaning because there is no efficient method of diagnosis, and it impacts children's ability to respond to oral vaccines, medicines, and nutritional supplements [11,12]. For these reasons, it is not prudent to consider delaying administration of a combined ETEC/*Shigella* vaccine until after six months of age.

Given these facts, the most appropriate dosing regimen would be to conduct a primary series of vaccinations in early infancy, with a homologous booster dose given at a later point. The ideal dosing schedule for a combined ETEC/*Shigella* vaccine would be two to three doses in the 0 to 6 month age range, with a booster given in the 9 to 18 month age range (see Table 1). There is currently insufficient rationale to consider boosting with only one vaccine component or to look at mixed modality in a heterologous prime-boost approach (e.g., vector prime, subunit boost) unless it was determined to be absolutely necessary.

4.3 Support for vaccine development and uptake

A strong case needs to be made in endemic countries that a vaccine or vaccines against ETEC and *Shigella* are needed to complement the current treatment and prevention methods, and that simply waiting for improvements to water and sanitation will result in a high toll of mortality and morbidity in the meantime. Despite recent incremental improvements in water and sanitation in some endemic areas, the overall diarrhea morbidity rates have changed very little during the past 20 years [16]. Since this meeting was held, the results of a study were published that indicated that the introduction of improved sanitation facilities in India did not improve child health outcomes, further underscoring the need for vaccines [17]. As the GEMS data recently demonstrated, episodes of moderate-to-severe diarrhea, which by themselves may not result in an acute fatality, do increase the risk of death and stunting over the following 60 days of life [5]. In addition, preventing severe disease from ETEC and *Shigella* is preferable to late treatment with oral rehydration therapy and zinc, given the high risk for post-infectious sequelae and the accepted risk of poor physical and cognitive development due to recurrent diarrhea [2,3]. The push by WHO and others to restrict the use of antibiotics to treat recurrent diarrhea and increased antibiotic resistance also underscore the value of prevention.

It is also critical to raise awareness about the disease burden of ETEC and *Shigella* and advocate for increased funding to develop both single-agent and combined vaccines. It would be helpful to inform potential investors, as well as endemic country officials, that there are candidates in the pipeline, and for all of the players in the enteric vaccine development field to present a united front with the same messages and asks. In addition, there is a strong need for disease burden information at the country level to understand and communicate the need for enteric vaccines. Global numbers for diarrheal disease deaths appear to be dropping, but it is not clear to most

stakeholders that these numbers are only the lower-bound for disease burden estimates. In the near term, it would be helpful to have a small set of core slides that convey key messages about enteric disease burden and funding in the context of all major causes of morbidity and mortality in children younger than the age of five years, which could be used across the enteric vaccine field. This would present a united front for attracting funding for these efforts.

5. Clinical and regulatory recommendations

5.1 Prioritizing development options

It is clear that a combined ETEC/*Shigella* vaccine is an optimal product and should be pursued. However, from a public health perspective, if a standalone ETEC vaccine is available to be licensed while the combined vaccine still remains in early development, it would be best to save lives and license the ETEC vaccine while the combined vaccine is in development. The combined vaccines currently in use were all developed from components that were already licensed vaccines. This also suggests that it may be advantageous to develop ETEC and *Shigella* vaccines separately, then combine them and/or use a currently licensed vaccine as a platform. In addition, because there is no definite correlate of protection for efficacy with an ETEC or *Shigella* vaccine, proof-of-concept becomes even more important.

Given the current landscape of ETEC and *Shigella* vaccine candidates, it is most likely that an ETEC vaccine will be ready for licensure sooner and development should not be delayed to wait for a *Shigella* component in order to make a combined vaccine. An ETEC vaccine candidate should continue to be moved forward as a standalone vaccine candidate along the clinical

development pathway in order to obtain field efficacy data. Only once field efficacy is established, should the *Shigella* component be added and tested. While ETEC field efficacy is being obtained, preclinical and clinical activities can occur in parallel with the ETEC/*Shigella* combined vaccine to establish efficacy against both ETEC and *Shigella* in a Phase 2b challenge study (see Figure 1). ETEC vaccines should be moved forward as quickly as possible with or without *Shigella* vaccines, because even an ETEC-alone vaccine would help establish a path for a combined vaccine and provide a significant benefit to children's health.

Given the lack of developed-world market demand for a standalone *Shigella* vaccine, a reasonable strategy may be for manufacturers in this market to hold off on licensing a *Shigella* vaccine until another component was ready to be combined. However, some studies have suggested that interest in a *Shigella* vaccine may be relatively strong at the local stakeholder and community levels in South Asia and China [18,19]. Nevertheless, if a *Shigella* vaccine was further along in development, the same general process outlined above would be followed until an ETEC component could be combined (i.e., *Shigella*-only field efficacy data would be obtained and then the two vaccines combined). *Shigella* single-agent vaccines should be developed to the point of a Phase 2b trial, and then consider pausing their development to add an ETEC component. However, it should be noted that manufacturers in the developed world may have a different perspective on moving towards licensure with a standalone vaccine against *Shigella* because their focus may not be on public markets in developing countries.

In addition, it is essential to determine the potential adverse effects of each component separately before combining them for testing; otherwise, it will be impossible to know which component is

causing any negative reactions. A step-wise approach for combination is suggested to help elucidate the cause of any negative interactions. It also may be that the best approach to using attenuated vaccines in a combination is to make one vaccine the vector for the protective antigens of the other agent. This approach is already in development for a *Shigella* vector for ETEC antigens [20].

5.2 Determining proof-of-concept

The existing animal models for ETEC and *Shigella* are less than ideal for answering many proof-of-concept questions about a combined vaccine. Human challenge models exist, and it is important to conduct challenge studies early on in development to eliminate risk for both components. It remains debatable how well these challenge models predict efficacy in an endemic population, as it is hard to know how well the response in the gut of a healthy adult in North America or other relatively developed country can predict the response in the leaky gut of a child in a resource-limited setting. Nevertheless, it is important to move rapidly to human testing because it provides the best opportunity for making early go/no-go decisions with each component as well as the combined vaccine (see Table 2). A potentially practical alternative to a challenge study may be to evaluate the vaccine in a travelers' population where the incidence of disease is fairly high, such as testing the vaccine in travelers or military personnel from developed countries that are going to endemic areas.

It should be noted that challenge studies are not a regulatory requirement or basis for licensure on their own, so some vaccine development companies may not see the value in conducting a challenge study. However, these types of studies could be utilized by vaccine developers as a

developmental stage-gate toward further vaccine development at their own discretion. Also, a challenge model for establishing the efficacy of a combined ETEC/*Shigella* vaccine is recommended for moving forward into field efficacy trials for a combined vaccine (see Figure 2). The combined vaccine efficacy trial should be conducted in a different country or geographic region than the antecedent standalone ETEC vaccine trial, which would afford broader data on the efficacy of the ETEC vaccine component and negate the potential ethical issues that could arise by conducting a placebo-controlled trial in an area where the ETEC vaccine efficacy has already been established. In addition, given that existing animal models are inadequate and there are no defined correlates of protection for ETEC or *Shigella* vaccines, larger clinical studies will likely be required, especially for determining efficacy. Consequently, the early use of challenge models in support of various combined vaccine development strategies will help to mitigate the risk of these later Phase 3 efficacy studies.

5.3 Clinical efficacy strategies

One of the key findings from GEMS was that the odds of dying during follow-up were 8.5-fold higher in patients with moderate-to-severe diarrhea than in the control cases [5]. Repeated infections of diarrhea from ETEC and *Shigella* (as well as other pathogens) lead to enteropathy, malnutrition, and deficits in cognitive development which contributes to severe and long-term consequences in terms of health, development, and poverty. The goal of a combined ETEC/*Shigella* vaccine is to decrease the number as well as the severity of enteric infections in order to prevent the sequelae of enteropathy. Given this, the long-term consequences of enteric infections will drive the primary vaccine efficacy endpoint for a combined vaccine trial. Due to the issues of post-infectious sequelae and enteropathy, the primary efficacy endpoint for a

combined vaccine trial should be all cases of moderate-to-severe diarrhea caused by ETEC and/or *Shigella* (not just the first or only episode) over at least 12 months after vaccination, with further follow up for two to three years.

The vaccine research field for both ETEC and *Shigella* suffers from a dearth of functional immune assays and putative correlates of immune protection. In addition, WHO has moved away from the use of reference centers in this area for enteric vaccine development. Given this, during an ETEC-alone efficacy study (while *Shigella* is still in early trials; see Figure 1) it would be extremely useful to examine correlates of protection during that trial, as this could help with establishing correlates later in the process, which could be incredibly beneficial to the field overall.

On a positive note, there is currently some work underway in the area of immunological assays and protective immune correlates associated with ETEC and *Shigella* disease and immunization, but large gaps remain and further investment is certainly needed. PATH and the US National Institutes of Health (NIH) are working together to produce a reference antigen bank, making recombinant antigens and putting them into the NIH resource bank (producing at least CFA/I, CS1, CS2, CS3, CS5, CS6, and possibly more). These will be available for all researchers in the field to help with consistency/standard reference across laboratories. In addition, PATH is working with the University of Alabama-Birmingham to develop functional assays for *Shigella*, starting with *Shigella flexneri* 2a, to evaluate the potential of lipopolysaccharide (LPS)-specific antibodies to mediate bactericidal activity. Development of a high-throughput assay for heat-labile toxin (LT) neutralization to replace current conventional assays is also being considered.

370 PATH will share these assays with the rest of the scientific community when the work is
371 completed. An existing functional assay that could be important is the hemagglutination
372 inhibition assay developed by the US Naval Medical Research Center, but it is not currently
373 high-throughput, which is key to all these functional assays and diagnostic tools. In addition, the
374 University of Gothenburg developed monoclonal antibodies that attempt to address the need for
375 a standard set of immunological reference reagents that can be used to help identify the
376 colonization factor/coli surface (CF/CS) phenotype of strains, quantitate the specific CF/CS
377 antigen load in vaccine doses, and serve as positive control sera in future assay development.

378

379 There is clearly a strong need and interest in developing shared resources in this area, as well as
380 a list of the assays that are currently available and those under development that could be shared
381 across the enteric vaccine field. It would also be helpful to clearly identify the current gaps,
382 including: methods to identify individual strains within a polyvalent vaccine as part of the entire
383 combination; well characterized common reagents and strains for use by researchers across the
384 field; a bank of standardized antiserum; and improved animal models. As noted earlier, it may
385 also be necessary to develop additional human challenge models using ETEC strains more
386 relevant for evaluating the efficacy of a combined vaccine moving forward into field efficacy
387 trials.

388

389 Another critical need is for a simple but sensitive point-of-care diagnostic for both ETEC and
390 *Shigella*. These tools could help determine better national and regional burden of disease
391 estimates, as well as increase awareness among stakeholders about the potential impact of a
392 combined vaccine. Improved diagnostics could also provide a means for rapidly determining

whether cases of illness occurring during a Phase 3 study are the result of strains not covered by the vaccine or other infectious agents. Some work is underway to evaluate molecular-based diagnostics in this area, but it would benefit significantly from better coordination and more consistent funding.

5.4 Regulatory considerations

The regulatory pathways for cellular versus subunit vaccine candidates are the same—both types of formulations would follow the same basic clinical pathway for testing and submission to a regulatory authority for licensure. However, it is important for vaccine developers to establish a dialogue with the relevant regulatory authorities throughout the clinical testing process. Having a sound scientific rationale and clinical development plan (with a clear goal and milestones) is the best way to work with all national regulatory authorities. In many cases, the country of final vaccine manufacture will also be the country of initial licensure, which will inform the clinical development and regulatory plans. This will also drive where the clinical studies should take place.

In addition to early involvement with regulatory authorities, it is absolutely essential to reach out to WHO as early as possible to make them aware of the combined vaccine product in development and understand the factors that will impact the goal of obtaining WHO prequalification. Drawing on experiences with typhoid and cholera vaccine development, engaging early with WHO and keeping them updated on the vaccine's progress is critical. This step does not supersede reaching out to national regulatory authorities, but it provides a hedge against the possibility of dealing with less advanced national regulatory authorities in countries

where the vaccines are most needed. It should be noted that a regulatory liaison with WHO does not currently exist for ETEC/*Shigella*, which is a broader issue that needs to be addressed with WHO as soon as possible.

In addition to maintaining communications with WHO, the clinical development plan needs to incorporate the basic clinical requirements for WHO prequalification (see Figure 3). These requirements include a Phase 3 pivotal efficacy trial and a separate EPI non-interference and lot-to-lot consistency study (which can be done in parallel to the Phase 3 trial).

6. Manufacturing recommendations

6.1 Potential incompatibilities across components

Standalone ETEC and *Shigella* vaccines are already complex and will include several antigenic components. Vaccine component interactions will generally be impossible to detect until administered *in vivo*. Each antigen has its own characteristics and all vaccine components, including excipients and potential adjuvants, may interact with each other. The most important manufacturing consideration in developing a combined ETEC/*Shigella* vaccine is to remember that these are not inert ingredients, and it is critical to determine how they will all interact with each other. In this vein, the greatest chance for successfully combining ETEC and *Shigella* vaccines would be to combine the same types of products. For example, a killed whole cell ETEC vaccine would best be combined with a killed whole cell *Shigella* vaccine. As such, vaccine developers should try to keep single-agent candidates as simple as possible to preserve

their potential for being compatible for combinations in the future. In general, the more complicated the combined product is, the more manufacturing risk will be involved.

Cellular, protein subunit, and conjugate vaccine approaches each pose very different challenges. Cellular vaccines are difficult to produce consistently and it is impossible to completely define the characteristics of these vaccines. This could impact the lot consistency testing requirements, and it may be difficult to find manufacturing sites willing to work with vaccine strains with high endotoxin levels. High concentrations of cells to achieve efficacy, especially in a volume-limited dose population like infants can also make formulation difficult. Subunit vaccines are easier to fully characterize and produce consistently, but biochemical interactions between components may affect stability, efficacy, and/or safety of the product. Conjugate vaccines can also be difficult to produce consistently and, therefore, have some of the same challenges related to lot consistency testing requirements as cellular vaccines. At this time, there is no clear benefit to pursuing one approach over another for a combined ETEC/*Shigella* vaccine, other than to ensure that both components are plausibly compatible.

6.2 Scale-up requirements

It is estimated that the scale of manufacturing required for the Phase 3 development and product launch of a combined ETEC/*Shigella* vaccine would be hundreds of millions of doses per year. Given that a combined vaccine is likely to have many components, several manufacturing sites may be needed and significant capital expenditures will be required to build appropriate facilities. As such, early scale-up process development should occur to ensure that consistent and well-characterized vaccines can be produced.

461

462 *6.3 Formulation options*

463 Fill-finish will likely be the highest manufacturing cost associated with a combined vaccine,
464 especially if it is orally administered, so larger (multi-dose use) vials are recommended for either
465 liquid or dry formulations. Dry formulations have advantages for thermostability; however, there
466 are challenges related to the need for clean water for rehydration and the added cost of
467 preparation could add to the overall cost for the vaccine product. Although liquid vaccines
468 require cold chain storage, there is the potential for pursuing post-licensure approval for use in
469 the “controlled temperature chain” (where the vaccine can be out of the cold chain for a certain
470 amount of time and will still be effective), following the example of the meningococcal vaccine,
471 MenAfriVac® [21]. At this point, both parenteral and oral approaches and liquid and dry
472 formulations should be pursued, since neither offers a clear advantage over the other at this
473 point. In general, multi-dose vials for the final combined vaccine product would be preferable.

474

475 *6.4 Optimal packaging*

476 While innovative technologies for product packaging are also a consideration, they may pose a
477 challenge for regulatory acceptance, which would add cost and time to the development process.
478 In general, it is not worth the added time and cost to wait for one of these breakthrough
479 technologies to be proven and ready for use with a combined ETEC/*Shigella* vaccine, unless
480 there is an already proven technology that is suitable for use with the product.

481

482 *6.5 Adjuvants*

The use of adjuvants in a combined ETEC/*Shigella* vaccine could play an important role in enhancing the immune response, as well as dose-sparing, which is critical when immunizing infants. Dose-sparing may also help to reduce the content of a combined vaccine, which is already likely to have a lot of components. A risk of using an adjuvant for a developing-country infant population is that there is not suitable pharmacovigilance that can track potential side effects. In addition, it is unclear what impact a mucosal adjuvant may have on children with inflamed intestines. Some work in the area of adjuvants is already underway, with several challenges and gaps remaining.

The double-mutant of ETEC heat-labile toxin (dmLT) is one of the most advanced mucosal adjuvants currently under development, and it may benefit a variety of vaccines for which mucosal immunity is important [22]. The dmLT may be able to improve immune responses to vaccines delivered orally [10] or to other mucosal surfaces, as well as those given parenterally by the intradermal route [15]. It is critical to optimize the ratio of adjuvant to vaccine (i.e., dose finding of the adjuvant) as early as possible in the development process for a combined vaccine. It is also important to consider how adjuvants might be viewed from a regulatory perspective, in order to determine if it will result in more time and risk to the development of the vaccine.

Specific to a combined vaccine, it is critical to ensure that the cost of adding an adjuvant would be offset by the lower cost afforded by reducing amount of antigen needed. It would also have to demonstrate an actual clear clinical benefit to push the adjuvant forward, and it will need extensive safety testing when the vaccine goes into larger numbers of subjects in order to ensure good vigilance for outlier side effects. Finally, vaccine developers must be sure to use

compatible adjuvants in stand-alone vaccines through the development process, as well as to screen individual vaccines with possible adjuvants, so that the true effect of the adjuvant can be determined early on.

7. Conclusions

This article presents key issues and recommendations from a two-day expert workshop on the potential merits and challenges of developing combined ETEC and *Shigella* vaccines for children in endemic countries. The workshop participants recommend the approach of advancing single-pathogen vaccines towards licensure, rather than holding up the late-stage development of a vaccine candidate that is progressing well in order to wait for another candidate with which it could be combined. Components for vaccines against different enteric pathogens should be developed individually to the point that extensive clinical safety and, to a lesser degree, efficacy data are produced. Manufacturing of a combined vaccine will require significant investment as each individual vaccine will already contain multiple components. Given that difficulties with intellectual property are likely to exist, a consortium approach should be considered and early investment and buy-in will be required. Robust manufacturing and consistent testing processes should also be established as early as possible. In addition, the dosing schedule and route of delivery for a combined vaccine should consider both the nature of the pathogens to be covered as well as the current childhood immunization schedule. These high-level recommendations aim to guide future vaccine development efforts and investment strategies in developing a combined enteric vaccine to address ETEC and *Shigella*.

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Important note: This article summarizes the views of an international group of experts as presented at an invitation-only scientific workshop in a given timepoint and context, and does not necessarily represent the opinion of any institution or corporation.

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639

640 **Figure legend**

641 Figure 1: Recommended clinical pathway for a combined ETEC/*Shigella* vaccine (part 1)

642

643 Figure 2: Recommended clinical pathway for a combined ETEC/*Shigella* vaccine (part 2)

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645 Figure 3: Clinical requirements for WHO prequalification

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Table 1: Ideal timing for administering combined ETEC/*Shigella* vaccine in coordination with EPI schedule

	Birth to 1 week	6 weeks/ 2 months	10 weeks/ 4 months	14 weeks/ 6 months	9 months	15 to 18 months
Today's EPI schedule	BCG dose	DPT+ ^a 1 st dose	DPT+ ^a 2 nd dose	DPT+ ^a 3 rd dose		
Potential EPI additions in the near future					MCV1 ^b	MCV2 ^b
Proposed ETEC/<i>Shigella</i> combination		2 to 3 doses, as determined by specific vaccine product			Back-up timing for booster dose	Ideal timing for booster dose ^c

^a DPT+ represents the combined diphtheria, pertussis, tetanus vaccine, as well as potentially *Haemophilus influenzae* type b, hepatitis B, polio, rotavirus, pneumococcal, and other vaccines (depending on location).

^b MCV1 and MCV2 represent potential first and second doses of a “measles-containing vaccine.”

^c Administer booster dose here if MCV2 is routine immunization rather than a mass campaign and if coverage is high; otherwise administer with MCV1. This assumes that the combined vaccine would be tested for non-interference with measles prior to introduction.

Table 2: An ideal streamlined approach to proof-of-concept for a combined vaccine

Chronological order	Action step
1	Secure necessary business agreements.
2	Preclinical assessments of immune interference and Phase 1 studies for single agents.
3	Early human challenge studies with single agents.
4	Phase 1 and challenge studies with combined vaccine.
5	Three-step descending-age study in endemic population for safety only (immunogenicity confounded by pre-existing immunity).
6	Proof-of-concept in target population.

Figure 1 (color)

Figure 1 (b&w)

Figure 2 (color)

**Note: With this primary endpoint, the sample size should provide sufficient power to evaluate ETEC outcomes in the single and combined vaccine groups as a secondary endpoint. This will help determine whether the combined formulation impacts the field efficacy of the ETEC vaccine.*

Figure 2 (b&w)

**Note: With this primary endpoint, the sample size should provide sufficient power to evaluate ETEC outcomes in the single and combined vaccine groups as a secondary endpoint. This will help determine whether the combined formulation impacts the field efficacy of the ETEC vaccine.*

Figure 3 (color)

Figure 3 (b&w)