

Enteric Bacterial Vaccines: Shigella and ETEC - Challenges of Vaccine Development in Endemic Countries

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ADVAC Alumni Refresher Course
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Gates Foundation

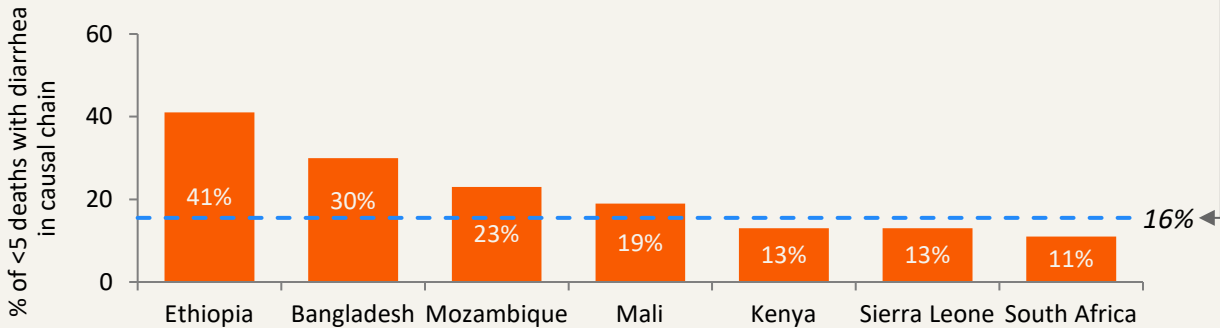


Diarrhea is an important contributor to the mortality burden in children <5, and among diarrheal deaths *Shigella* is a leading etiology

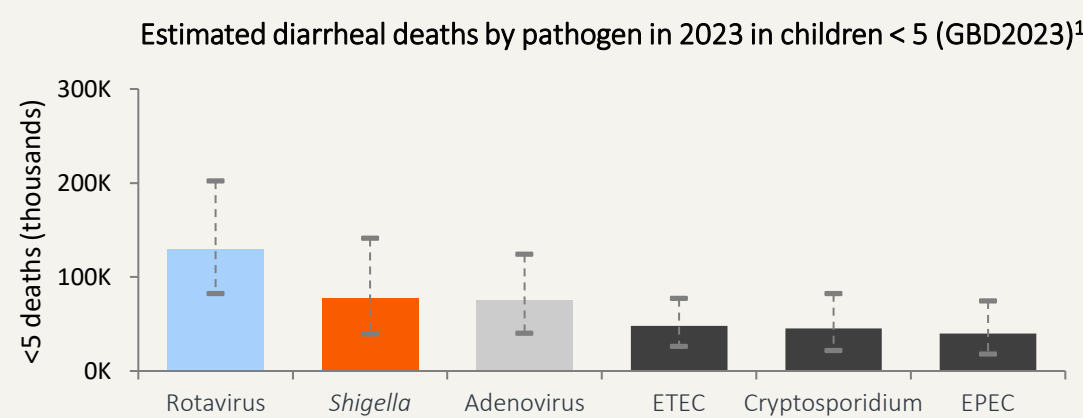
GBD 2023 estimates diarrhea accounts for ~8% of deaths in children <5 in low SDI countries

Cause of death	Number (%) of <5 deaths by cause in low SDI geographies (GBD2023) ¹	Number (%) of <5 deaths by cause in CHAMPS ²
Neonatal disorders	950,692 (32%)	-
Lower respiratory infections	373,837 (13%)	663 (40%)
Malaria	345,438 (12%)	364 (22%)
Diarrheal diseases	237,215 (8%)	271 (16%)
Congenital birth defects	218,027 (7%)	147 (9%)

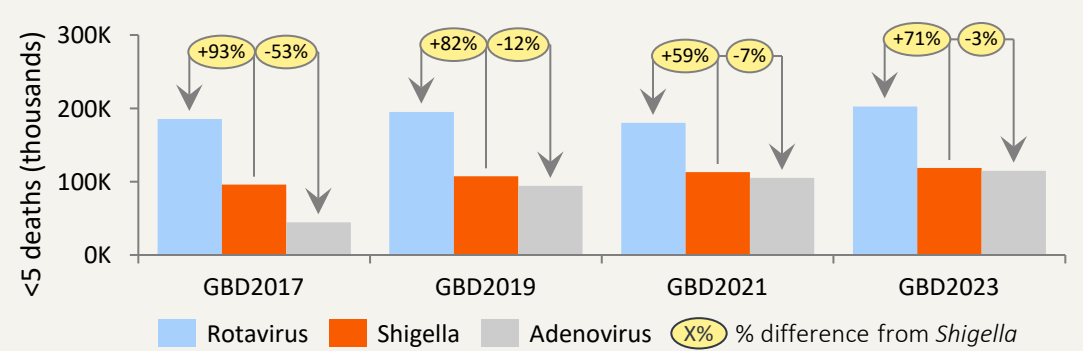
There is significant variability in CHAMPS sites, with some sites having 30 – 40% of deaths with diarrhea in the causal chain



Among diarrheal deaths, *Shigella* is frequently the second highest etiology after rotavirus



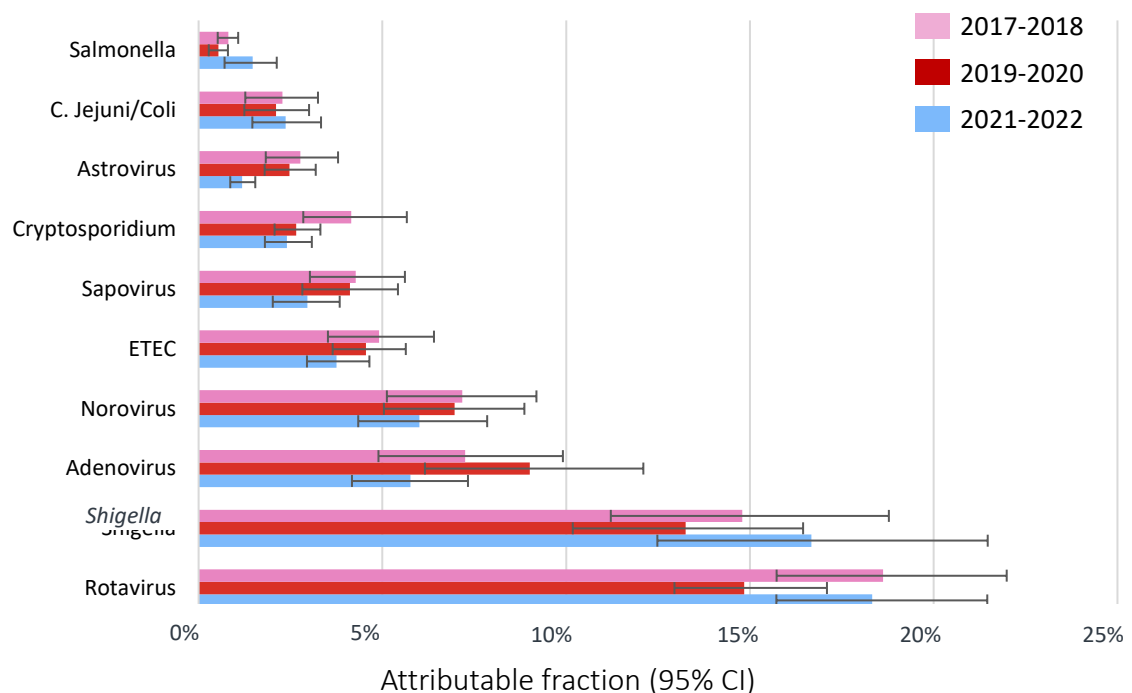
GBD mortality estimates for diarrheal pathogens have fluctuated
Comparison of 2017 <5 mortality burden of rotavirus & adenovirus compared to *Shigella* over progressive GBD rounds³



1. IHME GBD2023 2. CHAMPS 3. IHME analysis of GBD results keeping data constant (2017)

As rotavirus vaccines are introduced in more countries, the importance of diarrhea attributed to *Shigella* is increasing

Attributable fraction of diarrhea hospitalization across countries with Rotavirus vaccination in the national schedule (GPDS)¹



As rotavirus vaccines are rolled out in LMICs, diarrhea hospitalization attributed to *Shigella* is approaching rotavirus

In some countries with rotavirus vaccination, the incidence rate of MSD² for children 6-35 months attributed to *Shigella* is higher than rotavirus

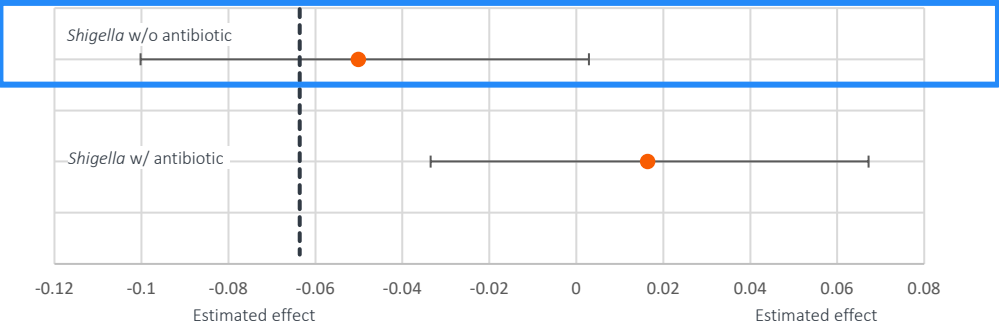
1. GPDS 2. MSD = medically attended acute diarrhea with dehydration, dysentery, or hospitalization

There is evidence that *Shigella*, more than other diarrrheal pathogens, is associated with growth faltering in children, with long-term consequences

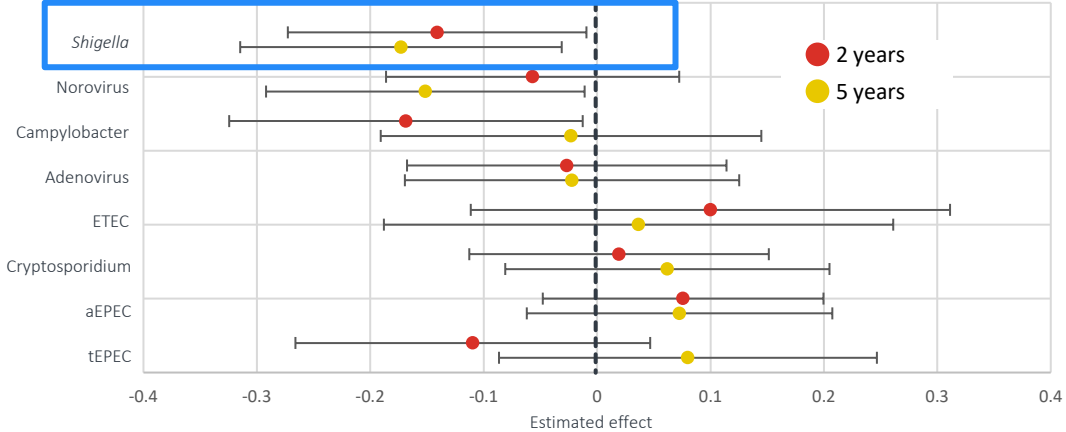
Studies consistently show a detrimental effect of *Shigella* on linear growth, particularly in the absence of appropriate antibiotics

Effect of untreated *Shigella* on change in HAZ ranged from -0.17 to -0.04

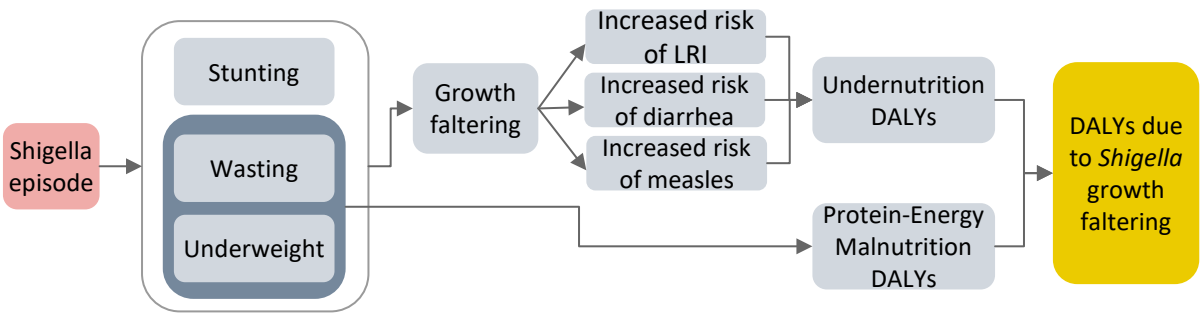
Estimated effect of *Shigella* on change in HAZ from enrollment to 3 months (ABCD)^{1,2}



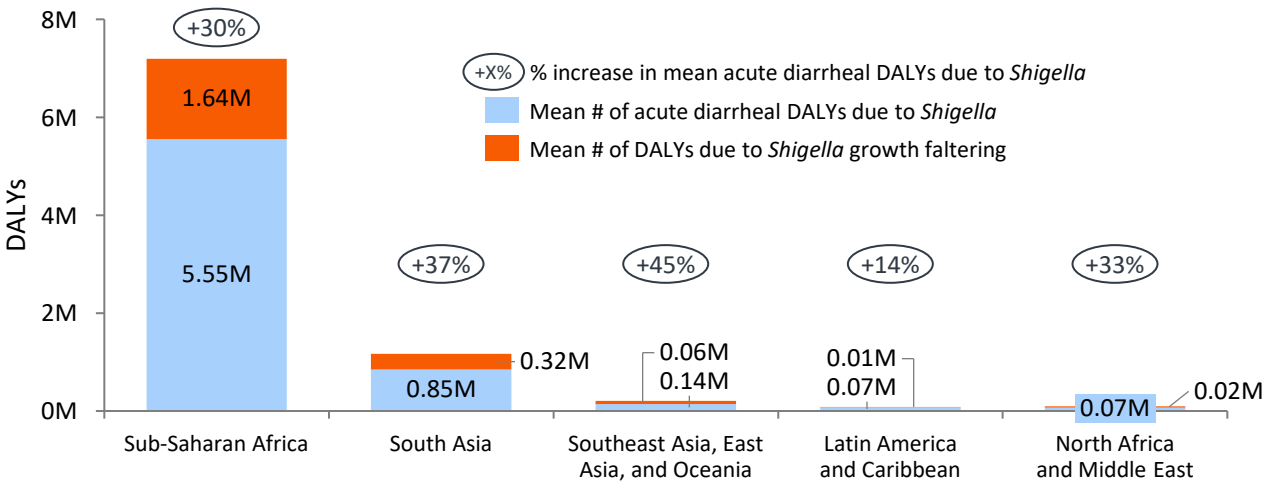
Estimated difference in HAZ across enteropathogen infections at 2 & 5 years of age (MAL-ED)^{5,6}



Growth faltering is a risk factor for other adverse health consequences⁷



Increase in mean acute diarrrheal *Shigella* DALYs due *Shigella* growth faltering⁷

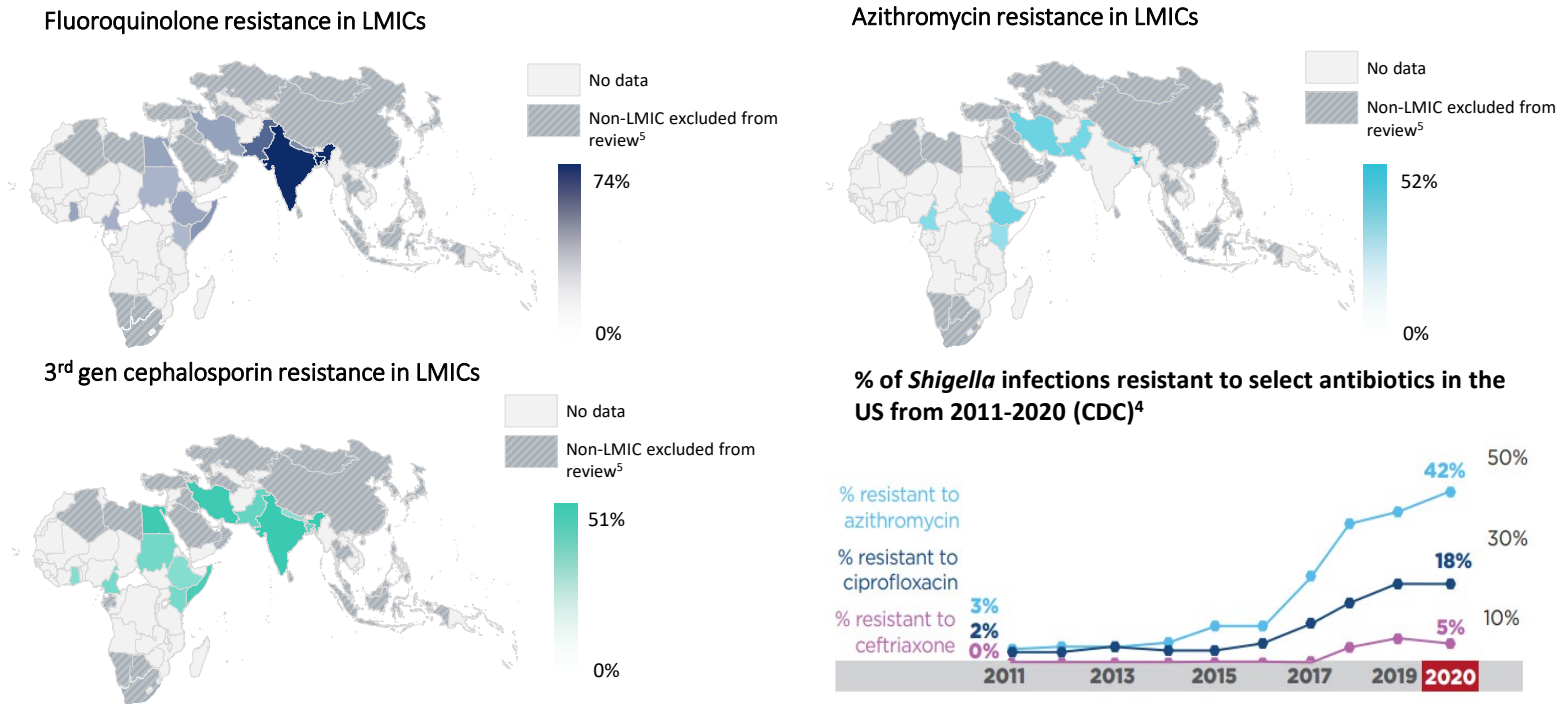


1. ABCD 2. Relative to other diarrrhea 3. EFGH 4. Relative to no pathogen detected 5. Rogawski, et al. Lancet 2018 392(10159):1789-1858 6. Relative to no *Shigella* detection in non-diarrrheal stools 7. IHME

Growing AMR burden of *Shigella* is affecting both high- and low-income countries, potentially worsening clinical severity

Preliminary data suggest clinical severity worsens with AMR *Shigella*, which is becoming more prevalent globally^{1,3,4}

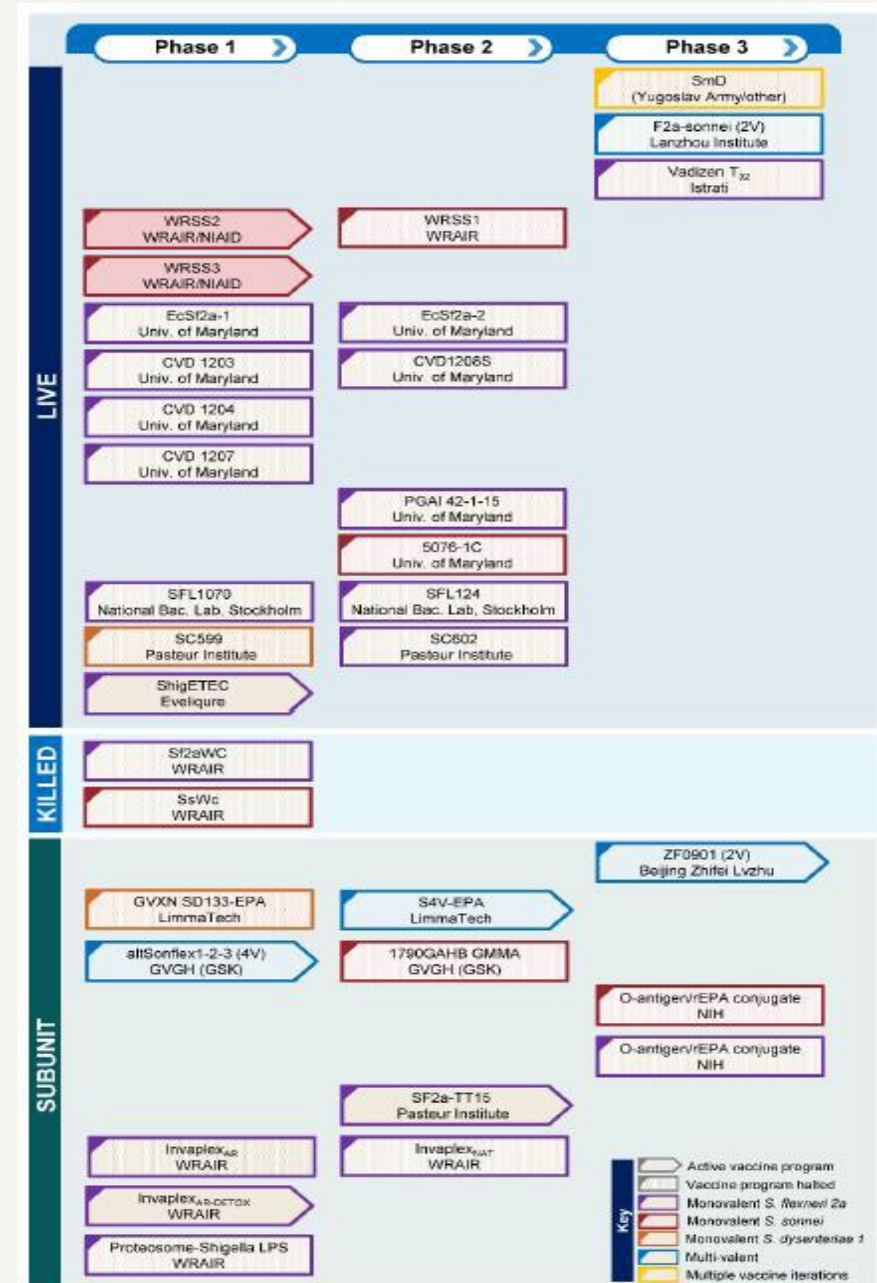
Proportion of *Shigella* isolates resistant across 3 antimicrobial treatments³



Defined as $\geq 70\%$ susceptibility 3. UW START Systematic review and meta-analysis to assess *Shigella* AMR 2025 4. CDC Special Report on COVID-19 & AMR 2022 5. Systematic review only included LMIC and LIC as defined by World Bank

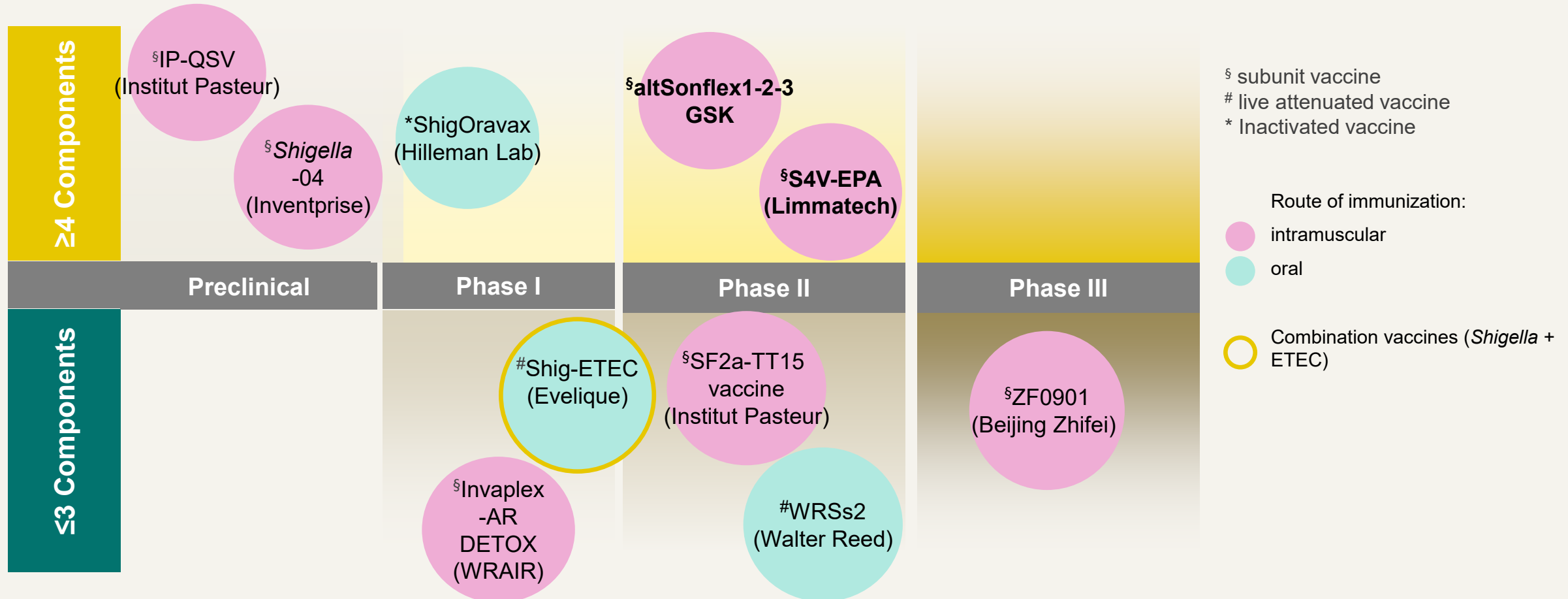
Shigella Vaccine Landscape

- > 100 years of Shigella vaccine development
- Graveyard of failed vaccine candidates – poorly immunogenic or high reactogenicity of live-attenuated Shigella vaccines or killed WC vaccines
- Proof of concept for a glyco-conjugate vaccine developed by US NIH and tested in Israel (1997)
- New emphasis on O-antigen based parenteral Shigella vaccine candidates



Source: MacLennan CA et al. Vaccines 2022.

Shigella vaccines in development



WHO Preferred Product Characteristics for *Shigella* vaccines



Indication

Prevention of moderate to severe diarrhea caused by *Shigella*



Efficacy

≥ 60% efficacy against moderate to severe diarrhea episodes caused by *Shigella* strains in the vaccine



Route of administration

Oral or injectable (IM, ID or SC), using standard volumes of administration



Durability of protection

Duration of protection for 24 months following the last vaccine dose in the primary series, with protection up to 5 years desirable



Population

Children, 6 months to 5 years
(the peak of incidence is between 12–24 months of age)



Safety & reactogenicity

Similar to licensed vaccines for the given age group

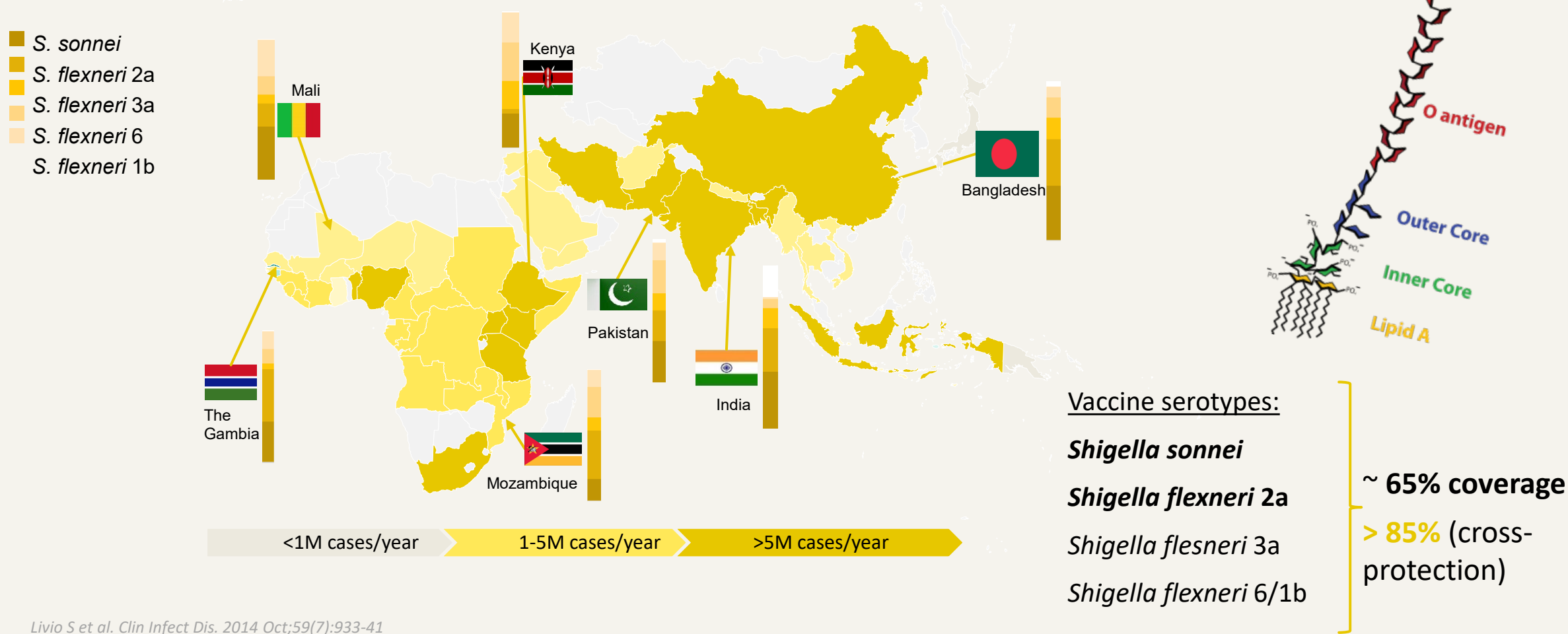


Dose & schedule

1–2 doses for primary immunization, given during the first 12 months of life. An additional booster dose may be required to maintain effective, protective immunity through the first 5 years of life

Multiple *Shigella* strains circulate globally, so vaccines need to be multi-valent vaccines

- 4 major *Shigella* species and more than 50 different serotypes
- Immunity to *Shigella* appears to be serotype-specific (driven by O-antigen diversity)

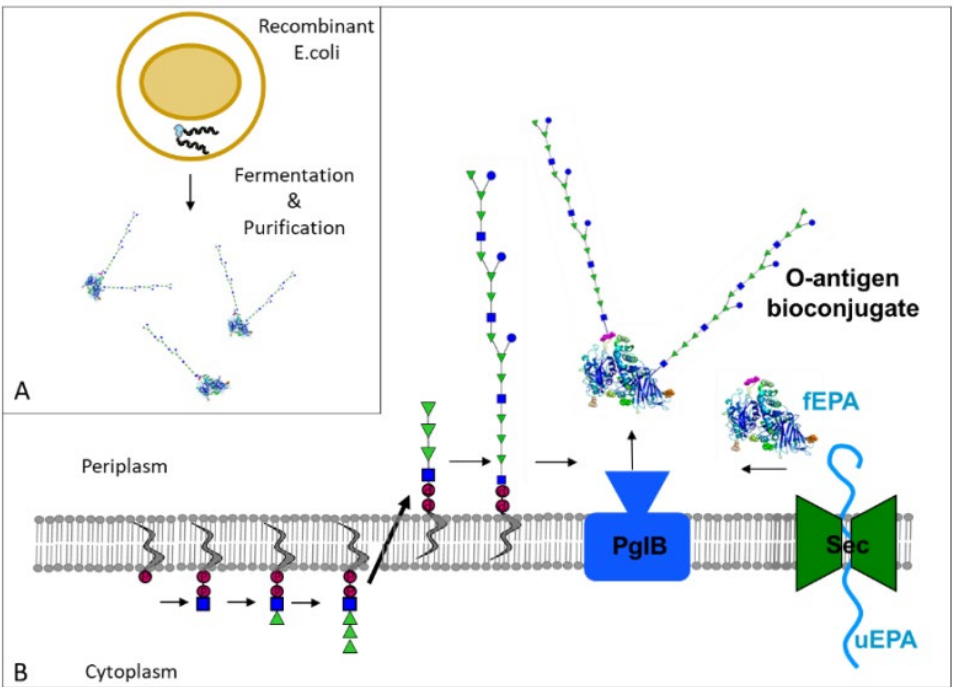


Limmunech S4V

Bioconjugation technology

O-antigens of *S. flexneri* serotypes 2a, 3a, 6 and *S. sonnei* bioconjugated to the EPA carrier protein

Bioconjugates are glycoconjugates **produced *in vivo*** in bacterial cells; the *Escherichia coli* glycan biosynthesis machinery is genetically modified to produce the target polysaccharides and transfer them to defined sites on the protein moiety



Martin et al. *Vaccines (Basel)*. 2022;10(2):212

Shigella serotype	Isolate ID	Fold increase ^a in antibodies induced in rabbits after immunization with:					
		Monovalent				Quadrivalent	
		<i>S. flexneri</i> 2a	<i>S. flexneri</i> 3a	<i>S. flexneri</i> 6	<i>S. sonnei</i>	4V	4V-Adj

Shigella serotypes not within the vaccine formulation							
<i>S. flexneri</i> 2b	SBA-K-S.f2b-131	67	—	—	—	—	41
<i>S. flexneri</i> 2b	SBA-K-S.f2b-132	62	—	—	—	—	25
<i>S. flexneri</i> 4a	SBA-K-S.f4a-032	—	—	—	—	—	7
<i>S. flexneri</i> 4a	SBA-K-S.f4a-039	—	—	—	—	—	7
<i>S. flexneri</i> 4b	SBA-K-S.f4b-068	—	—	—	—	—	9

- Cross-functional antibodies elicited in rabbits

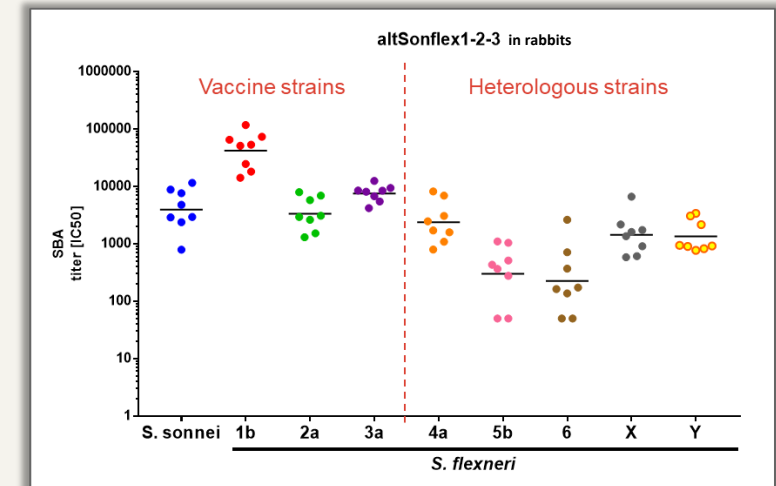
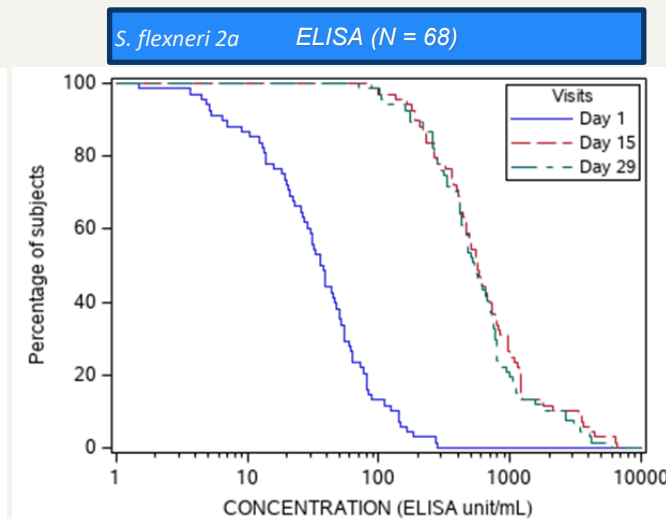
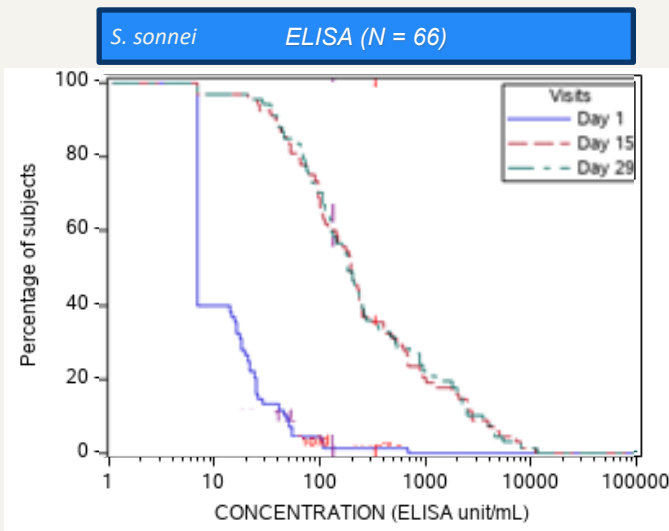
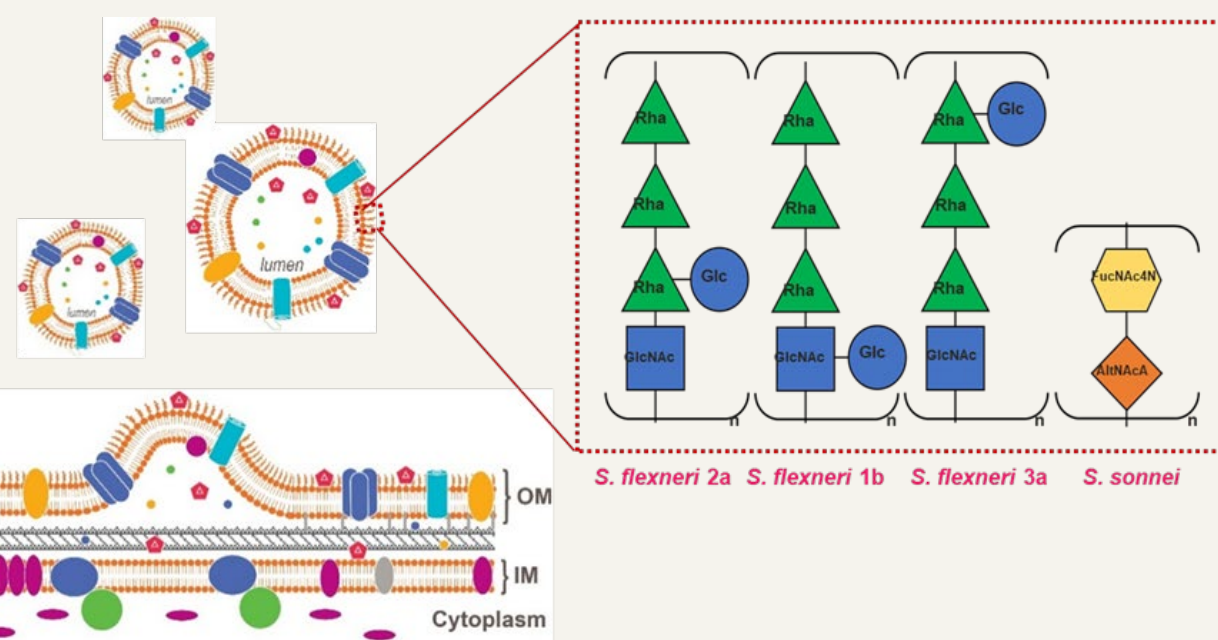
- A phase 1/2 dose-finding study in Kenya showed that the vaccine is **safe and immunogenic in 9-month-old infants** (*results presented at BactiVac 2023 and ASM Microbe 2024*)
- In August 2024 **Valneva in-licensed S4V2** as part of an exclusive worldwide development, manufacturing, and commercialization agreement with LimmaTech
- Ph2b CHIM study (NCT06615375) is ongoing and a study to expand safety and immunogenicity data with S4V2 in 9-month-old Infants (NCT06523231) is planned
- In October 2024, the U.S. Food and Drug Administration (FDA) has granted Fast Track designation to S4V2

GVGH altSonflex1-2-3

GMMA technology

Outer membrane vesicles shed from Gram-negative bacteria genetically modified to **enhance yields** and produce less-acylated lipid A for **reduced endotoxicity**

Rossi, Citiulo et al. NPJ Vaccines. 2023;8(1):130
Leroux-Roels et al. JID, 2024, DOI: 10.1093/infdis/jiae273



- **High anti-OAg IgG response and increase in antibody functionality post 1 in European adults** (numerically higher response for *S. sonnei* and *S. flexneri* 2a)
- Ph1 results allowed **progression to Phase 2** descending-dose finding/schedule finding studies in Kenya (ongoing)
- **Cross-functional antibodies elicited in rabbits**

Institut Pasteur IP-QSV & Inventprise *Shigella*-4

Two additional 4-valent vaccine candidates in preclinical

Institut Pasteur IP-QSV

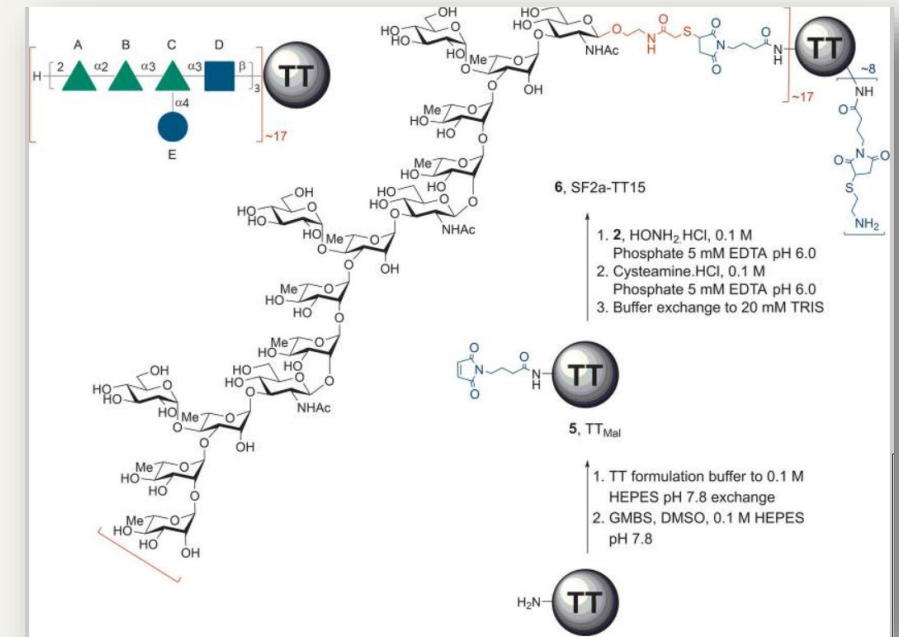
S. flexneri serotypes 2a, 3a, 6 and *S. sonnei* **synthetic** O-antigens conjugated to TT carrier protein

- Sf2a-TT15 (1V) vaccine candidate tested in Ph2 descending dose-finding study in Kenya showing to be safe and highly immunogenic (results presented at ASM Microbe 2024)
- IP-QSV (4V) GMP DS/DP manufactured

Inventprise *Shigella*-4

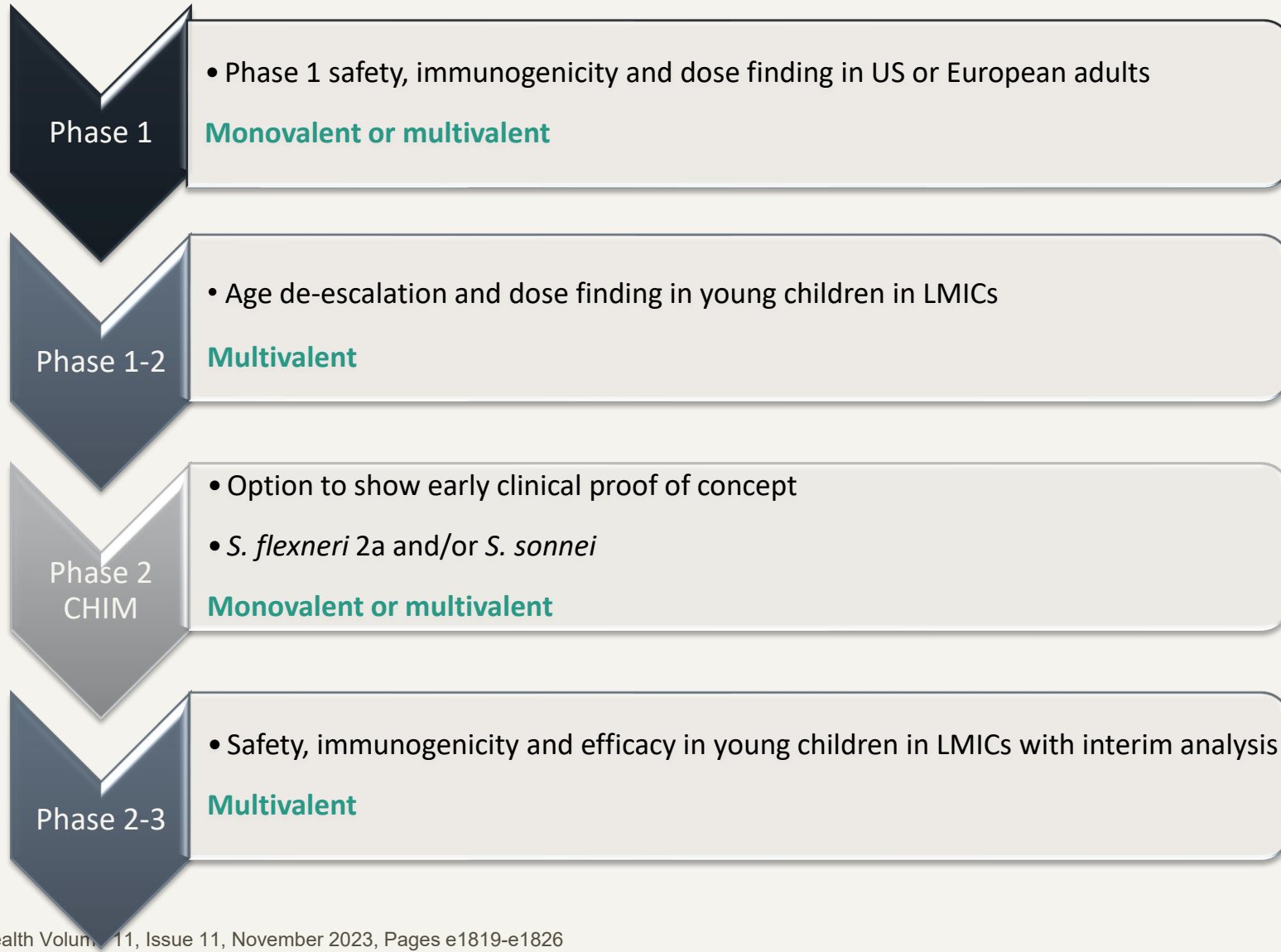
S. flexneri serotypes 2a, 3a, 6 and *S. sonnei* O-antigens conjugated to **IpaB carrier protein**

- *Shigella*-4 (4V) GMP DS/DP manufactured



Phalipon et al. *J Immunol.* 2009;182(4):2241-7

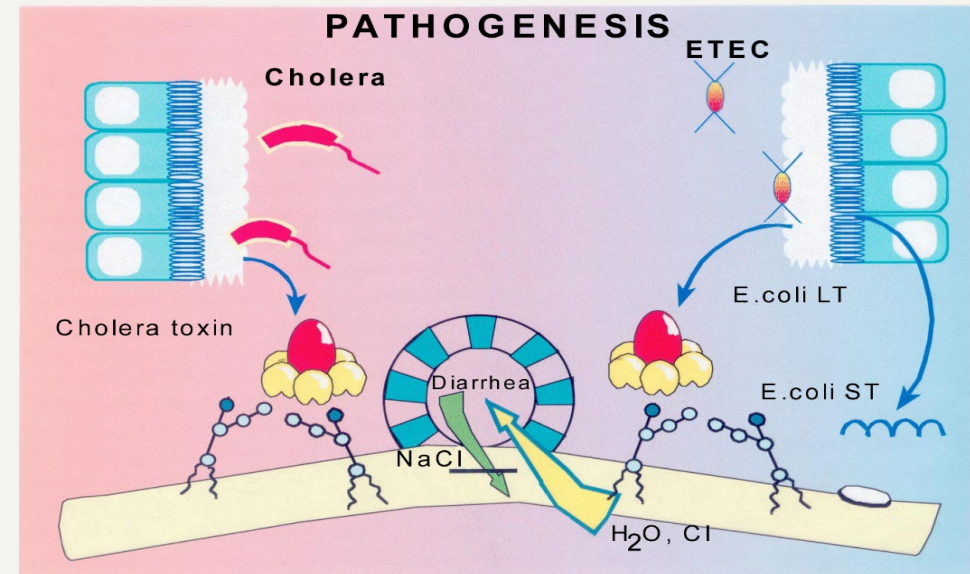
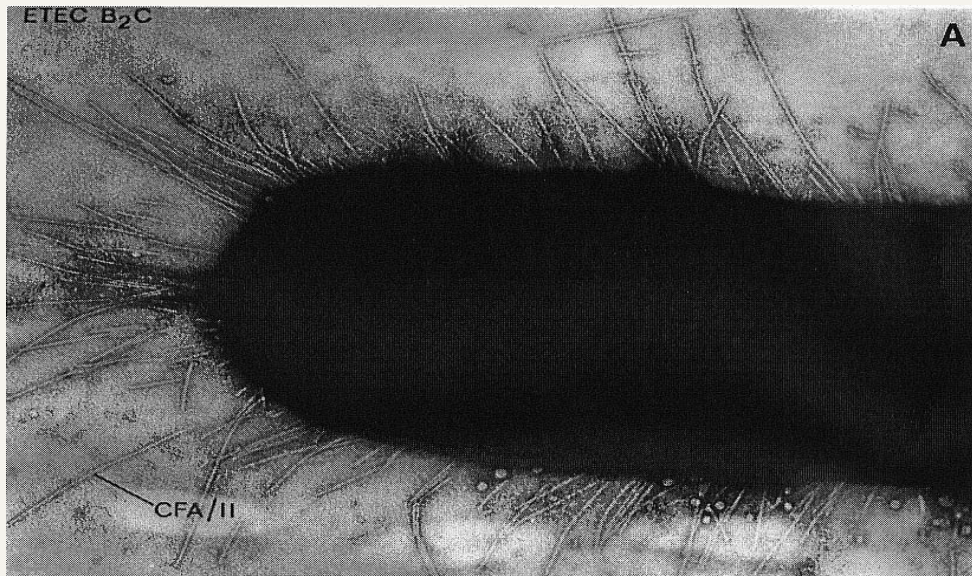
Clinical and regulatory development strategies for *Shigella* vaccines intended for children younger than 5 years in low-income and middle-income countries



Enterotoxigenic E. coli (ETEC)

Enterotoxigenic *Escherichia coli* (ETEC) and *Vibrio cholerae* are leading bacterial causes of severe dehydrating diarrhea in LMICs: Both share similar pathogenic mechanisms

ETEC (LT and ST) and cholera (CT) entero-intoxication is mediated by increasing intestinal cell cAMP (LT/CT) or cGMP (ST) levels leading to decreased intestinal absorption of NaCl and increased secretion of water and Cl⁻ from tissues into the intestinal lumen, resulting in the onset of watery diarrhea



- For ETEC, colonization and toxin delivery to intestinal epithelial cells is facilitated by bacterial mucinases and colonization factors that enable close bacterial contact and binding at the mucosal surface.
- Primary signs/symptoms of ETEC/Cholera: mild-severe diarrhea, abdominal pain/cramps, urgency, nausea, anorexia, dehydration.
- LT in addition to its toxicity also drives other enteropathic/inflammatory changes in the small intestine that contribute to stunting and susceptibility of the host to ETEC as well as infection with other enteropathogens.

ETEC burden overview in LMICs and among International Traveler's

- ETEC is estimated to cause about 220 million diarrhoea episodes globally, with about 75 million of those episodes occurring in children under 5 years of age.
- Repeated ETEC infections (even asymptomatic) can induce/exacerbate stunting and other forms of malnutrition with adverse consequences on growth and cognitive development, as well as an increased risk of death due to other ID causes
- Based on recent data, ETEC also likely contributes to neonatal diarrhoea ([Pajuelo MJ, et al.2024. *Front. Public Health* 12:1332319.doi: 10.3389/fpubh.2024.1332319](#)).
- Among Traveller's, ETEC remains the leading cause of traveler's diarrhea, associated with 15-40% of cases; with ~10-14% of episodes going on to develop persistent functional bowel disorders.
- Regarding mortality, between 18,700 (IHME) to 42,000 deaths (MCEE) are estimated to occur annually among children younger than 5 years. ETEC is also associated with significant mortality in older age-groups, particularly the elderly.
- In the most recent evaluation, ST ETEC (both ST and LT/ST strains) remains among the leading causes of diarrheal disease associated mortality in LMICs ([GBD 2021 Diarrhoeal Diseases Collaborators, 2025. *Lancet Infect Dis* 2025; 25: 519–536](#))

Inflammatory nature of enteric infections: WHO ETEC vaccine roadmap (2021) – even asymptomatic ETEC infections (both ST and LT pathotypes) can contribute to the pathogenic pathway leading to EED/Stunting

I. Khalil, R. Walker, C.K. Porter et al.

Vaccine 39 (2021) 4266–4277

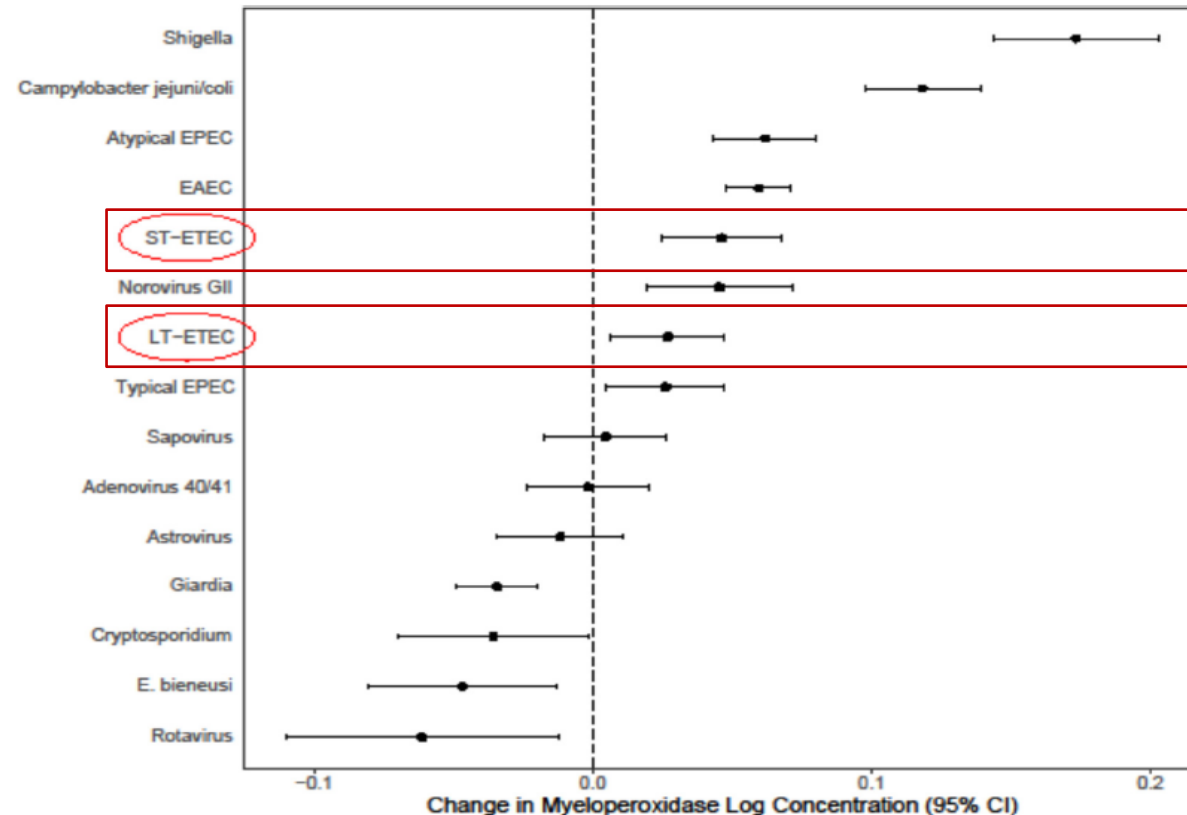


Fig. 1. Association between enteric pathogens detected by quantitative PCR in monthly non-diarrheal stool samples and stool myeloperoxidase in the multisite MAL-ED birth cohort study. Estimates are per tenfold increase in pathogen quantity from a single linear mixed-effects model including the quantity of each pathogen, sex, and age as fixed effects and site and subject as random effects. A total of 18,365 monthly non-diarrheal stools from 1715 children followed until 2 years of age were included, all of which had valid qPCR results for all included pathogens and were tested for myeloperoxidase (MPO; measured in nanograms per milliliter), a marker of neutrophil activity in the intestinal mucosa (Alpco). (Unpublished data provided courtesy of James Platts-Mills, University of Virginia).

ETEC and Shigella associated diarrheal disease morbidity in LMICs: Impact of longer-term effects on children health and development

Burden of enterotoxigenic *Escherichia coli* and shigella non-fatal diarrhoeal infections in 79 low-income and lower middle-income countries: a modelling analysis



John D Anderson IV*, Karoun H Bagamian*, Farzana Muhib, Mirna P Amaya, Lindsey A Laytner, Thomas Wierzb, Richard Rheingans



Summary

Background Enterotoxigenic *Escherichia coli* (ETEC) and shigella are two major pathogens that cause moderate-to-severe diarrhoea in children younger than 5 years. Diarrhoea is associated with an increased risk of stunting, which puts children at risk of death due to other infectious diseases.

Lancet Glob Health 2019;
7: e321–30

See [Comment](#) page e284

*Joint first authors

Summary:

- ETEC and Shigella infections of children younger than 5, is **estimated to results in ~3.5 million episodes of moderate to severe stunting annually.**
- **Infectious disease deaths due to stunting are projected to results in a 28% and 24% increase respectively in Shigella and ETEC associated deaths over those directly attributed to these two pathogens**
- The distribution of this direct/indirect morbidity/mortality was highest in the **WHO's African and Eastern Mediterranean regions.**

Conclusions: The longer-term effects of non-fatal Shigella and ETEC-related diarrheal episodes can have lasting health consequences. **Prevention of these infections could reduce the risk of direct deaths and stunting, as well as deaths due to other ID causes.**

Current ETEC vaccine and funding landscape

Oral administration

Clinical candidates

ETVAX inactivated
(SBH, UG, DoD, EDCTP)

ShigETEC (EveliQure)
(Horizon 2020, NIH)

CVD GuaBA mutants
expressing ETEC Ags.;
CVD1208S-122 (CVD, NIH)

Parenteral injection

FTA (NMRC, NIH)

- **CfaEB** (CFA/I)
- **CssBA** (CS6)

Potential ETEC Vaccine Donors:

- **Europe/UK**—2020, EDCTP, DFID/FCDO, Wellcome Trust
- **USA**—NIAID, NIH, DoD

Preclinical candidates

STM expressing heterologous enteric antigens
(IVI, NMRC/WRAIR, PATH)

MEFA (ILLU, JHU, NIH)

- MecVax (ETEC adhesins + toxins)
- ShecVax (ETEC + Shigella antigens)

LT/ST conjugate toxoids
(Univ. of Bergen, Tulane)

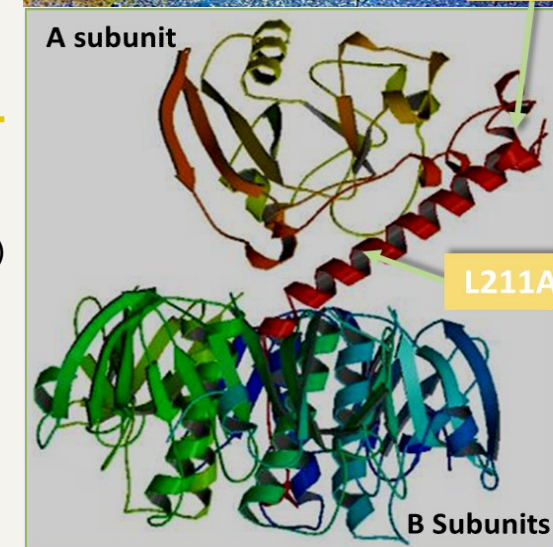
ETEC proteins Flagellin, EtpA, EatA,
YghJ, EaeH
(WASHU, Univ. of Bergen, GlyProVac)

STM = inactivated mutant *Shigella* with truncated O-PS side chain optimizing exposure of conserved protein antigens

MEFA = Multiple-epitope fusion antigen recombinantly produced on protein backbone; may also include dmLT

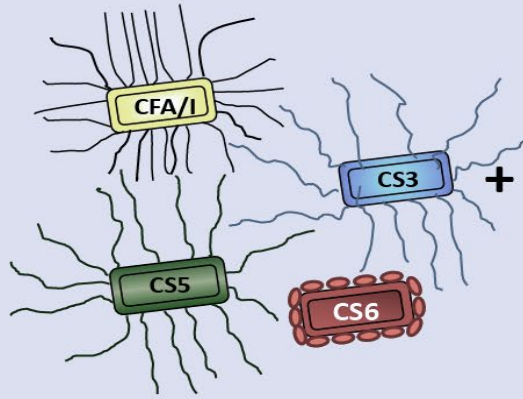
FTA = Fimbrial tip adhesins are recombinantly produced antigen fragments from adhesin protein

LT/ST conjugate and EtpA, EatA, YghJ and EaeH proteins are not viewed as standalone vaccines but as additional components to broaden coverage



ETVAX vaccine overview

Multivalent vaccine



Adjuvant

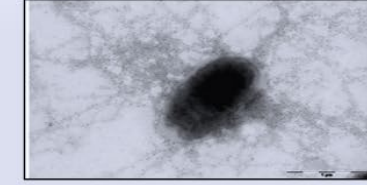


ETVAX generates immune responses to Colonization Factors and heat labile (LT) enterotoxin

ETEC Expressing CFA/I



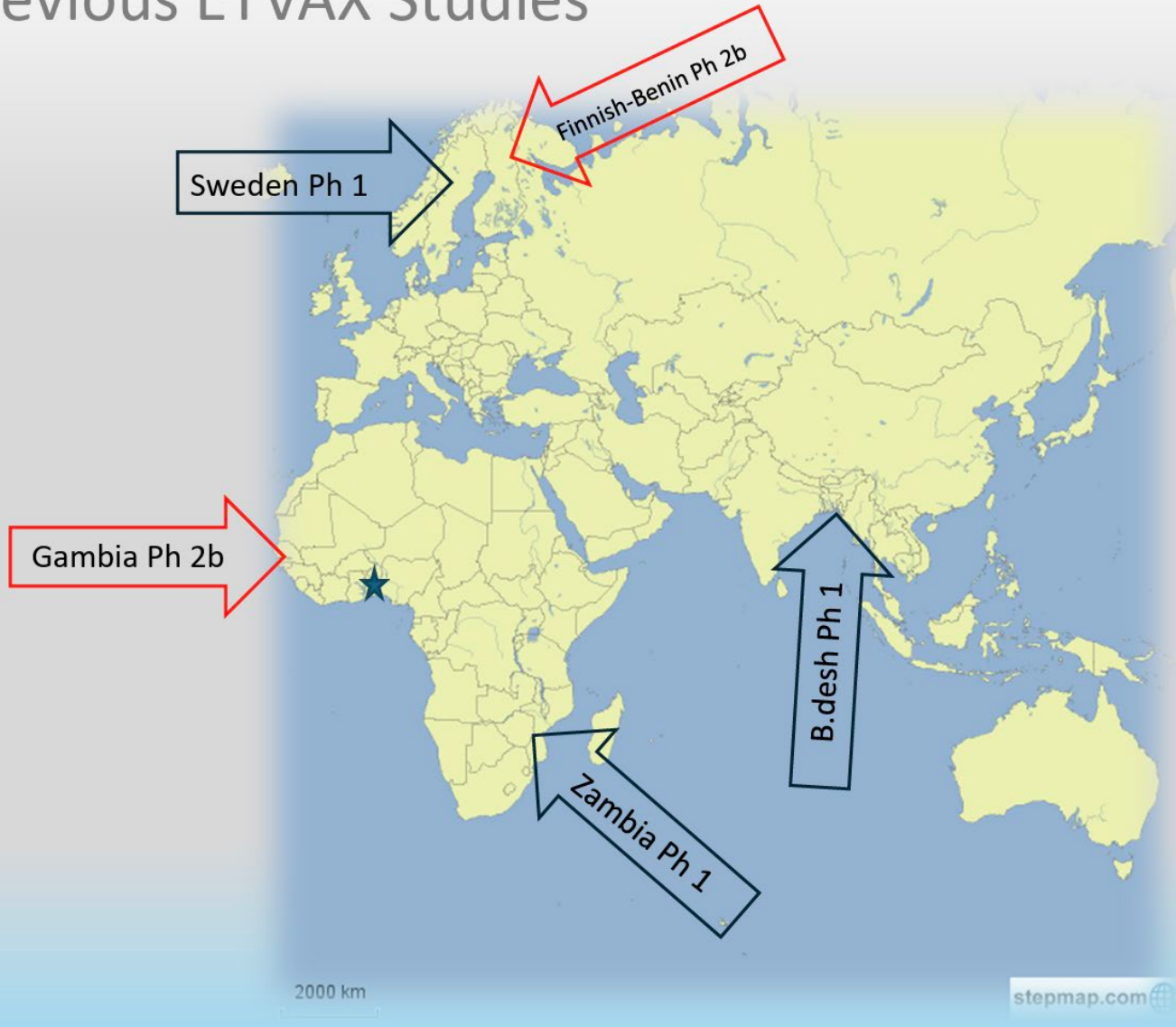
Normal Bacterium



E. coli over expressing CFA/I



Previous ETVAX Studies



Sweden: Phase 1, 129 adults, placebo-controlled. Adverse events were few, mostly mild, immunogenic, dmLT enhanced immunity (Lundgren, Vaccine, 2014)

Bangladesh: Phase 1, 45 adults, placebo-controlled. ETVAX was safe, well tolerated, and highly immunogenicity (Akhtar, Vaccine, 2019)

Phase 1/2, placebo-controlled, dose-escalation, age-descending in children 24-59 (n=130), 12-23 (n=100), 6-11 (n=200) months with half, quarter, eighth adult dose with/without dmLT. Fecal IgA, mucosal IgA responses were robust. dmLT enhanced immunity. (Qadri, Lancet Infect Dis, 2020)

Zambia: Phase 1, placebo-controlled, age-descending, dose-finding, $\frac{1}{4}$ or $\frac{1}{8}$ adult dose, among 40 adults, 60 children 10-23 months, 146 children 6 to 9 months. ETVAX was safe and Immunogenic in adults and children. One-quarter dose was selected for Gambia trial. (Nsofwa, Vaccine, 2023)

ETVAX pediatric study in The Gambia



A phase 2b, double-blind, placebo-controlled trial, 4,936 healthy Gambian children aged 6 to 18 months randomized (1:1) to receive ETVAX or placebo at study days 1, 15, and 90 (i.e., 3 doses).

- As in previous ETVAX trials, the vaccine was found to be safe and well-tolerated
- ETVAX showed good immunogenicity
- ETVAX offers broad protection against ETEC when allowing for any co-infection: 50.9%, $p=0.02$ and has the potential to become a valuable public health tool
- ETVAX offers significant protection against ETEC when allowing for any co-infection except parasites: 80.6%, $p=0.0092$.
- ETVAX may offer broader protection than solely against ETEC cases
 - 21.4% ($p=0.03$) significant protection against moderate-to-severe diarrhea independent of etiology over the 2-year study period
 - Based on the study results ETVAX. Number needed to vaccinate is only 32.
- ETVAX is currently undergoing scientific advice procedures with the EMA to support its progression towards registration for both international travelers and children in LMICs.

Acknowledgement: Bjorn Sjostrand, Scandinavia Biopharma, Sweden

FTA vaccine approach overview

Other vaccines in development

ETEC adhesin vaccine (FTA) components: Candidate status and parenteral delivery proof-of-concept

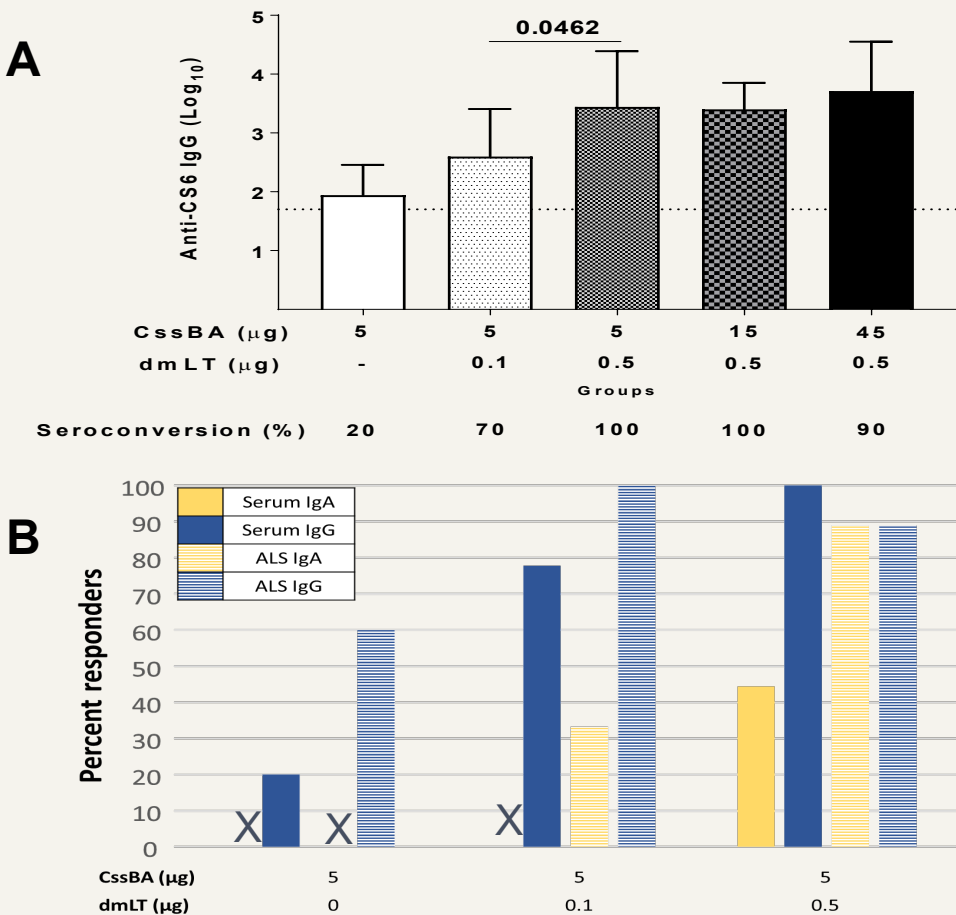
ETEC FTA program overview

CF class	Molecule	Biochemical	Immunogenicity	NHP efficacy	Clinical trials
5a	CfaEB	Structure	+	++	Ph 1, 2b
5b	CsbDA-CooA	Structure	+	+	
5c	CotDA	Structure	+	+	
CS6	CssBA	Modeled	+	+	Ph 1, 2b

CssBA+dmLT Phase 1 trial (IM) (Lee et al 2021 *Vaccine*; <https://doi.org/10.1016/j.vaccine.2021t.08.032>)

- Addition of dmLT had significant impact on serum (IgG and IgA) and mucosal anti-CssBA IgA responses (see panels A and B)
- Increasing doses of CssBA up to 45 µg + 0.5 µg of dmLT lead to 100% of subjects being positive for anti-CssBA IgA α4β7 positive PBMCs and fecal IgA
- Anti-CssBA antibody responses persisted for over 1 year in the highest dosing group and had increased avidity
- **Note: Ph2b efficacy evaluation of CssBA + dmLT using a CHIMs with ETEC strain B7A is underway with support from the IDCRC Program, NIH**
- **Also, CfaE + mLT given by the ID route showed efficacy in an earlier CHIMs study** (Gutierrez, R et al. 2024. *Microorganisms* **2024**, 12, 288; <https://doi.org/10.3390/microorganisms12020288>)

CssBA Phase 1 results: Safe at all dose levels



Additional clinical reference for CfaE + mLT Ph1 - Gutierrez R et al. 2023. *Microorganisms*; <https://doi.org/10.3390/microorganisms11112689>

S. flexneri 2a CVD 1208S: CFA/I-LThA2B (CVD 1208S-122)

S. flexneri 2a strain CVD 1208S

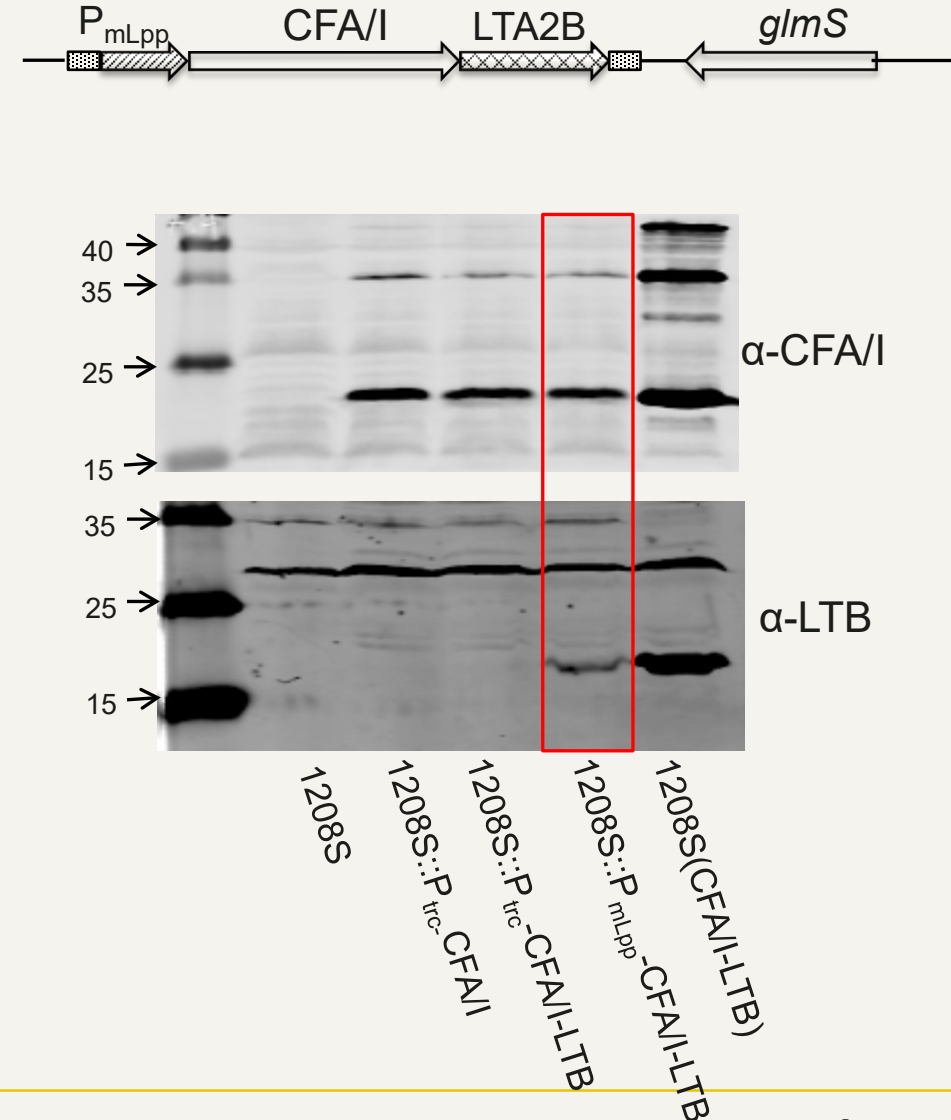
- $\Delta uaBA$, Δsen , Δset
- Safe and immunogenic in volunteers (Kotloff, 2007)

Engineered to express CFA/I and LTA2B subunit from
genes inserted at a chromosomal site

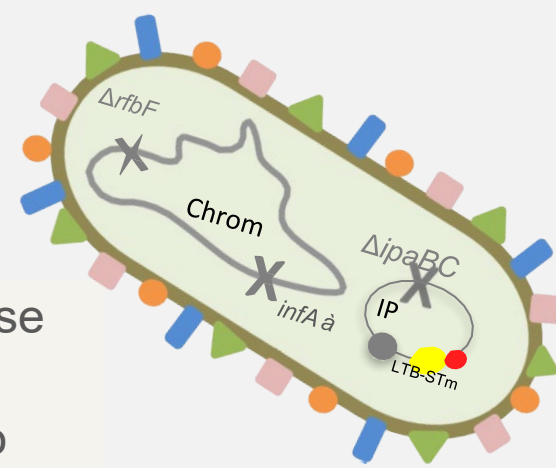
Manufactured as cGMP product

Phase 1: November 2022

Funding: NIH NIAID AI132257 and U01 AI14393



Universal Shigella-ETEC Combination Oral Vaccine (ShigETEC)



- **Shigella vaccine platform:** Removal of LPS O-antigen induces broad antibody response against conserved structures to protect against all types of Shigella
- **ETEC coverage:** LT-B/STm(N12) fusion protein expressed from the invasion plasmid to induce protective antibody response (toxin neutralizing antibodies)

Phase 1: concluded in Europe in 2021

- **Safe, well tolerated**
 - Induction of systemic and mucosal immune response against ShigETEC vaccine strain and LTB and ST

Phase 2: challenge studies in the US

- Three separate controlled human challenge studies, starting mid 2023
- Two different Shigella species (*S. flexneri* 2a, *S. sonnei*)
- One LT+/ST+ ETEC strain
- ETEC challenge study pending; **S.flexneri 2a Ph2b challenge currently ongoing**

Phase 2 supported by NIAID Contract, partners:

Johns Hopkins University

Cincinnati Children's Hospital Medical Center

WRAIR, NMRC, Antigen Discovery



Q&A

Gates Foundation