# TABLE OF CONTENTS

## Global burden of disease, epidemiology, and health economics

### ORAL PRESENTATIONS

- GB054 A novel diagnostic tool to aid surveillance and vaccine evaluation for diarrhea due to enterotoxigenic *E. coli* and *Shigella* spp. ................................................................. 8
- GB059 The unrecognized consequences of ETEC and *Shigella* non-fatal infections: Burden in 79 low- and lower-middle-income countries................................................................. 8
- GB065 Impact of enteropathogen infection on linear growth using quantitative molecular diagnostics: Results from the MAL-ED study......................................................................... 9
- GB069 Etiology, burden, and characteristics of diarrhea in children in low-resource settings using quantitative molecular diagnostics: Results from the MAL-ED study .................................................. 10
- GB081 Changes in incidence, etiology, and consequences of moderate-to-severe diarrhea among children under 5 years in sub-Saharan Africa: The VIDA study........................................... 11

### POSTER PRESENTATIONS

- GB017 *Shigella*-associated diarrheal disease in Ghana: An underestimated disease burden...............................................................................................................................12
- GB028 Prevalence of malnutrition and diarrhea among under-five children of Malayali tribe Tamil Nadu, India..............................................................................................................12
- GB030 Serotypes and phylogenetic groups of ETEC from different sources in Honduras........... 13
- GB032 Prevalence of colonization factors and other virulence genes among enterotoxigenic *E. coli* strains from Latin America, Africa and Asia .............................................................. 14
- GB043 Cost-effectiveness of a reactive oral cholera immunization campaign using Shanchol™ in Malawi .................................................................................................................. 14
- GB044 Cost-of-illness of cholera to households and health facilities in rural Malawi.................. 15
- GB073 Susceptibility to symptomatic enterotoxigenic *Escherichia coli* infections in non-secretor Nicaraguan children ........................................................................................................16
- GB076 Pedestal formation by enteropathogenic *Escherichia coli* is triggered by different condition *in vitro*.................................................................................................................................16
- GB080 Comparative genomics of CFA/I and CS6-producing ST-only enterotoxigenic *Escherichia coli* associated with human diarrhea............................................................. 17
- GB082 Prevalence of ETEC adhesins, EtpA, CFA/I and TibA among isolates from children hospitalized because of diarrhea and from community.........................................................18
- GB089 Epidemiology and risk factors of *Cryptosporidium* infection in rural Gambian children ........................................................................................................................................19
- GB090 *E. coli* O153 from diarrhea cases in different developing countries present STh, STp, CS21 pertains to the phylogenetic group D ............................................................................... 20
- GB092 Etiology of diarrhea and the effect co-infection on risk of severe disease in children under 5 in Zambia ................................................................................................................... 21
GB102  Prevalence of enterotoxigenic *Escherichia coli* among Mexican children with acute diarrhea and isolates toxin gene profiles.................................................................22

GB103  Zinc deficient infants at increased risk of bacterial diarrhea in a Bangladeshi birth cohort...............................................................................................................................23

GB104  Potential impact and cost-effectiveness of ETEC and *Shigella* vaccines in 79 low- and lower middle-income countries.............................................................................24

GB105  Characterization of ETEC and *Shigella* among low-cost specimen preservation samples from patients in Cameroon with diarrhea and/or dysentery ..........................25

**Host parameters and genomics that predict responses to infection and disease.................................................................................................................................26**

**ORAL PRESENTATIONS.................................................................................................................................26**

GEN016  New insights regarding the interplay between *Shigella* and human lymphocytes.......26

GEN072  Immune-profiling of fecal IgA samples on ETEC protein microarray..........................26

GEN078  Development of *Shigella* proteome microarrays and analysis of human antibody responses following vaccination and challenge..........................................................27

**POSTER PRESENTATIONS.................................................................................................................................28**

GEN019  Glyco-engineered cell line and computational docking studies reveal that ETEC CFA/I fimbriae bind to human Lewis a glycans.................................................................................28

GEN031  Development and inter-laboratory evaluations of a simple, high-throughput *Shigella* serum bactericidal assay .............................................................................................29

GEN038  Diarrhea caused by enterotoxigenic *Escherichia coli* induces T-cell responses in the circulation.................................................................................................................................29

GEN046  Human microbiota mice: New animal models for enteric *Escherichia coli* infections....30

GEN047  Efficacy of a commercially available hyperimmune bovine colostrum product for the prevention and treatment of shigellosis in rhesus macaques.....................................................30

GEN056  Monitoring the human gut microbiota during a *Campylobacter jejuni* challenge ........31

GEN062  Serological profiling by proteomic microarray of culture-confirmed ETEC-associated travelers’ diarrhea .............................................................................................................................32

GEN068  Quantitation of enterotoxigenic *Escherichia coli* strain TW11681 in stools of experimentally infected human volunteers............................................................33

GEN085  Synergistic effect of fecal markers of environmental enteropathy on stunting among Zambian children..................................................................................................................34

GEN087  A novel transgenic mouse with a hyperactive mutation in receptor guanylyl cyclase is a model for familial diarrhea syndrome................................................................................34

GEN101  Comprehensive characterization of circulating T follicular helper cells and plasmablasts in human volunteers experimentally infected with enterotoxigenic *Escherichia coli* ....35
Novel adjuvants and immunization strategies for vaccination........................................................................36

POSTER PRESENTATIONS..........................................................................................................................36

ADJ023  Presentation of Skp in outer membrane vesicles protects mice against enterotoxigenic
*Escherichia coli* challenge..................................................................................................................36

ADJ055  U-OMP19 from *Brucella abortus* increases dmLT immunogenicity and improves
protection against heat-labile toxin (LT) oral challenge *in vivo*.........................................................36

ADJ077  Design and validation of heat-labile toxin (LT) neutralization assay......................................37

ADJ086  PGT new generation of cross-protective human vaccines..........................................................38

Preclinical evaluation of vaccine candidates and models of enteric disease........................................39

ORAL PRESENTATIONS ..........................................................................................................................39

PRE012  ST-secreted conjugates are non-toxic and induce ST-neutralizing antibodies in
immunized mice.........................................................................................................................................39

PRE051  Evaluation of class 5a fimbrial adhesin-pilin fusion vaccines in *Aotus nancymaee*
against diarrhea caused by CS14 STh+ expressing ETEC..................................................................39

PRE052  A synthetic conjugate toxoid vaccine candidate targeting heat-stable toxin-producing
*Escherichia coli*. .........................................................................................................................................40

PRE064  ETEC strain TW10722 may be suitable for use in vaccine challenge studies for testing
heat-stable toxoid based vaccine candidates..........................................................................................40

PRE066  Human experimental infection with ST-only enterotoxigenic *Escherichia coli* wild-type
strain TW11681 ........................................................................................................................................41

PRE088  Towards bacterial glycoprotein antigens for vaccine development............................................43

PRE097  MACE, Multi-Antigen Combination Enteric, vaccine for broad protection against ETEC
and *Shigella* infections............................................................................................................................43

POSTER PRESENTATIONS..........................................................................................................................44

PRE013  Enhancing *in silico* peptide-based vaccine discovery for enterotoxigenic *Escherichia
Coli* using molecular docking and molecular dynamics simulation approach.................................44

PRE018  A study of serotypic differences in innate and adaptive immune responses in
*Shigellosis* in humans..............................................................................................................................45

PRE021  Immunogenicity and characterization of enterotoxigenic *Escherichia coli* (ETEC)
toxoid fusion and adhesin MEFA antigens in intradermally or intramuscularly
immunized mice........................................................................................................................................45

PRE022  Computational characterization and functional annotation of hypothetical proteins
of *Shigella* using *in-silico* tools: A bioinformatics study..................................................................46

PRE029  Immunogenicity and protective efficacy of inactivated *Shigella* multivalent vaccines......46

PRE039  Establishment of a human colonoid model to study *Shigella* pathogenesis and
evaluate vaccine candidates.....................................................................................................................47
| PRE048  | A comparative immunogenicity study of enterotoxigenic *Escherichia coli* heat-stable toxin (STA) toxoid genetic fusion versus chemical conjugates | 47 |
| PRE050  | A human-associated colonization factor in enterotoxigenic *E. coli* recognizes mucosal receptors present in human and pig small intestines | 48 |
| PRE053  | Purification and characterization of native and vaccine candidate mutant enterotoxigenic *Escherichia coli* heat-stable toxins | 48 |
| PRE061  | Immune assays to evaluate ShigETEC, a live, attenuated combination vaccine against shigellosis and ETEC diarrhea | 49 |
| PRE070  | Evaluation of inactivated derivatives of the bi-valent *Shigella*-ETEC vaccine candidate CVD 1208S-122 | 49 |
| PRE071  | Characterization of the Invaplex vaccine using dynamic light scattering | 50 |
| PRE084  | An integrated mathematical and immunological approach to optimizing the functional immune responses in an adjuvanted subunit vaccine for ETEC | 51 |
| PRE091  | Heat-killed multi-serotype *Shigella* immunogens induce cell mediated adaptive immunity and protective efficacy in animal model | 51 |
| PRE096  | Expression, characterization and immunogenicity of CS21 subunit-based enterotoxigenic *E. coli* vaccine candidates | 52 |
| PRE098  | Identification and characterization of human monoclonal antibodies for oral immunoprophylaxis against enterotoxigenic *Escherichia coli* infection | 53 |
| PRE100  | Microbiological surveillance of *Shigella* spp. and the enterotoxigenic *Escherichia coli* pathotype in Cuba | 53 |

**Strategies for broader coverage of combination/co-administered vaccines**

**ORAL PRESENTATIONS**

CMB083  Developing WHO preferred product characteristics for ETEC and *Shigella* vaccines | 54 |

**POSTER PRESENTATIONS**

CMB020  ETEC adhesin-toxoid MEFA CFA/I/II/IV-3xSTA12S-mnLTG192G/L211A induces antibodies protecting against ETEC diarrhea | 54 |

**Vaccine candidates in clinical trials and human challenge models**

**ORAL PRESENTATIONS**

CL033  Functional antibodies and cytokine responses to live oral *Shigella sonnei* vaccine strain WRSS1 in Bangladeshi adults and children | 56 |

CL035  Immune response characterization after controlled infection with a lyophilized *Shigella sonnei* 53G, (cGMP Lot 1794) | 56 |
Immune response profiles following vaccination with a *Shigella* bioconjugate vaccine that correlate with a reduction in shigellosis severity .................................................. 58

A Phase I/II trial of the oral inactivated ETEC vaccine (ETVAX; OEV 122) in descending age groups in Bangladesh .................................................................................. 59

Mucosal immune responses to an oral inactivated ETEC vaccine (ETVAX) among descending age groups in Bangladesh .................................................................................. 60

Safety and immunogenicity study of SF2a-TT15, a synthetic carbohydrate-based conjugate vaccine against *S. flexneri* 2a in healthy adult volunteers .................................................. 60

*Shigella*-specific serum bactericidal and opsonophagocytic killing antibodies induced by oral *S. flexneri* 2a whole cell killed and live attenuated vaccines ........................................... 62

Experimental oral challenge with B7A ETEC induces proliferative CD4+ T cell responses to CS6 and LT, which are associated with enhanced systemic and mucosal B cell responses ......................................................................................................................... 62

**POSTER PRESENTATIONS** .................................................................................................................. 64

Serotype and antigen specificity of serum bactericidal activity after intranasal immunization with *S. flexneri* 2a artificial Invaplex ........................................................................... 64

Parenteral immunization with the *Shigella flexneri* 2a bioconjugate vaccine induces LPS-specific memory b cell responses .......................................................................................... 65

Immune responses to oral vaccination: Reduced dosages of a late booster dose .......... 66

Establishment the dot blot for the identity of polysaccharide vaccines ......................... 66

Development and validation of the method for determining Vi polysaccharide molecular integrity by HPLC in *Salmonella typhi* conjugated vaccine ........................................ 67

Phase I clinical studies of a bivalent, LPS-based conjugate vaccine against *Shigella flexneri* 2a and *S. Sonnei* in China .......................................................................................... 67

A Phase 2b clinical trial of ETVAX, an oral whole-cell inactivated vaccine against enterotoxigenic *Escherichia coli*, in Finnish travelers to Benin ................................................................ 68
Global burden of disease, epidemiology, and health economics

ORAL PRESENTATIONS

GB054  A novel diagnostic tool to aid surveillance and vaccine evaluation for diarrhea due to enterotoxigenic E. coli and Shigella spp.

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Enterotoxigenic Escherichia coli (ETEC) and Shigella spp (ES) are the leading causes of moderate-to-severe diarrhea among children < 5 years of age in impoverished areas of the world. Recurrence of infection can result in death, stunting, impaired cognitive development and long-term disability. Due to the huge impact of ES in children, vaccine development has been prioritized and accelerated in recent years. As promising vaccine candidates for ES move toward field trials in endemic areas, an improved understanding of the epidemiology of ES will be critical when planning Phase 3 trials. A critical constraint is the complex, time constraining and expensive diagnostic methods currently required for detecting ES infections such as bacterial culture tests for Shigella, PCR of selected E. coli colonies for ETEC etc. These methods are neither sufficiently sensitive nor standardized to provide an understanding of country-specific burden of ES, and the current methods are not feasible for assessing vaccine efficacy in the resource poor settings (RPS) where field trials need to be conducted. Thus, a simple, as well as sensitive, detection assays for ES are critical to fill this gap.

We developed a novel simple, rapid (~60 minutes), sensitive and inexpensive assay, ES-RDT (ETEC-RDT and Shigella-RDT) at JHU which is able to detect ETEC (LT, STh and STp genes) and Shigella (ipaH gene) within about one hour. The assay involves a novel 6-minute sample preparation method directly from stool with our unique lyophilized reaction strips and uses an established loop mediated isothermal amplification (LAMP) platform.

To determine analytical sensitivity, the ES-RDT was performed with stool samples spiked with 10-fold serial dilutions of ETEC H10407 and S. flexneri 2457t separately. The sensitivity was tested with our novel ES-RDT kit directly from stool and compared with quantitative PCR (qPCR) using DNA extracted with Qiagen kit. The lowest detection limit (LOD) of the ES-RDT was ~ 1X10^5 CFU/gm of stool for all the genes (mentioned above), which we could consistently detect 15 times. This LOD was similar to as found in qPCR. We then evaluated the diagnostic sensitivity and specificity of ES-RDT using 261 frozen stool samples collected from patients with diarrhea from our study in Bangladesh and 136 frozen stool samples collected from a traveler’s study in Guatemala and Mexico. Compared to qPCR the sensitivity and specificity were LT (98%, 100%), STh (100%, 100%), STp (100%, 100%) in the samples from Bangladesh and LT (100%, 100%), STh (100%, 100%), STp (97%, 99%) in the samples from travelers. Due to very low prevalence of Shigella (~2%) in these samples, we didn’t have sufficient power to evaluate the ipaH gene. However, results from the two assays correlated well.

Given, ES-RDT is rapid, sensitive, specific, easy to use, using minimal, battery powered equipment which is easy to scale up and appropriate for RPS, we believe this assay is an ideal tool to fill the gap for ES disease surveillance and vaccine evaluation in the endemic countries.

GB059  The unrecognized consequences of ETEC and Shigella non-fatal infections: Burden in 79 low- and lower-middle-income countries

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**Background:** Enterotoxigenic *Escherichia coli* (ETEC) and *Shigella* are two major pathogens causing moderate-to-severe diarrhea (MSD) in children less than five years of age. Diarrhea is associated with an increased risk of stunting, which puts children at risk of death due to other infectious diseases.

**Methods:** We model the ETEC and Shigella mortality and impact of MSD episodes to determine the additional number of children stunted due to these infections in 79 low- and lower middle-income countries. We then apply a population attributable risk for increased number of deaths due to other infectious diseases in children who are stunted.

**Findings:** In children under 5 years of age, we estimate 196 million episodes of ETEC and Shigella diarrhea occur annually, resulting in 168,000 cases of moderate-to-severe stunting and 32,990 total ETEC and 78,500 total Shigella deaths in 2015. Additional infectious disease mortality due to stunting resulted in a 48% and 59% increase over direct deaths due to ETEC and Shigella diarrheal episodes, respectively. The distribution of mortality and morbidity is heterogeneous with AFRO and EMRO countries bearing the greatest burden.

**Interpretation:** The expanded effects of non-fatal ETEC and Shigella diarrheal episodes can have lasting consequences. Preventing these infections may reduce the risk of direct death as well as stunting and deaths due to other infectious diseases. It is important to capture the expanded effects of infection to capture the full value of prevention. Understanding the countries and populations with the highest disease risk helps target interventions for the most vulnerable populations.

**GB065 Impact of enteropathogen infection on linear growth using quantitative molecular diagnostics: Results from the MAL-ED study**

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**Background:** Exposure to enteropathogens early in life not only causes diarrhea but may also contribute to poor linear growth. We used highly sensitive quantitative molecular diagnostics to assess whether particular causes of diarrhea and asymptomatic carriage of pathogens in the first two years of life were associated with linear growth across seven diverse low-resource settings.

**Methods:** In the multi-site longitudinal birth cohort, MAL-ED, we measured enteropathogen exposure in monthly asymptomatic stools and during diarrhea with qPCR assays for 30 pathogens. Length was measured monthly. We estimated the effects of etiology-specific diarrhea and asymptomatic enteropathogen carriage on linear growth in 3-month intervals, at 2 years of age, and using a longitudinal model that accounted for temporality and time-varying confounding.
**Findings:** Among 1,469 children who completed follow-up to two years of age, 35,622 stool samples were tested and yielded valid results. Diarrhea episodes attributed to bacteria and protozoa, but not viruses, were associated with small decrements in LAZ after 3 months, but these associations were not maintained at 2 years of age. Conversely, high asymptomatic burden of *Shigella/EIEC* (LAZ difference: -0.15, 95% CI: -0.28, -0.02), EAEC (-0.21, 95% CI: -0.37, -0.05), *Campylobacter* (-0.18, 95% CI: -0.34, -0.03), and Giardia (-0.18, 95% CI: -0.31, -0.06) were associated with significant decrements in LAZ at 2 years. *Shigella/EIEC* had the highest impact per quantity in stool. Subtyping was performed on a subset, and a majority of *Shigella/EIEC* detections were *S. flexneri* or *S. sonnei*. Based on these models, interventions that successfully prevent all exposure to these four pathogens would be expected to increase mean length at 2 years by 0.24 LAZ (95% CI: 0.08, 0.41; 0.75 cm), and up to 0.37 LAZ in high burden sites.

**Interpretation:** Episodes of diarrhea in isolation did not have sustained impact on length, while asymptomatic carriage of enteropathogens was common and comparatively more important for linear growth. The use of molecular diagnostics has revealed a greater burden of these enteropathogens, particularly of *Shigella/EIEC*, which is under-detected by culture methods. A successful *Shigella* vaccine would not only reduce diarrhea but may ameliorate stunting.

**GB069 Etiology, burden, and characteristics of diarrhea in children in low-resource settings using quantitative molecular diagnostics: Results from the MAL-ED study**


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**Background:** The application of quantitative molecular diagnostics to etiologic studies of diarrhea has substantially improved estimates of disease burden but has not been applied to a study of diarrhea at the community level.

**Methods:** We re-analyzed archived stool specimens from an eight-site birth cohort study using quantitative PCR (qPCR) for 30 enteropathogens. We calculated attributable burdens of diarrhea and assessed relationships between specific etiologies and clinical characteristics.

**Findings:** We analyzed 6625 diarrheal and 33781 monthly non-diarrheal stools. Overall, 64.1% (95% confidence interval: 62.8 – 72.0) of diarrhea episodes could be attributed to an infectious aetiology by qPCR. Ten pathogens accounted for 94.6% of all attributable diarrhea (157.8/166.8 attributable episodes per 100 child-years): *Shigella/EIEC* (25.4 attributable episodes per 100 child-years; 95% CI: 22.8 - 28.8), sapovirus (23.5; 18.2 - 28.3), rotavirus (21.0; 19.6 - 23.4), adenovirus 40/41 (18.7; 15.8 - 21.7), ETEC (18.0; 16.1 – 23.0), norovirus GII (14.6; 11.3 - 17.8), astrovirus (14.5; 11.8 - 18.4), *Campylobacter* spp. (12.4; 10.3 - 16.7), Cryptosporidium (5.9; 4.2 - 8.1), and typical EPEC (3.9; 1.9 - 8.5). Subtyping was performed on a subset, and a majority of *Shigella/EIEC* detections were *S. flexneri* or *S. sonnei*. ETEC colonization factor typing by PCR revealed that 88.4% of ETEC diarrhea were positive for LT, CFA/I, CS5, CS6, and CS3. Pathogen quantity cut-offs could be derived for episode-level assignment of etiology, and using clinical characteristics as well as child age, we derived model-based scores to identify etiology-specific diarrhea. A score largely based on age and blood
in stool could identify diarrhea attributable to *Shigella* with reasonable accuracy (AUC ROC 0.784), while ETEC diarrhea could not be clinically discriminated.

**Interpretation:** A vaccine against *Shigella* and ETEC would reduce 2 of the 5 leading etiologies of community-based diarrhea and approximately 43.4 diarrheal episodes per 100-child years.

**GB081 Changes in incidence, etiology, and consequences of moderate-to-severe diarrhea among children under 5 years in sub-Saharan Africa: The VIDA study**

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**Background:** The Vaccine Impact on Diarrhea in Africa (VIDA) study is a 36-month prospective, population-based case-control study of moderate-to-severe diarrhea (MSD) in three countries in sub-Saharan Africa – The Gambia, Mali, and Kenya. The study is a follow-on to the Global Enteric Multicenter Study (GEMS) at these same sites and aims to provide a detailed assessment of the incidence, etiology, and adverse clinical consequences of MSD among infants and children 0-59 months of age following the introduction of rotavirus vaccine.

**Methods:** This ongoing study uses similar methods to GEMS. We enroll children with MSD (diarrhea (>3 loose stools/24h) plus either dysentery, sunken eyes, decreased skin turgor, IV rehydration, or hospitalization seeking care at study site sentinel health centers along with matched community controls without diarrhea. We collect clinical data and anthropometry at enrollment. A follow-up visit is conducted ~60 days later to determine vital status and to repeat anthropometric measurements. Parents complete a home diary to document diarrhea duration. Each participant provides a fecal sample at enrollment to identify enteropathogens by conventional methods (culture, ELISA, multiplex PCR). In parallel, all specimens are assessed using quantitative PCR (qPCR).

**Results:** During the first 2 years of the study, we have enrolled 3,505 children with MSD and 4,155 controls. Whereas in GEMS rotavirus was the predominant pathogen in infancy, in VIDA the attributable fraction (AF) of rotavirus for MSD is now lower or comparable to that of other pathogen AFs at all three sites. In 0-11-month-old infants, the AF of *Cryptosporidium* is equal to or higher than rotavirus, and in Kenya norovirus GII is equivalent to rotavirus. Whereas in GEMS rotavirus continued to predominate in the 12-23-month age stratum, *Shigella* now predominates in The Gambia and Kenya, and is the most important pathogen in the oldest age groups in The Gambia and Kenya (also Mali if using qPCR). Adjusting for age, baseline height-for-age z score, and duration of follow-up, MSD cases have exhibited poorer linear growth between enrollment and follow-up compared to their matched controls. Although post-MSD mortality has diminished since GEMS, cases continue to exhibit a higher odd of death than controls in VIDA (1.9% versus 0.4%, OR: 39.0, 95% CI: 8.5-infinity). Progression to persistent diarrhea was significantly higher in VIDA vs GEMS in The Gambia, but significantly lower in Mali in all ages and lower only among infants in Kenya.

**Conclusions:** Rotavirus continues to cause diarrheal morbidity among infants and young children in sub-Saharan Africa following vaccine introduction, but is no longer the leading pathogen. Other pathogens such as *Cryptosporidium* and norovirus are now predominating in infancy and *Shigella* in 12-59 months of age. In the face of this shifting landscape of pathogens, and trends toward decreasing morbidity and mortality, MSD continues to be significantly associated with linear growth faltering and mortality during the 60 days after an episode.
POSTER PRESENTATIONS

GB017  *Shigella*-associated diarrheal disease in Ghana: An underestimated disease burden

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**Background:** Shigellosis, caused by enteroinvasive *E. coli* or *Shigella* species, plays an important role in the morbidity and mortality of children <5 years of age. Between 2012 and 2013, children <5 years of age with bloody diarrheal stool samples seeking medical care at 2 sentinel sites in Ghana were recruited and enrolled in diarrheal disease research study. This study was a collaboration between Noguchi Memorial Institute for Medical Research, University of Ghana and Tel Aviv University, Israel under the Stopenterics program. A preliminary screening using conventional culture methods in our laboratory in Ghana yielded no results. Samples were retested in Tel Aviv University using molecular methods. The present study describes the prevalence of *Shigella* from diarrheal stool samples of children <5 years of age, using invasive plasmid antigen H-based (ipaH-based) PCR technique.

**Methods:** DNA was extracted from the diarrheal stool samples (n=400) using the PowerSoil® DNA isolation kit as per the manufacturer’s protocol. The extracted DNA were subjected to ipaH-based PCR technique.

**Results:** The invasive *Shigella* plasmid antigen H (ipaH) gene was detected in 34% (134/400) of the stool samples screened. This was in sharp contrast to a previous study conducted in Ghana in which *Shigella* was detected in 9.2% of diarrheal stool samples tested by culture. *Shigella* infection was significantly higher among males than females (51% vs 49%; *P*<0.017). Infection was common in the 0-18 months age group (85%, 80/94) with peak infection (33%, 19/57) documented in the 13-18 months age group. There was no significant association between infection and diarrhea status (*P*<0.539).

**Conclusions:** IpAH-based PCR for the detection of *Shigella* must be carried out in conjunction with the traditional stool culture method as the former is more sensitive than the conventional culture method.

GB028  Prevalence of malnutrition and diarrhea among under-five children of Malayali tribe Tamil Nadu, India

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**Introduction:** Health is a state of complete wellbeing free from any discomfort and pain. Despite remarkable worldwide progress in the field of diagnostic, curative and preventive medicine, still there are large populations of people living in isolation in natural and unpolluted surroundings far away from civilization, maintaining their traditional values, customs, beliefs and myths. India has the second largest tribal population of the world next to the African countries. About half of the world’s autochthonous people live in India, thus making India home to many tribes which have an interesting and varied history of origins, customs and social practices. The present study was conducted to assess prevalence of malnutrition and diarrhea among under-five children of Malayali tribe Tamil Nadu, India.

**Materials and Methods:** A cross-sectional study was conducted in 14 villages of the Yelagiri hills. A total of 400 under-five year children were identified. Demographic details, episodes of diarrhea among under-five children and treatment/care seeking behavior were collected from mothers/care givers by interview. Nutritional status was assessed using new WHO standards.

**Results:** Of the total 400 children, 58% were girls and 42% were boys. In the last 1 month, 24% reported diarrhea. The prevalence of underweight among under-five children was 47% . Majority (70%) sought treatment for illness in modern
Conclusion: These tribal children had high prevalence of malnutrition and diarrhea. Efforts need to be strengthened for social inclusion of tribes into mainstream.

**GB030  Serotypes and phylogenetic groups of ETEC from different sources in Honduras**

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**Introduction:** *Escherichia coli* is a facultative anaerobic bacterium that is part of the normal microbiota of the human intestine. However, *E. coli* strains that cause diarrhea in humans are known as diarrheagenic *E. coli* (DEC) and are divided into 6 pathogenic categories: ETEC, EIE, EPEC, EHEC, EAEC and DAEC. Due to the importance of contaminated water and dairy products have been the focus of studies to determine its involvement in the transmission of disease associated to human. The aim of this novel descriptive study was to know, virulence genes and microbiological features of *E. coli* strains from different sources in Honduras.

**Materials and methods:** There were 3 main sources of samples: handmade dairy products (cheese), water (superficial, underground and lake), human (from a previous study on children under 3 years). The bacteria identification was performed with biochemical tests prepared in the laboratory and serotyping was determinated with 187 anti-O and 53 anti-H rabbit sera. The phylogenetic groups of the *E. coli* strains were determinated by PCR assay with Clermont`s system. Virulence genes of EPEC, ETEC,(eae, bfp, eaf, lt, st) pathotypes were analyzed by PCR assays.

**Results:** A total of 166 isolated were identified as *E. coli* strains, 32, 86 and 48 from water, humans and handmade cheese. A hundred strains amplified for the different virulence factors being EPEC the main pathotype in human and handmade cheese source, in second place ETEC was present in all sources. A 29% of *E. coli* strains amplified for one, two or both toxins of ETEC. On water source, 6 ETEC strains were found in natural lake (Yojoa Lake); the 6 strains amplified for both gene toxins (LT and STh). The serotypes found were O6:H16 (n=4, phylogroup A), O8:H9 and O159:H21 (phylogroup C and D respectively). On human source, ETEC (n=10 strains); the serotype O20:H30 (A, n=1). amplified for both genes (LT and STh). Serotypes O?:H10 (n=2), O159:H21 (n=1), O81:H31 (n=1) also amplified for both toxins, belong to phylogroup B1. Finally, O8:H19 (n=3) of group B1 amplified STp. The STp gene also found in O25:H4 (n=1, group B2); serotype O73:H18 of phylogroup D only amplified STh. With a total of 13 strains described as ETEC on handmade cheese source has a variety of serotypes. Common serotypes with other sources were O8:H9 (n=3) from phylogroup C amplified for STh only. Serogroup O159 with flagellar variants H- (non-motile), H11, H12 and H21 present either LT, STh or both gene toxins, there were present on phylogroups B1, D or U (unknown).

**Conclusions:** This is the first study detailing and giving information of DEC strains from different sources in Honduras. ETEC is the second pathotype on every source described. There are common serotypes on each source for example O159:H21; phylogroup B1 was predominant in handmade cheese and human source but no in water source were phylogroup A was predominant in ETEC strains.
Prevalence of colonization factors and other virulence genes among enterotoxigenic

*E. coli* strains from Latin America, Africa and Asia

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Enterotoxigenic *Escherichia coli* (ETEC) are a leading cause of acute diarrheal disease among children and travelers in low- and middle-income countries. ETEC diarrhea is also a leading cause of mortality in children less than five years of age, living in these resource-poor countries. The high incidence of acute infection and dramatic burden of diarrheal disease emphasize the significance of prevention, yet there is no licensed vaccine for prevention of ETEC diarrheal disease. Phylogenetic characterization of virulence genes among ETEC clinical isolates is the foundation for selection of the most prevalent surface antigens for vaccine development. These virulence genes include classical colonization factors (CF), heat-labile enterotoxin (LT), heat-stable enterotoxin (ST) and non-classical virulence genes (NCVG). CF are surface antigens that adhere to intestinal cells, enterotoxins are exotoxins that induce secretory diarrhea, and NCVG are surface antigens with diverse functions, including promotion of ETEC gut colonization.

The objectives of this study are to measure the prevalence of virulence genes among ETEC clinical isolates from Colombia, Peru and Mexico, and compare them with ETEC strains from African and Asian countries. We also aim to evaluate genetic diversity by sequencing housekeeping genes. Recognizing prevalent ETEC clonal groups and antigens may help prioritize vaccine development strategies.

ETEC clinical isolates were derived from stool samples of subjects less than five years from Colombia (n=52), Peru (n=80) and Mexico (n=143), as part of previously IRB-approved childhood diarrhea surveillance studies. Genomic DNA from ETEC clinical isolates and *E. coli* controls was isolated and processed for multiplex PCR assays for identification of virulence genes. Multiple locus sequencing typing (MLST) was conducted and phylogenetic trees were constructed for the evaluation of genetic diversity and recognition of sequence type (SeqT) and clonal groups. Virulence gene profiles from African and Asian ETEC strains were accessed from public databases for comparison.

LT was the most common toxin profile in Colombia (61.5%) and Peru (57.5%). LT-ST was most common in Mexico (40.6%). CS21 was the most common CF in Colombia (42.3%) and Peru (23.8%). CS6 was most common in Mexico (21.0%). Irp2 was the most common NCVG in Colombia (65.4%). EtaA was most common in Peru (45.0%) and Mexico (44.8%). MLST analysis showed that ST2332, ST10 and ST443 were the most common SeqT in Colombia (13.5%), Peru (12.5%), and Mexico (16.1%), respectively. Twelve novel SeqT were found among these Latin American ETEC strains.

Latin American ETEC virulence gene profiles were compared to African and Asian strains from public databases of ETEC global distribution studies. We found striking similarities in CF distribution. In Asia, specifically Bangladesh, Indonesia and India, CS21 and CS6 are most prevalent. In Africa, specifically Egypt, Kenya and Morocco, CS6 and CS21 are most prevalent.

Our study suggests that ETEC clinical isolates from Colombia, Peru and Mexico share strong similarities with ETEC strains from Africa and Asia regarding virulence gene profiles, despite genetic diversity evaluated by MLST. CS21 and CS6 antigens should be considered for vaccine development strategies against ETEC diarrhea, not only in Latin American, but also in Africa and Asia.

Cost-effectiveness of a reactive oral cholera immunization campaign using Shanchol™ in Malawi

Patrick G. Ilboudo⁵, Martin A. Mengel⁵, Bradford D. Gessner⁴, Bagrey Ngwira⁵, Philippe Cavailler⁴, Jean-Bernard Le Gargasson⁴
Background: Oral cholera vaccines (OCV) have been recommended as additional measures for the prevention of cholera. However, little is known about the cost-effectiveness of OCV use in sub-Saharan Africa, particularly in reactive outbreak contexts. This study aimed to investigate the cost-effectiveness of the use of OCV Shanchol in response to a cholera outbreak in the Lake Chilwa area, Malawi.

Methods: A cost-effectiveness model was developed in Excel 2010 to assess the cost-effectiveness ratios with and without indirect protection. Model input parameters were obtained from cost evaluations and epidemiological studies conducted in Malawi and the published literature. One-way sensitivity and threshold analyses of cost-effectiveness ratios were performed.

Results: Compared with the reference scenario i.e. treatment of cholera cases, the immunization campaign would have prevented 64% and 80% of cholera cases without and with indirect protection, respectively. The cost-effectiveness ratios were US$16,778 per death, US$436 per case, and US$645 per DALY averted without indirect protection. They were US$10,276 per death, US$267 per case, and US$395 per DALY averted with indirect protection. The net cost per DALY averted was sensitive to five input parameters, including case fatality rate, discount rate, duration of immunity (vaccine’s duration), vaccine delivery cost per fully immunized person and cholera incidence.

Conclusion: Relative to the Malawi gross domestic product per capita, the reactive OCV campaign represented a cost-effective intervention, particularly when considering indirect vaccine effects. Results will need to be assessed in other settings, e.g., during campaigns implemented directly by the Ministry of Health rather than by international partners.

GB044 Cost-of-illness of cholera to households and health facilities in rural Malawi

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Cholera remains an important public health problem in many low- and middle-income countries. Vaccination has been recommended as a possible intervention for the prevention and control of cholera. Evidence, especially data on disease burden, cost-of-illness, delivery costs and cost-effectiveness to support a wider use of vaccine is still weak. This study aims at estimating the cost-of-illness of cholera to households and health facilities in Machinga and Zomba Districts, Malawi. A cross-sectional study using retrospectively collected cost data was undertaken in this investigation. One hundred patients were purposefully selected for the assessment of the household cost-of-illness and four cholera treatment centers and one health facility were selected for the assessment conducted in health facilities. Data collected for the assessment in households included direct and indirect costs borne by cholera patients and their families while only direct costs were considered for the assessment conducted in health facilities. Whenever possible, descriptive and regression analysis were used to assess difference in mean costs between groups of patients. The average costs to patients’ households and health facilities for treating an episode of cholera amounted to US$65.6 and US$59.7 in 2016 for households and health facilities, respectively equivalent to international dollars (I$) 249.9 and 227.5 the same year. Costs incurred in treating a cholera episode were proportional to duration of hospital stay. Moreover, 52% of households used coping strategies to compensate for direct and indirect costs imposed by the disease. Both households and health facilities could avert significant treatment expenditures through a broader use of pre-emptive cholera vaccination. These findings have direct policy implications regarding priority investments for the prevention and control of cholera.
Susceptibility to symptomatic enterotoxigenic *Escherichia coli* infections in non-secretor Nicaraguan children

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Enterotoxigenic *Escherichia coli* (ETEC) is an important causative agent of Diarrhea in Children and adults from all over the world, including Nicaragua. A study conducted in Bangladesh have suggested that children with Lewis blood group “a” antigen (Lea) have more often symptomatic than asymptomatic Enterotoxigenic *Escherichia coli* (ETEC) infections (P<0.001). Furthermore, another study carried out with the same population showed that two non-synonymous FUT2 single nucleotide polymorphisms (rs200157007-TT and rs601338-AA) are associated with symptomatic but not asymptomatic ETEC infection irrespective of the child’s Lewis secretor status. Based on the above observations, we conducted the present study in order to investigate if a Non-Secretor Status makes also Nicaraguan children more susceptible to symptomatic ETEC infections. A total of 234 children ≤5 years of age (91 symptomatic and 143 asymptomatic) participated in a community- and hospital-based study of acute diarrhea in León, Nicaragua, during 2014–2017. In brief, clinical cases were evaluated according to the World Health Organization strategy for diarrhea management and fecal and saliva samples were collected from each child. PCR was used to detect the ETEC pathotype. The Lewis phenotype and Secretor status was determined by an ELISA based assay. In general, ETEC was detected in 14.1% (33/234) of the children, with 14.3% ETEC positive cases in the symptomatic and 14.0% of the asymptomatic children. We found no association between the Lewis phenotype and symptomatic ETEC infection. However, we could see a higher proportion of symptomatic than asymptomatic ETEC infections in Non-secretors Children (23.1% symptomatic and 12.0% asymptomatic), and similar proportions of ETEC infections in Secretors children (12.5% symptomatic and 14.7% asymptomatic). In conclusions, our results also suggest that a Non-secretor status is a host feature affecting susceptibility to ETEC infection and have implications in the current vaccine efforts.

Pedestal formation by Enteropathogenic *Escherichia coli* is triggered by different condition *in vitro*

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**Introduction:** Both typical and atypical Enteropathogenic *Escherichia coli* (tEPEC, aEPEC) are important etiological diarrheal agents among children from middle and low-income countries. EPEC produces a characteristic attaching and effacing lesion (A/E) on intestinal epithelium cells. A/E lesion results from the interaction between a bacterial surface molecule known as intimin and its receptor Tir (Translocated intimin receptor), which is translocated through the bacterial Type Three Secretion System to the enterocyte, first to the cytoplasm and then is anchored to the enterocyte membrane. This interaction between intimin and its translocated receptor results in actin recruitment underneath the attached bacteria, and the formation of a structure known as pedestal. Standard protocols to assess pedestal formation in vitro have been established for typical and atypical EPEC reference strains. However, most EPEC clinical isolates do not induce pedestal formation when assessed using these standard protocols.

**Methods:** We model the ETEC and *Shigella* mortality and impact of MSD episodes to determine the additional number of children stunted due to these infections in 79 low- and lower middle-income countries. We then apply a population attributable risk for increased number of deaths due to other infectious diseases in children who are stunted.
**Findings:** In children under 5 years of age, we estimate 196 million episodes of ETEC and *Shigella* diarrhea occur annually, resulting in 168,000 cases of moderate-to-severe stunting and 52,990 total ETEC and 78,500 total *Shigella* deaths in 2015. Additional infectious disease mortality due to stunting resulted in a 48% and 59% increase over direct deaths due to ETEC and *Shigella* diarrheal episodes, respectively. The distribution of mortality and morbidity is heterogeneous with AFRO and EMRO countries bearing the greatest burden.

**Interpretation:** The expanded effects of non-fatal ETEC and *Shigella* diarrheal episodes can have lasting consequences. Preventing these infections may reduce the risk of direct death as well as stunting and deaths due to other infectious diseases. It is important to capture the expanded effects of infection to capture the full value of prevention. Understanding the countries and populations with the highest disease risk helps target interventions for the most vulnerable populations.

**GB080 Comparative genomics of CFA/I and CS6-producing ST-only enterotoxigenic *Escherichia coli* associated with human diarrhea**

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Enterotoxigenic *Escherichia coli* (ETEC) is a significant cause of diarrhea each year worldwide, and is a leading cause of traveler’s diarrhea. A recent epidemiological study demonstrated that ETEC with the heat-stable enterotoxin (ST) were more likely to be associated with diarrhea than were ETEC that had only the heat-labile enterotoxin (LT). Colonization factors (CFs) also have an important role in ETEC virulence, and CFA/I and CS6 were identified as two of the most prevalent CFs. To investigate the genetic diversity of the CFA/I and CS6 ST-only ETEC, we used functional characterization and comparative genomics to analyze 269 ST-only ETEC strains that had CS6 or CFA/I, and were associated with human diarrhea. The CFA/I and CS6 ST-only genomes exhibited considerable genomic diversity as these genomes were identified in 13 ETEC lineages; however, 85% (229/269) of the genomes were identified in only six lineages. Complete genome sequencing of 26 of the ST-only ETEC strains demonstrated that the genes encoding ST and CFA/I were located on highly conserved plasmids, while at least four distinct plasmids types contained the genes encoding ST and/or CS6. Gene-based comparisons demonstrated there were differences among the CFA/I and CS6 genomes at the phylogroup and lineage levels, as well as among strains within a lineage that were isolated from different geographic locations. Thus, insights into the genomic diversity of CFA/I and CS6 ST-only ETEC from this study may aid investigations of virulence, and the development of improved diagnostics and a vaccine protective against the ST-only ETEC.
Enterotoxigenic *Escherichia coli* (ETEC) is an important aetiologic agent of weanling diarrhea in children from low and middle-income countries and of traveler’s diarrhea. ETEC strains are specie-specific and they produce diarrhea by secreting heat-stable (ST) and/or heat-labile (LT) enterotoxins. Attachment to the small intestinal epithelium by specie-specific adhesins is the first step in ETEC pathogenesis. Up to now among human ETEC isolates at least 23 immunologically distinct adhesins, known as coli surface antigens (CS), have been identified, in addition to CFs other adhesins as TibA, EtpA and Tia have been described. CFs, participate in ETEC adhesion to human intestinal epithelial cells. Colonization factor antigen I (CFA/I), a pilus adhesin, was the first CFs described, which seems to be the most frequently human-ETEC pilus adhesin identified among ST clinical ETEC isolates. TibA and EtpA are nonpilus adhesins. TibA mediates ETEC adhesion to surface epithelial cells, auto aggregation, and biofilm formation, while EtpA acts as a molecular bridge, binding the exposed regions of FliC at the flagellar tip and host surface structures. However, little is known of the prevalence of these two distinctive nonpilus adhesin among human ETEC isolates. The aim of the present study was: 1) to establish the prevalence of cfa/I, tiba and etpa adhesin genes among ETEC isolates from Mexican children using two Multiplex PCR developed in our laboratory MPCR 1 simultaneously identifies lt, st and cfa/I genes while MPC2 tiba and etpa genes.

**Material and Methods:** *E. coli* strains collected from two diarrheal aetiology studies, one from community-acquired diarrhea and the other from hospitalized children with diarrhea, conducted at the Mexico City Valley in 2001 and 1998-2001, respectively. From the first study per patients maximum three *E. coli* strains were isolated and from the second study five, all strains were characterized in our laboratory for the presence of lt and st genes. Results In table we analyzed the ETEC strains isolated from 24 patients with acute diarrhea requiring hospitalization and from 66 children with symptomatic and asymptomatic ETEC infections (17 children had diarrhea) of the community. We identified significantly (0.0022, CI 2.069 to 29.02, OR 8) more ETEC adhesins among isolates from children hospitalized than in isolates from children from a low socioeconomic community (even in ETEC isolates from diarrhea cases). Conclusions our results suggest that ETEC strains causing more severe cases carry more adhesins genes increasing their capacity to adhere to the host intestine and cause disease.

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<tr>
<th>Children hospitalized because acute diarrhea, N=24</th>
<th>Community children, N=66</th>
<th>Gene identified in the ETEC isolates per patient</th>
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GB089 Epidemiology and risk factors of Cryptosporidium infection in rural Gambian children

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Background: Cryptosporidium was the third most common pathogen of moderate-to-severe diarrhea (MSD) in children less than 5 years in Global Enteric Multicenter Study (GEMS) and was associated with mortality and growth faltering. We investigated the risk factors for Cryptosporidium diarrhea among children enrolled in GEMS from an enumerated population in rural Gambia.

Methods: We recruited MSD cases for 3 years (2008-2010), and recruited both MSD and less severe diarrhea (LSD) cases for one year (November 2011-November 2012) presenting at sentinel health centers. We selected one or more diarrhea free controls at random for each case matched by age, sex and community within two weeks of recruitment of cases. We collected information on socio-demographics, water use, sanitation and the presence of animals in the compound from study participants. Each participant provided a stool sample to identify enteropathogens, including Cryptosporidium by immunoassay. A subset of randomly selected case-control pairs was tested for Cryptosporidium species by TaqMan assay. We calculated the prevalence of Cryptosporidium in MSD and LSD cases and their matched controls, by age, sex and season. Case-control analysis determined the association of potential risk factors with Cryptosporidium positive diarrhea, compared to their matched controls using conditional logistic regression.

Results: We enrolled 1938 cases (1381 MSD, 557 LSD) and 2969 matched controls; 231/1929 (12.0%) of diarrhea cases and 141/2962 (4.8%) controls were positive for Cryptosporidium. Most Cryptosporidium diarrhea cases (85.7%, 198/231) were aged 6-23 months, and most (81.4%, 188/231) occurred in pre-monsoon and during the rainy season, the months May-October. Cryptosporidium prevalence was similar in MSD and LSD (167/1372, 12.2% vs. 64/557, 11.5%, p=0.68). Cryptosporidium hominis was the predominant (83.3%, 75/90) species. Factors associated with Cryptosporidium-positive MSD or LSD, or both, were number of household members [expressed in units of 5 individuals; adjusted odds ratio (OR) 0.85, 95% confidence interval (CI) 0.81-0.91], consumption of stored drinking water (OR 4.6, 95% CI 2.1-9.9), cow living in the compound (OR 2.2, 95% CI 1.2-4.1 for MSD; OR 11.2, 95% CI 2.8-44.0 for LSD), cat living in the compound (OR 3.8, 95% CI 1.9-7.4 for MSD), rodents living in the compound (OR 4.3, 95% CI 1.5-12.3 for LSD), fowl living in the compound (OR 0.31, 95% CI 0.16-0.63), and presence of Giardia infection (OR 0.25, 95% CI 0.13-0.48). Except for specific animals living in the compound, associations with Cryptosporidium-positive diarrhea were similar for MSD and LSD.

Conclusion: Cryptosporidium-associated diarrhea is common in this setting. The prevalence of Cryptosporidium-positive diarrheal infection is highest at 6-23 months of age. Water-borne transmission of Cryptosporidium is suggested by the preponderance of Cryptosporidium infection in the rainy season and the increased risk of Cryptosporidium-positive diarrhea with consumption of stored water. Improved hygienic practices and sanitation, water storage and treatment of drinking water should reduce Cryptosporidium-associated diarrhea. The role of animals in the transmission of Cryptosporidium requires further investigation.
**GB090 E. coli O153 from diarrhea cases in different developing countries present STh, STp, CS21 pertains to the phylogenetic group D**

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**Introduction:** Our laboratory has participated in different epidemiological studies on diarrhea in children; when performing an analysis of our database we founded that enterotoxigenic (ETEC) and enteropathogenic (EPEC) *Escherichia coli* from diarrheagenic pathotypes, were the strains more frequently associated with the disease. Previous studies in Brazil, Argentina, and Spain refer that *E. coli* of O153 serogroup depicts ETEC characteristics.

**Objective:** To know the frequency and characteristics of *E. coli* O153 strains isolated during different epidemiological studies of diarrhea in children of different countries.

**Material and Methods:** From 52 diarrhea cases in children under 5-years of age were isolated 65 *E. coli* strains from Mexico (33), Egypt (22) and Thailand (10). The strains Identity was confirmed by metabolic tests and the serogroups with rabbit serum (SERUNAM) against the somatic (O) and flagellar (H) antigens. By PCR assays using arpA chuA, yjaA, and STp4 primers were defined the phylogroups and the pathotype ETEC identifying the presence of CFAI, CFAII, CFIII, sc3, CS21, LT, STh, and STp genes. Results: All the isolates evaluated showed the typical biochemical profile of *E. coli*, in the serological typing were identified O153:H45 (66.2%) and O153:Non-motile (33.8%) serotypes. The phylogroups D (60%) and A (32.3 %) were the most commons, B1 (6.2%), and B2 (1.5%) with less frequency. CFAI (50.8%) and sc21 (69.2%) were most frequently identified for colonization factors. The sc21 antigen in the different countries evaluated showed a frequency of 36.9% in Mexico, 21.5% in Egypt and 10.8% in Thailand. In relation to the genes for the expression of enterotoxins, their presence was 13.8% and 4.6% for sth and stp, respectively.

**Discussion:** As previously has been reported *E. coli* O153 is a serogroup of the pathotype ETEC associated with diarrhea, our observations confirm it and show their presence in developing countries like ours. The phylogroup assay showed that these strains are mainly included in the virulent group D with STh, STp, and sc21 genes, confirming that O153 can be included within the ETEC pathotype related to the etiopathogenesis of diarrhea with presence in developing countries.

We thank DGAPA-PAPIIT for their support to project IN216417.
Etiology of diarrhea and the effect co-infection on risk of severe disease in children under 5 in Zambia.

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Background: The historical presumption that rotavirus is the leading cause of severe gastroenteritis in infants and children now requires accurate evidence in the post vaccine introduction era. We aimed to document enteric pathogens among children presenting with moderate to severe diarrhea at outpatient clinics in Zambia and investigated the effect of multiple enteric infections on risk of severe diarrhea following country-wide introduction of rotavirus vaccination.

Methods: Clinical data and stool samples collected between July 2012 and October 2013 from children <5 years presenting to outpatient clinics in Lusaka with presenting with moderate-to-severe diarrhea. The study was conducted during the early months post rotavirus vaccine introduction in Zambia. We used Luminex x-TAG® gastrointestinal pathogen panel to simultaneously detect enteric viruses, bacteria and protozoa from the stool samples. Log-binomial regression was used to estimate the effect of co-infection on incidence of severe diarrhea, adjusted for inadequate water sanitation and hygiene. P-values were calculated using likelihood ratio test.

Results: The top 5 leading enteric infections detected among children were rotavirus (67.6%), adenovirus (42.2%), ETEC (41.7%), salmonella (38.0%), and giardia (37.5%). Among rotavirus infected children, 39% (368/933) were co-infected with Shigella, whereas among children infected with adenovirus, 44% (255/582) were also co-infected with Shigella. The incidence of severe diarrhea was estimated as 3.9% (54/1,380) (95%CI=3.0, 5.1). There was strong evidence that children who were infected with at least two enteric pathogens had about three times the risk of severe diarrhea compared to those without any multiple infection (adjusted RR = 2.61; 95%CI = [0.37, 18.57]; p=0.008).

Conclusion: Our study documents specific enteric pathogens with potential to emerge as important causes of diarrhea in Zambia in the post rotavirus vaccine era. This information is essential for prioritization of new enteric vaccines. The observed higher risk of severe diarrhea resulting from co-infecting enteric pathogens highlights the need to consider these infections in diarrhea management and treatment strategies.
Prevalence of enterotoxigenic *Escherichia coli* among Mexican children with acute diarrhea and isolates toxin gene profiles

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Introduction: Diarrheal disease is the second leading cause of death in children under five years old and each year diarrhea kills around 525,000 children of this age group. However, diarrheal illness is both preventable and treatable. Enterotoxigenic *E. coli* (ETEC), is one of the most important causes of diarrheal illness in less developed areas of the world, causing 15-20% of cases in children aged <5 years, and it is the main etiological agent of traveler’s diarrhea among persons from industrialized countries who have visited Africa, Asia and Latin America. In 2015 it was estimated that among children aged <5 years from less developed areas of the world, ETEC caused approximately 84,000 deaths and 128 million cases, with a 5% attributable fraction of all diarrheal cases in this group children. ETEC pathogenesis is mainly mediated by the presence of its enterotoxins: heat-stable toxin (ST) and heat-labile toxin (LT), accordingly detection of one or both toxins in the isolates by molecular methods is used to diagnose the infection.

Methods: In all studies, three to five *E. coli* strains were collected from each child stool with acute diarrhea and all *E. coli* strains were analyzed for the presence of LT and ST toxins genes, by a multiplex-PCR. The diarrheal etiology studies were conducted in the states of Mexico City, Tabasco and Yucatan.

Results: ETEC prevalence was evaluated in three transversal studies of children hospitalized because of acute diarrhea. The first study was conducted between 1998-2001 in a mild weather region (Mexico City), the other two studies were conducted in tropical regions (Villahermosa 2004-2006 and Mérida 2010-2014), and in two cohort studies of children from a low socioeconomic community of the outskirts of Mexico City (1998 and 2001). According to year of the study, the prevalence of ETEC in the children hospitalized because of acute diarrhea were 2.4%, 3.9% and 5.3%, respectively, while the prevalence in the low socioeconomic community studies were 6.9% and 14.7%, respectively. The distribution of toxins genes among the patients isolates from hospitalized children was for LT toxin gene of 74% in the study conducted in the mild weather region and of 50% in both of the tropical region studies. In contrast, all ETEC isolates from the community studies were LT positive. In the studies of Villahermosa and Mérida, the prevalence of ST-positive strains was the same (33.3%), in the Mexico City study it was 14.8%, the percentage of patients carrying LT/ST-positive isolates was 16.6, 10.4, and 7.4, respectively, and we also identified two patients with ETEC isolates that had different gene toxin profiles.

Conclusions: ETEC remains and important etiological agent of diarrheal illness among Mexican children, LT strains not only cause community diarrheal illness, but as well disease requiring hospitalization, ST-positive ETEC strains were only identified among children with diarrheal illness requiring hospitalization.
GB103  Zinc deficient infants at increased risk of bacterial diarrhea in a Bangladeshi birth cohort

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Today, diarrhea ranks second only to pneumonia as a leading cause of global under-five mortality. Among the top diarrheal pathogens in the first year of life, only rotavirus has a licensed vaccine, leaving a dearth of currently-available preventive options for diarrhea of other etiologies. Alternatives to prevent childhood diarrhea are imperative while vaccines against Shigella spp, pathogenic E. coli spp, and Campylobacter spp move through the vaccine development pipeline. Toward identifying potential non-vaccine interventions to prevent diarrhea in the first year of life, we examined the role of zinc deficiency in diarrheal outcomes in a birth cohort in Dhaka, Bangladesh (PROVIDE Study). We evaluated 1,367 diarrheal specimens contributed by 608 infants from age 18–52 weeks to determine diarrheal etiology by quantitative polymerase chain reaction (PCR) TaqMan Array Card (TAC) assay. Pathogens included Shigella spp/entero invasive E. coli (EIEC), heat-stable enterotoxigenic E. coli (ST-ETEC), Campylobacter jejuni, rotavirus, adenovirus 40/41, and norovirus. Previously published diarrhea-associated Quantification Cycle (Cq) thresholds were used to determine pathogen attribution. Zinc deficiency was defined as week 18 plasma zinc concentration (PZC) < 65µg/dL. Among infants in the pre-protocol population, 16.5% were zinc deficient (ZD) at 18 weeks. Comparing risk of bacterial diarrhea in infants with and without zinc deficiency, 66% of ZD infants had bacterial diarrhea vs 48% of non-deficient (p=0.008, Chi-Square), and ZD infants had increased odds of bacterial diarrhea (OR 2.09, 95% CI 1.20 – 3.64). Similarly, the odds of viral diarrhea were nearly 4 times higher among ZD infants (OR 3.94, 95% CI 1.55 – 10.03). For viral etiology, we found a strong correlation between zinc deficiency and time to first episode of viral diarrhea (median survival 27 vs 33 weeks in zinc deficient vs non-deficient infants, p <0.0001, Kaplan Meier), with zinc deficient infants at 55% greater risk (HR 1.55, 95% CI 1.21 – 1.99, Cox Proportional Hazards). Similar analyses for bacterial diarrhea will be presented. Our results indicate further consideration of zinc as a critical and modifiable co-factor in ameliorating the burden of childhood diarrhea. Carefully designed trials to evaluate the effect of zinc supplementation, to address underlying zinc deficiency, on diarrheal outcomes could determine whether zinc may fill the gap in protection against childhood diarrhea.
GB104  Potential impact and cost-effectiveness of ETEC and Shigella vaccines in 79 low- and lower middle-income countries

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Globally, diarrhea remains the second leading cause of mortality in children under 5-years-old. While diarrheal mortality has declined over the last two decades, there has been a slower decline in the number of diarrheal episodes. Thus, there are many children that are subject to a lower quality of life because of repeated exposure to diarrheal pathogens and related morbidity. Current studies have shown associations between frequent diarrheal episodes and an increased probability of childhood stunting. The development of a vaccine for enterotoxigenic Escherichia coli (ETEC) and Shigella, two of the main diarrhea-causing pathogens in children in low income countries, is an intervention that could have significant benefits in reducing child diarrhea burden and preventing other infectious disease mortality associated ETEC- and Shigella-induced stunting. We developed a cost-effectiveness analysis for future standalone ETEC and Shigella vaccines to evaluate vaccine impact on mortality, morbidity, number of stunted children, and stunting-associated deaths from other infectious diseases. We modeled the impact over the first ten years after vaccine introduction in children under five years of age living in 79 low and low-middle income countries. We also generated subnational estimates in four East African countries. Diarrheal mortality was calculated using midpoint values of country estimates from 2015 Global Burden of Disease and Maternal and the Group for Maternal Child Epidemiology Estimation and projected for the first ten years after introduction in 2025. The number of moderate-to-severe ETEC and Shigella episodes were used to estimated population shifts in z-scores to project the number of moderately or severely stunted children. Country-level results were calculated and then aggregated by WHO regions and for Gavi-eligible countries. According to our models, from 2025 to 2034, ETEC and Shigella would cause an estimated 289,600 and 397,400 deaths, respectively, in children under five without vaccination, with most deaths occurring in AFRO countries. ETEC and Shigella episodes would result in over 28.6 and 45.0 million stunted children, respectively. These cases of stunting would result in an additional 95,800 (ETEC) and 130,000 (Shigella) deaths due to other infectious diseases. Introducing ETEC or Shigella vaccines would prevent 92,000 ETEC and 126,600 Shigella direct deaths and 44,800 ETEC- and 60,800 Shigella-induced stunting deaths from other infectious diseases over the first 10 years. ETEC and Shigella vaccination would prevent 38 million cases of moderate or severe stunting. Regional ETEC ICERs range from $1,616/DALY in AFRO to $13,615/DALY in EURO. Regional Shigella ICERs range from $421/DALY in EMRO to $525,000/DALY in EURO. Other infectious disease deaths due to induced stunting accounted for 33% of the total deaths averted from ETEC and Shigella vaccination. Inclusion of other infectious disease mortality due to stunting provides a more accurate assessment of total ETEC and Shigella disease burden and significantly increased the projected impact and cost-effectiveness of vaccination. Introducing vaccines only in high burden countries and regions could substantially reduce cost, without substantially reducing health impact. Subnational models reveal variation across regional and socioeconomic subpopulations, supporting targeted efforts to reach the most vulnerable children.
Characterization of ETEC and Shigella among low-cost specimen preservation samples from patients in Cameroon with diarrhea and/or dysentery.

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**Background:** UNICEF reports diarrhea causes more than 600,000 death of children under 5 annually. Recent studies, including The Interactions of Malnutrition & Enteric Infections: Consequences for Child Health and Development (MAL-ED) and the Global Enteric Multi-Center Study (GEMS) have improved diarrheal disease surveillance. GEMS identified Shigella and Enterotoxigenic E. coli (ETEC) among the top-four diarrheal causative agents. Subsequently, the GEMS re-analysis used new molecular methods to demonstrate that Shigella and ETEC are two times greater than reported using culture-based methods of detection³.

**Surveillance:** While molecular methods have been developed for the rapid detection of enteric pathogens, the costs associated with establishing molecular capacities, cold storage, transport, and shipment of specimens in developing countries limits applicability. From 2015 - 2017, we have conducted cholera surveillance in Cameroon using simplified laboratory methods to rapidly detect cholera. We also found extremely high numbers of MSD that was not of cholera in origin. Cameroon was previously not included in the multi-center studies. Persons that presents to our surveillance sites reporting >3 loose stools in the previous 24 hours with moderate to severe dehydration and/or blood in their stool were eligible for enrollment in our nested sub-study.

**Hypotheses and Specific Aims:** We hypothesized that the use of dried filter paper specimen preservation will provide a low-cost, simplified and timely methodology to identify diarrheal diseases causing significant morbidity in resource constrained countries. Our aims were to evaluate a simplified specimen preservation methodology to improve detection of diarrheal pathogens; to apply this tool to determine the prevalence of these pathogens among diarrheal patients presenting to treatment centers; and to use quantitative real time PCR to attribute disease, if possible, and to correlate clinical severity of diarrheal illness.

**Results:** To date, we have screened 396 cholera negative-specimens for ETEC (LT, STh and STp) and Shigella (IpaH) using conventional PCR methods. We have found 22.5% (N=90) ETEC positive (among all types), of which there are 20% LT-STh positive, and 10% LT-STp positive. 21% of ETEC positive were in patients >5 years of age. We found 26.4% of persons presenting with diarrhea with MSD and/or bloody stool are IpaH positive, of which 21% are in older patients. We will further quantify the presence of these pathogens using sybergreen, and for a random sample, Taqman Array Card. We will compare amount of bacterial pathogen to severity of diarrhea, age, and sex.

**Potential Impact:** The ability to use dried filter paper fecal samples will help facilitate the identification and quantification of the enteric pathogens from children living in remote, medically underserved locations in resource poor countries. This study will enhance our ability to conduct enteric surveillance studies and an overall understanding of disease burden which is needed to guide vaccine development and intervention guidelines. In addition this work may yield a more practical and fieldable approach to diarrheal disease diagnosis. Additionally, this methodology eliminates the need for costly storage and maintenance of freezer stocks.
Host parameters and genomics that predict responses to infection and disease

ORAL PRESENTATIONS

GEN016  New insights regarding the interplay between *Shigella* and human lymphocytes

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Upon natural infection with *Shigella*, the elicited specific immunity is of short duration and requires several rounds of infection to peak. Lymphocytes, key immune cells for the generation of adaptive immunity, were thus the focus of our recent studies aimed at understanding the cross-talk between the bacterium and host. We demonstrated that *Shigella* targeting of human B lymphocyte subsets leads to their apoptosis via a mechanism dependent on the T3SS needle tip protein IpaD and TLR2 (1). Additionally, *Shigella* targeting of human T lymphocytes was shown to result in a T3SS-dependent arrest of their migration both in vitro (2) and in vivo (3). Here we present our latest findings revealing that: (i) T3SS effector injection not resulting in cell invasion is the main targeting mechanism towards human lymphocytes (4); (ii) this targeting, especially towards T cells, is dependent on their glycosylation pattern (5); (iii) such targeting impacts the formation of the immunological synapse (6). These new insights prompt us to revisit the paradigm of T3SS-mediated *Shigella* pathogenicity.

2. Konradt et al., Cell Host and Microbe, 2011.
4. Pinaud et al, PNAS, 2017
5. Belotserkovsky et al., in revision
6. Samassa et al., in preparation

GEN072  Immune-profiling of fecal IgA samples on ETEC protein microarray

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ETEC is a major cause of diarrheal disease associated mortality and morbidity among children in developing countries but there are no currently licensed vaccines against ETEC. A Phase2b clinical study, was conducted to evaluate the experimental efficacy of an oral live attenuated ETEC vaccine (ACES527) when administered in three doses, with or without the mucosal adjuvant dmLT. Clinical observations from this study demonstrate significantly higher protection afforded by the ACE 527 vaccine, when administered with dmLT. However, specific immune responses responsible for
protection and the role of dmLT in conferring protection are not well understood. Towards that objective, fecal IgA, a surrogate of mucosal immune response, was evaluated using ETEC proteome microarrays that are comprised primarily of proteins expressed using the In Vitro Transcription Translation (IVTT) system, but also contains a small set of purified proteins - colonization factors and other ETEC antigens such as CFA/I, CS1, CS2, CS3, CS4, CS6, CS14, CS17, CS19, LT-A, LT-B, EtpA, EatA, YghJ, Ag43, FliC and PCF071. IgA for the microarray analyses were isolated from fecal specimens collected on days -1, 28, 56, 63 and 84 with respect to the first vaccination, and days -1, 7 and 28 with respect to challenge with H10407. While strong and distinct responses were observed in the arrays to purified ETEC colonization factors, novel antigens, and toxins across all sample types, this abstract will only highlight responses observed to IVTT proteins.

Analysis of fecal IgA has always been a challenge, due to observed diurnal variations in concentration and composition of total IgA & IgG in stool samples that gets exacerbated especially during diarrheal episodes caused by enteric pathogens such as ETEC. These variations have led to difficulties in accurately measuring changes in total and antigen-specific IgA before and after immunizations and/or challenge. To counter these variations, fecal IgA samples were normalized using methods previously used for serum. This resulted in data that were congruent with ALS and serum results in longitudinal analysis for IVTT proteins. For e.g., in ALS and serum, following challenge with H10407 (serotype O78:H11), the clearest signals observed in frequency and magnitude were increased responses to the IVTT protein that corresponded to FliC serotype H11; the same was true with fecal IgA.

Responses to IVTT versions of CS3, EatA, YghJ and Ag 43 in ALS and serum following vaccination were similar in unprotected and protected subjects; however, the responses in fecal IgA to these antigens differ between unprotected and protected subjects. Fecal IgA responses against IVTT versions of CS3, EatA, YghJ, and the flagellar hook-associated protein 1 (ETEC_1147) were more frequent and higher in magnitude in protected volunteers than the unprotected volunteers. Conversely, more responders were observed to the IVTT fimbrial chaperone protein (ETEC_0583) in unprotected subjects than in protected subjects. In summary, the above observations have demonstrated a robust approach to evaluating intestinal secretory IgA responses to ETEC antigens following immunization and challenge using the ETEC proteome microarray.

**GEN078 Development of Shigella proteome microarrays and analysis of human antibody responses following vaccination and challenge**

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*Shigella* spp. cause severe diarrhea and dysentery in humans and is a significant contributor to mortality and morbidity in children under five years of age living in resource-limited countries. There is no licensed vaccine against shigellosis. Ongoing efforts to identify vaccine candidates would benefit from an improved understanding of immune responses to *Shigella* as well as the identification of target antigens and immune correlates of protection. In an effort to address this gap in knowledge, we developed a *Shigella* proteome microarray consisting of 2,177 proteins that represent the conserved core of all four *Shigella* species. To test the platform’s capacity to identify targets of antibody responses and to distinguish differences in host immunity in different settings (i.e. vaccination and pathogen exposure), the microarrays were probed with serum samples obtained from human volunteers pre- and post- three distinct interventions (i) vaccination with a formalin-inactivated Whole Cell *S. flexneri* 2a Vaccine (Sf2aWC), (ii) vaccination with a live-attenuated *S. flexneri* 2a Vaccine Strain (CVD 1204) and (iii) challenge with wild-type *S. flexneri* 2a 2457T strain (Sf2a challenge). In addition to serum, ALS samples from the vaccination study with the formalin-inactivated Sf2aWC vaccine were evaluated on the *Shigella* proteome arrays.
Ongoing efforts to identify vaccine candidates would benefit from an improved understanding of immune responses to *Shigella* as well as the identification of target antigens and immune correlates of protection. In an effort to address this gap in knowledge, we developed a *Shigella* proteome microarray consisting of 2,177 proteins that represent the conserved core of all four *Shigella* species. To test the platform’s capacity to identify targets of antibody responses and to distinguish differences in host immunity in different settings (i.e. vaccination and pathogen exposure), the microarrays were probed with serum samples obtained from human volunteers pre- and post- three distinct interventions (i) vaccination with a formalin-inactivated Whole Cell *S. flexneri* 2a Vaccine (Sf2aWC), (ii) vaccination with a live-attenuated *S. flexneri* 2a Vaccine Strain (CVD 1204) and (iii) challenge with wild-type *S. flexneri* 2a 2457T strain (Sf2a challenge). In addition to serum, ALS samples from the vaccination study with the formalin-inactivated Sf2aWC vaccine were evaluated on the *Shigella* proteome arrays.

Globally, responses to *Shigella* antigens on the arrays varied based on sample time points. In general, serum samples obtained at the pre-immunization or pre-challenge time point, showed a baseline response to the *Shigella* antigens on the array. In the case of volunteers from the live-attenuated CVD 1204 vaccine study and the challenge study with Sf2a, sera samples from the post- vaccination / post-challenge time point, elicited strong and broad responses to multiple *Shigella* vaccines.

**POSTER PRESENTATIONS**

**GEN019**  Glyco-engineered cell line and computational docking studies reveal that ETEC CFA/I imbriae bind to human Lewis a glycans

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As an infection prerequisite, enterotoxigenic *Escherichia coli* (ETEC) binds to the host’s small intestine using colonization factors (CFs), representing target candidates for vaccines or anti-infection therapeutics. The human intestinal binding receptor(s) for ETEC CFs however, remains less well defined. We have previously reported clinical data, as well as identified host genetic biomarkers to suggest that ETEC CFA/I fimbriae bind to the histo-blood group antigen Lewis a (Lea), a glycan epitope ubiquitous in the small intestinal mucosa of young children (less than two years of age), and individuals with a genetic mutation of FUT2.

In an attempt to further elucidate the physiological binding properties of this intestinal/CF interaction, we created human small intestinal cell line models by engineering Chinese Hamster Ovary (CHO-K1) cells to express Lea or Leb determinants on both N- and O-glycans. This was accomplished by expressing the Lewis antigens’ immediate precursor, the type 1 chain glycan (Galβ3GlcNAc) using the B3GALT5 enzyme. Then to generate the O-glycan precursor, the extended core 1 O-glycan chain (GlcNAcβ3Galβ3GalNAca) was generated by co-expression of the B3GNT3 enzyme. Expression of the Lewis gene-encoding enzyme FUT3 alone generated the Lea antigen, and when expressed together with the H gene-encoding enzyme FUT1, Leb antigens were expressed on the CHO-K1 cell surface.

We demonstrate that CfaB, the major subunit of ETEC CFA/I fimbriae, as well as four related ETEC CFA/I fimbriae (CS1, CS2, CS4 and CS14), bind significantly more to our glycan-engineered CHO-K1 cell-line expressing Lea, compared to cells carrying Leb or the CHO-K1 wild-type glycan phenotypes. Moreover, using computational docking analysis, we predict that up to three amino acids (Glu25, Asn27, Thr29) found in the Ig-like groove region of CfaB and related fimbriae, could be important for the preferential and higher affinity binding of CFA/I fimbriae to the Lea glycan.
GEN031 Development and inter-laboratory evaluations of a simple, high-throughput Shigella serum bactericidal assay

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Shigella is an important cause of diarrhea worldwide. Current vaccine development efforts are focused on Shigella lipopolysaccharide (LPS)-based candidates, as O-antigen specific conjugate vaccines have proved immunogenic and highly efficacious in preventing disease. Immunization with LPS-containing Shigella vaccines elicit antibodies capable of killing Shigella in a serotype specific manner, and candidate vaccines often include LPS from Shigella flexneri 2a, S. flexneri 3a, and S. sonnei for their high epidemiologic prevalence. To facilitate Shigella vaccine development, we have developed a serum bactericidal assay (SBA) specific for three Shigella serotypes by directly measuring killing of target bacteria at multiple dilutions of samples. The SBA has a very high analytical throughput but uses simple technologies and readily available reagents. The SBA was characterized with human sera which have bactericidal antibodies against S. flexneri 2a, S. flexneri 3a, and S. sonnei. Free LPS of homologous serotype, but not heterologous serotype could completely inhibit bacterial killing in a serotype specific manner. Assessment of precision found median intra-assay precision to be 13.3% and median inter-assay precision to be 19-30% for the three serotypes. The SBA is linear with slight deviation for samples with low (~40) killing indices. The SBA was sensitive enough to require about 100-fold pre-dilution of immune serum samples. Repeat assays yielded results with less than 2-fold deviations, indicating the robustness of the assay. Indeed, the SBA is simple and robust enough so that multiple independent laboratories could produce highly correlated results. When the SBA results were normalized with a reference serum, the results from four different laboratories were highly comparable. Using our Shigella SBA along with a reference serum by multiple laboratories should facilitate development of Shigella vaccines.

GEN038 Diarrhea caused by enterotoxigenic Escherichia coli induces T-cell responses in the circulation

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Protective immune response occurs after an enterotoxigenic Escherichia coli (ETEC) infections and there is a decline in ETEC diarrheal incidence with growing age. However, the mechanism of protective immunity in natural ETEC infection is not well understood that speed up the efforts to understand the mechanism of adaptive immune responses. Therefore, the main goal of the study is to characterize the systemic humoral and cellular immune responses in natural ETEC infection. Flow cytometrical approach has been performed to evaluate the T (CD4+) cells, memory T-cells (CD4+CD45RO+) and cytokine responses to different ETEC specific antigens that are vital in protective mechanisms against this pathogen. We observed significant increase of memory T cell responses at early convalescent (day 7) to LTB and dmlT in compare to acute stage (day 2) and healthy participants. However, there was no significant T cell proliferation observed for ST or EtaA in natural ETEC infection. Increased IFN-g and IL-13 responses were observed at day 7 in compared to day 2 of infection that suggest both Th1 and Th2 type of response. Meanwhile, significantly elevated plasma anti-LTB IgG, IgA, and moderate IgM responses at early convalescent stage were recorded in ETEC infected patients. Therefore, this study demonstrates that antigen specific memory T cell responses develop at early convalescent stages after infection that also correlates concurrent development of antigen specific systemic B-cell responses. This suggests that T-cell responses to ETEC antigens may be important for the generation and a stable B-cell response. Further analyses of subtype of T cells are needed to understand more mechanistic responses in natural ETEC infection.
**GEN046  Human Microbiota Mice: New Animal Models for Enteric Escherichia coli infections**

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**Introduction:** Conventional mice are resistant to infection with human enteric bacterial pathogens such as Enterohemorrhagic *E. coli* (EHEC) and Entertoxigenic *E. coli* (ETEC). This is, in part, because of host species-specific bacterial virulence factors, but also because of colonization resistance conferred by the normal microbiota of the mouse gut. Our aim was to determine whether gut microbiome composition influences infectivity of mice with either human EHEC or ETEC isolates. Methods C57BL/6 mice with either a mouse microbiome (MMB), or stably colonized with a human gut microbiome (HMB) were gavaged with EHEC strain 86-24 (Stx1, Stx2) or ETEC strain H10407 (ST, LT). Two groups were pretreated with streptomycin (SM) to disrupt their microbiota (MMBsm and HMBsm) before gavage. Mice were observed for weight, signs of infection and fecal shedding of the challenge strain, then euthanized to measure fluid secretion into total gut, small intestine (SI), cecum (CE) and colon (CO) by gut/carcass wt. ratios, and intestinal colonization by 86-24 or H10407 in small intestine and cecum. To define microbiome composition, DNA from fecal pellets was analyzed for 16srDNA sequences. Results For EHEC, a colonic pathogen, secretion was significantly increased over controls in HMB in total gut, SI and CO but was not increased in MMB without SM pretreatment. Secretion was increased to the same degree in SM treated HMBsm and SMBsm mice. Fecal shedding was increased in HMB, but not MMB, mice, as well as in both HMBsm and SMBsm SM-treated mice. Colonization was increased in CE and SI of HMB mice vs. MMB mice, and was equally increased in the CE and SI of both MMBsm and HMBsm treated with SM. For ETEC, a small intestinal pathogen, secretion, fecal shedding and colonization of intestinal segments was not observed in either MMB or MMB mice, unless they were treated with streptomycin. The development of intestinal secretion, colonization and fecal shedding in HMB mice challenged with EHEC 86-24 without SM pretreatment occurred without major changes in microbiome composition. In MMB mice increased secretion, colonization and shedding of either EHEC 86-24 or ETEC H10407 required SM treatment which induced major microbiome alteration.

**Discussion:** The presence of a human microbiota in C57BL/6 mice (HMB) rendered the “humanized “ mice susceptible to EHEC infection without the need for microbiome disruption with streptomycin. In contrast, a human microbiota was not sufficient to induce susceptibility to infection by ETEC which required pretreatment with streptomycin. Increased secretion colonization and shedding following EHEC or ETEC challenge of conventional C57BL/6 (MMB) mice was only seen after microbiome disruption with streptomycin. We conclude that a human microbiota in C57BL/6 mice renders them susceptible to infection by the colonic pathogen EHEC 86-24, but not by the small intestinal pathogen ETEC H10407.

**GEN047  Efficacy of a commercially available hyperimmune bovine colostrum product for the prevention and treatment of shigellosis in rhesus macaques.**

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Entero-invasive Gram-negative bacteria are a global issue affecting travelers traveling to and children living in endemic areas. Infectious diarrhea is a WHO public health priority area due to a lack of effective vaccines and the accelerating global antimicrobial resistance (AMR) crisis. In the absence of a licensed vaccine, alternative, non-antibiotic, prophylactic modalities would serve a definitive role in mitigation of disease burden.

A hyperimmune bovine colostrum powder (HBC) has been generated in dairy cows hyperimmunized with vaccines composed of antigens from selected homogenized Enterotoxigenic Escherichia coli (ETEC) bacterial cells. The commercially available HBC (Travelan™, Immuron, Melbourne, Australia) neutralizes ETEC by blocking their attachment to the intestinal wall and has demonstrated efficacy in controlled human infection model (CHIM) studies using ETEC H10407. Polyclonal antibodies in the HBC can target antigens in a less specific manner, which improves their efficacy against mutating pathogens. Moreover, immunoglobulins also have non-specific activity which improve gut health and function, and thus provide indirect support to gastrointestinal health and resilience. Prophylactic administration of HBC may provide an effective treatment alternative to antibiotics for the development of gastroinestinal (GI) infections in humans. Additionally, it may have specific advantages that include better formulation and stability and can be derived to provide broader protection against a variety of enteric pathogens which are encountered globally.

Recently, Western blot analysis using Travelan™ has revealed that the product is cross-reactive with other Gram negative enteric bacteria, not contained within the ETEC vaccine used for immunizations, to include various serotypes of Shigella spp., ETEC and Campylobacter spp. These finding suggest that Travelan™ may be effective as an immunoprophylactic against multiple enteric organisms.

Human and nonhuman primates (NHP) share susceptibility to many pathogens, creating an invaluable intragastric animal model for studying human infectious diseases, including enteric pathogens such as Shigella spp. The rhesus macaque diarrhea challenge model will be used to assess the immunoprophylactic potential of Travelan™ as a diarrhea prevention and treatment modality. A seven-day course of orally administered Travelan™ will be used to assess HBC tolerability and efficacy in juvenile, naive rhesus macaque as a prevention and treatment for shigellosis. The rhesus macaque model closely represents the human disease of shigellosis, and will provide efficacy and safety data to support a future human clinical trial of Travelan™.

**GEN056 Monitoring the human gut microbiota during a Campylobacter jejuni challenge.**

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Campylobacter is recognized as one of the most common cause of foodborne disease worldwide. Campylobacteriosis is a self-limiting disease which usually does not require intervention. Nonetheless, some cases require antibiotic treatment. Vaccine development is one strategy to reduce the burden of disease; however, vaccines are a long-term solution. A potential alternative for short-term travel settings is chemoprophylaxis; however, unfortunately, as with many other bacterial infections, antibiotic resistance is on the rise. As an alternative, we studied the potential of rifaximin, a poorly absorbed oral antibiotic as chemoprophylaxis for the prevention of campylobacteriosis in a controlled human infection model. During this study we monitored the evolution of the subjects’ gut microbiota before, during and after C. jejuni challenge.
Three days pre-challenge, 28 subjects (1:1 randomization) received 550 mg of rifaximin or placebo for 4 days. On day 0, subjects were challenged with $1.8 \times 10^5$ CFU dose of *C. jejuni* strain CG8421. Stools were collected prior challenge (baseline), daily during the inpatient phase until antibiotic treatment and then during follow up visits on days 21, 56 and 84. Stool aliquots were placed on dry ice immediately following collection and stored at -80°C until processing. Whole DNA were extracted from the samples using Qiagen DNeasy PowerSoil Kit. The stool DNA served as template for Polymerase Chain Reaction using primers targeting the variable region 4 of the 16s rRNA. Amplicons were pooled and subjected to Next-Generation Sequencing on a Miseq (illumina) sequencer. Microbiota profiling was done using the Greengenes 16 sRNA database. Statistical analyses were performed using JMP 10.0 (SAS Institute, Cary, NC).

The *C. jejuni* challenge resulted on a wide array of outcomes providing us the opportunity to study the potential role of gut enterotypes on disease severity. Rifaximin appeared to have a negligible impact on the gut resident microbiota. Additionally, analyses suggest that the majority of the subjects return to their original “baseline” microbiota composition following *C. jejuni* infection and azitromycin/ciprofloxacin treatment.

Human clinical trial challenges provide an ideal controlled setting to study the short and long-term effects of pathogens on the host microbiota. Natural infections lack the most crucial sample, pre-infection, which serves as baseline. Monitoring the host microbiota evolution during human clinical challenges can highlight effects of the pathogen on the short and long-term host resident microbiota.

**GEN062  Serological profiling by proteomic microarray of culture-confirmed ETEC-associated travelers’ diarrhea**

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**Background:** To date, assessment of the immune responses to enterotoxigenic *E. coli* (ETEC) infection have been limited to a small set of antigens based on the heat-labile enterotoxin (LT) and a small subset of colonization factors (CFs). Recently an *E. coli* pan-genomic protein microarray was developed containing 7,366 full-length or fragmented, In Vitro Transcription Translation (IVTT) expressed proteins and purified proteins representing diarrheagenic *E. coli* subspecies. The IVTT expressed proteins are comprised of core genome and ETEC-specific proteins ($n=5,001$) and unique proteins from EHEC ($n=729$), EPEC ($n=456$), EIEC ($n=452$), EAEC ($n=286$) and ExPEC ($n=241$). The purified proteins are thirty-nine ETEC purified proteins that include such proteins as LT-A, LT-B, CS1, CS3, CS6, CFA/I, and EtpA. This expansive microarray was used to assess antibody responses in subjects with culture confirmed ETEC-associated watery diarrhea (AWD) participating in a multi-site travelers’ diarrhea (TD) treatment trial.

**Methods:** Subjects included active-duty military personnel (or beneficiaries), deployed to Afghanistan, Djibouti, Kenya, or Honduras, aged ≥18 years, who presented with AWD and were ambulatory. To be included in the study, subjects had to meet the definition of TD (≥3 loose stools in 24 hours or ≥2 loose stools in 24 hours with associated symptoms, such as nausea, vomiting, abdominal cramps, or tenesmus) of ≤96 hours duration and be able to comply with follow-up procedures. Stools were considered to be loose if they took the shape of the container. Subjects taking antibiotics within 72 hours before presentation (excluding malaria prophylaxis) were excluded as were those taking >4 mg loperamide (total) or any amount of loperamide for >24 hours before enrollment, or those with dysentery or documented fever. Serum samples were collected on day of clinical presentation with travelers’ diarrhea (acute sample) as well as 21 days after initial presentation (convalescent sample). Stool samples were also collected for pathogen identification. Acute and convalescent serum samples were selected from 15 subjects with culture confirmed ETEC infection for testing using the *E. coli* pan-genomic protein microarray to assess for IgA and IgG responses. Immune responses were compared across ETEC phenotypes (by toxin and CF) and severity of disease at initial clinical presentation.
Results: Among the 15 subjects whose serum samples were selected for analysis, 6 (40.0%) had an LT+ infection, while 11 (73.3%) were positive for STp-expressing ETEC and 5 (33.3%) were positive for STh ETEC. ETEC-specific antigen responses correlated with ETEC toxin/CF phenotype. Within the toxin/phenotypes, several novel antigens had significant rises from the acute to convalescent samples.

Conclusions: Using a *E. coli* pan-genomic protein microarray it was possible to profile antibody response to over 7,000 proteins simultaneously, drastically reducing the traditional timeline. Using this platform, we were able to identify novel antigens that are associated with travelers’ diarrhea. We were able to correlate results from the protein microarray to the known CF/toxin profile. These data highlight the utility of the microarray in assessing proteomic immune responses in the setting of travelers’ diarrhea and may supplement culture independent diagnostic methods.

**GEN068  Quantitation of enterotoxigenic *Escherichia coli* strain TW11681 in stools of experimentally infected human volunteers**

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Background: As part of an effort to develop an enterotoxigenic *Escherichia coli* (ETEC) human challenge model for testing new vaccines based on the heat-stable toxin, we experimentally infected nine human volunteers with wild-type ETEC strain TW11681. To improve our understanding of how the volunteers respond to the infection we measured the amount of TW11681 DNA in daily passed stools, and assessed its association with clinical symptoms and strain-specific immune responses.

Methods: Three groups of three volunteers were given 1×106, 1×107, and 1×108 colony-forming units of ETEC strain TW11681 (O19:H45, STh-CFA/I CS21, ETEC8 family), respectively, and followed for 9 days with daily stool specimen collection and clinical examinations. Blood specimens were drawn before ingesting the strain and 10 and 28 days afterwards. We quantified TW11681-specific DNA in stool DNA by using a qPCR probe assay that targets the O19-specific O-antigen polymerase gene. We used a multiplex bead flow cytometry assay based on Colonization Factor Antigen I (CFA/I) subunits and on the *E. coli* mucinase YghJ to evaluate the humoral immune responses to the infection.

Results: For six of the nine volunteers, TW11681 DNA peaked sharply between 3% and 8% of total stool DNA, 2-5 days after orally ingesting the dose. For the remaining three volunteers, TW11681 DNA did not exceed 0.5%. The peaks often spontaneously decreased before onset of the antibiotic treatment and before an increase in CFA/I-specific antibodies became apparent. The size or timing of the peaks did not seem to be associated with dose or strength of the immune responses, but the two volunteers who had diarrhea and the five who had abdominal pains or cramps had these peaks. The three volunteers who did not have peaks displayed few and mild symptoms relevant to the infection. Two of them still developed immune responses, although the responses tended to be weaker than those seen for the other volunteers.

Conclusion: While all volunteers became colonized with TW11681, the colonization appeared to be substantially more prolific in six of the volunteers, and this proliferation was associated with experiencing clinical symptoms and stronger immune responses. When analyzing data from human challenge or experimental infection studies, accounting for stool strain proliferation may help to explain variation in clinical outcomes and immune responses.
GEN085  Synergistic effect of fecal markers of environmental enteropathy on stunting among Zambian children

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Background: The effect of environmental enteropathy (EE) on stunting is rarely studied, partly because the pathobiology of EE is complex. In this study, we elected three candidate stool biomarkers: myeloperoxidase (MPO) and calprotectin (CALP) to measure gut inflammation and alpha-1 anti-trypsin (AAT) to measure gut leakiness/permeability to investigate their individual or synergistic effects on risk of stunting.

Methods: We used data from existing stool samples collected from a case-control study conducted in Lusaka Province of Zambia among children under 5 presenting to health facilities with moderate-to-severe diarrhea. The study was conducted prior to and during the early months of widespread rotavirus vaccine implementation in Lusaka between July 2012 and October 2013. The primary outcome was stunting defined as children with height-for-age z-score below -2. The exposures of interest were the three fecal biomarkers of EE measured on continuous scale. We used generalized linear Poisson model with robust standard error to estimate the main and interaction effects of the biomarkers on risk of stunting. We estimated synergistic effects of biomarkers using relative excess risk due to interaction (RERI).

Results: A total of 219 children were included in the analysis with median age of 16 months (IQR=8, 25). The median concentration of MPO, CALP, and AAT was 2560.4 ng/ml (interquartile range (IQR)=806.1, 6522.9), 79089.1 ng/ml (IQR=362466.9), and 48570.4 ug/l (IQR=12130.7, 139520.8) respectively. There was no evidence of independent effects of MPO (Adjusted RR (aRR)=1.01; 95%CI=[0.94, 1.07]; p=0.962), CALP (aRR=0.97; 95%CI=[0.94, 1.01]; p=0.123), and AAT (aRR=0.97; 95%CI=[0.93, 1.02]; p=0.286) on risk of stunting. However, the data suggests strong evidence of synergistic effects of the fecal markers on risk of stunting: MPO-CALP combination had RERI=2.01 (95%CI=[1.99, 2.02]; p<0.0001), MPO-AAT combination had RERI=1.95 (95%CI=[1.94, 1.96]; p<0.0001), CALP-AAT combination had RERI=1.95 (95%CI=[1.94, 1.96]; p<0.0001), and MPO-CALP-AAT combination had RERI=2.01 (95%CI=[1.99, 2.03]; p<0.0001).

Conclusion: We found that stool biomarkers of EE synergistically played a role in increased risk of stunting among children under 5 in Zambia.

GEN087  A novel transgenic mouse with a hyperactive mutation in receptor guanylyl cyclase C is a model for familial diarrhea syndrome

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Guanylyl Cyclase-C (GC-C) is identified as the receptor for heat stable enterotoxin (ST), the causative agent of Enterotoxigenic E. coli mediated diarrhea. Activation of GC-C results in production of cGMP which regulates fluid and ion secretion, cell proliferation and gut immune responses. Absence of GC-C in knock out mice results in early lethality to oral Salmonella Typhimurium infection. Mutations in GC-C are seen in the human population, that result in hyperactive or inactive GC-C. One mutation (S840I) was the first hyperactive mutation reported in GC-C, and led to congenital
secretory diarrhea. Patients harboring this mutation had chronic diarrhea, micronutrient deficiencies, small bowel obstruction and intestinal inflammation leading to IBD. To understand the underlying physiology of hyperactive GC-C, a transgenic mouse harboring the corresponding mutation was generated. This mouse showed higher frequency of bowel movement and increased fecal water content and was hyper responsive to ST peptide. The transgenic mouse also showed intestinal anomalies, such as unusual intestinal architecture and inflammation, as well as differential gene regulation in regions of the gut. We believe that this mouse would not only serve as a good model to understand human disease, but also can be evaluated for testing the efficacy of toxoid vaccines for ST.

GEN101 Comprehensive characterization of circulating T follicular helper cells and plasmablasts in human volunteers experimentally infected with enterotoxigenic Escherichia coli

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Background: Infection with enterotoxigenic Escherichia coli (ETEC) induce protection against reinfection with the same or similar ETEC strains. Identifying immunological markers that correlate with protective immunity would benefit ETEC vaccine development. Plasmablasts and circulating follicular T helper (cTfh) cells seem to have important roles in acquired protective immunity, and quantifying these cell subsets after infection may help characterize the immune response. cTfh cells provide B cells with potent stimuli and are necessary for class switching. We here present a comprehensive mass cytometry panel for cTfh cells and plasmablasts, and pilot study findings from six subjects infected with one of two epidemiologically relevant ETEC strains.

Methods: Thirty healthy adult human volunteers ingested between 1×10^6 and 1×10^10 colony-forming units (CFU) of either TW10722 (O115:H5, STh, CS5, CD6, ETEC5 family) (n=21) or TW11681 (O19:H45, STh-CFA/I CS21, ETEC8 family) (n=9). Blood samples were obtained from the volunteers 0 and 7 days after inoculation. Peripheral blood mononuclear cells (PBMCs) were isolated and analyzed the same day, or frozen for later analysis. Additionally, whole blood samples from 12 of the TW10722 volunteers were fixed and frozen immediately 0, 3, 5, 7, 10 and 14 days after infection. PBMCs were analyzed with a 29-marker mass cytometry (CyTOF) panel enabling the identification of cTfh cells (CD3+ CD4+ CXCR5+) and plasmablasts (CD19+ CD20- CD38++, CD27+). cTfh cells were further characterized into gut-homing (Beta7+) and Th1, Th2, Th17 subsets based on their chemokine profiles (CXCR3+, CCR4+, CCR6+, respectively) and whether they had activated (ICOS+, PD1+, CD38+) or regulatory phenotypes (CD25+, CD127-). Plasmablasts were characterized into a gut-homing and IgA+ subtype. Fixed and frozen cells will be used for profiling the kinetics and characteristics of plasmablasts and cTfh over time in experimental infection.

Results: In six volunteers infected with TW10722, there was an 11-fold mean increase in the frequency of gut-homing plasmablasts from day 0 to day 7. The gut-homing IgA+ plasmablast fraction remained relatively stable from 39.7% to 46.3%. Total gut-homing cTfh cells showed only a 1.13-fold increase, but activated gut-homing cTfh cells increased 2.5-fold, from 0.09% to 0.22% of CD4+T cells. This increase was seen for all subtypes, with fold changes of 1.13 for Th1 cells, 1.13 for Th2 cells, and 1.19 for Th17 cells. Gut-homing cTfh regulatory cells constituted 0.16% of CD4+T cells at day 0 and increased 1.5-fold. The percentage of activated gut-homing cTfh cells at day 7 tended to correlate with gut-homing IgA+ plasmablasts (R=0.74, p=0.098).

Conclusion: The optimized CyTOF panel allows comprehensive examination of cTfh and plasmablasts in response to ETEC infection. The results from the full set of collected samples, including kinetics and correlation with clinical symptoms will be presented at the meeting. The work could improve our understanding of altered frequencies of cTfh subtypes and plasmablasts in ETEC infection, and how protective immunity to ETEC could be effectively engendered.
**ADJ023** Presentation of Skp in outer membrane vesicles protects mice against Enterotoxigenic *Escherichia coli* challenge

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Outer membrane vesicles (OMVs) are promising vaccine components because they combine antigen and adjuvant in a single formulation. Detoxified *Salmonella enterica* strains which express penta-acylated lipid A retain OMV immunogenicity but with reduced reactogenicity. Here we compared directly the efficacy of expressing the enterotoxigenic *Escherichia coli* (ETEC) seventeen kilodalton protein, Skp, in detoxified Salmonella OMVs to recombinant fusions of Skp to the glutathione-S-transferase (GST) epitope in ETEC challenge experiments in mice. We observed that the display of Skp on OMVs, in the absence of exogenous adjuvant, was equally effective as compared to the use of recombinant GST-Skp combined with cholera toxin in a mouse pulmonary challenge model.

**ADJ055** U-OMP19 from *Brucella abortus* increases dmLT immunogenicity and improves protection against heat-labile toxin (LT) oral challenge in vivo

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Enterotoxigenic *E. coli* (ETEC) is the most common cause of bacterial diarrhea in developing countries. Both naturally acquired infection and oral-mucosal vaccination against heat-labile toxin (LT), colonization factors or adhesins can induce protective immunity. So, LT is being used as oral adjuvant/antigen (Ag) in mice. Since its toxicity limits its practical use in humans, a double mutant of LT (dmLT) which is less toxic and retains its adjuvant properties is under clinical trial.

U-Omp19 is a protease inhibitor from *Brucella* spp. with immunostimulatory properties. We propose to use U-Omp19 as platform to deliver antigens (Ags) in oral formulations against infectious diseases. Previously, it has been shown that this protein can protect co-administered Ags from digestion and it can also trigger and direct the type of immune responses against the Ag. In this work our aim was to investigate the effect of U-Omp19 co-delivery on dmLT immunogenicity and protective efficacy in vivo. To this end inbred BALB/c or outbred CD1 mice were orally immunized, according to different protocols, with i) saline ii) dmLT or iii) dmLT+U-Omp19. Three doses of dmLT were studied alone or plus U-Omp19: 25µg, 12.5µg and 2.5µg. Fecal and serum α-LT antibodies (Abs) were evaluated by ELISA every week and after last immunization mice were challenged orally with LT enterotoxin (patent mouse gut assay). Results obtained indicated that co-delivery of U-Omp19 increased (P<0.05) mucosal and systemic IgA and IgG α-LT Abs and avidity of systemic IgA α-LT. Moreover, U-Omp19 when co-delivered with dmLT induced significant protection (P<0.05) against oral challenge with LT in BALB/c and CD1 mouse model, while dmLT alone did not. All together our results indicated that U-Omp19 can help to increase dmLT immunogenicity and produce neutralizing antibodies against LT *in vivo*. So, U-Omp19 would be a good candidate to be included in dmLT vaccine formulations against ETEC.
ADJ077  Design and validation of heat-labile toxin (LT) neutralization assay

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Diarrheal illness contributes to malnutrition, stunted growth, impaired cognitive development, and high morbidity rates, affecting 1/5th of children worldwide. In children under five, enterotoxigenic *Escherichia coli* (ETEC) accounts for 280 million diarrheal cases and 380,000 resultant deaths annually. Currently, there is no vaccine against ETEC although previous studies indicate that antibodies to LT can help protect against diarrheal secretion from LT+ ETEC strains. However, no standard laboratory test exists to detect functional anti-LT antibodies. The purpose of these studies is to design and optimize an *in vitro* LT neutralization assay that can be standardized across laboratories and can rapidly test human and animal serum samples.

The assay we designed evaluates the ability of LT toxin to reduce NF-κB signaling after Toll-like receptor (TLR) agonist treatment using HCT116-DualTM cell line (invivogen #hctd-nfis) in a 96-well plate format. These HCT116 cells secrete a quantifiable embryonic alkaline phosphatase (SEAP) in response to NF-κB transcription activity (via IL-12p40 promoter fused to NF-κB and AP-1 binding sites). In this assay, serum antibodies are pre-incubated with LT toxin to determine the dilution or titer of antibody that neutralizes toxin effects on SEAP secretion. We evaluated this assay for optimal NF-κB-SEAP including (1) the best TLR agonist to maximize SEAP levels, (2) quantity of LT toxin to inhibit TLR-induced SEAP, (3) culture incubation time, and (4) ability of immunized and naive animal and human serum to neutralize LT toxicity. We successfully optimized all assay components. Our results indicate that the most effective TLR agonist is 25 μg Poly I:C, whose SEAP levels are decreased when co-treated with 10ng -1μg LT that has been pre-incubated with test or control sera. The optimal culture time is 7 hours after which the supernatants were collected, frozen, and eventually quantified for SEAP using the Great EscAPE SEAP Chemiluminescence Kit 2.0 (Takara). Detected optical densities were quantified using the ImageQuant TL program. Serum from immunized human and rabbit samples (but not naive samples) were able to inhibit the LT-reduction in SEAP, indicating successful detection of serum neutralizing antibodies. In the future, we plan to further validate our assay using Phase 2 clinical trial serum samples (ClinicalTrials.gov Identifier: NCT01922856) from an NMRC ETEC vaccine study. The serum in this study is comprised of 3 cohorts, each containing vaccinated or naïve control individuals from whom serum samples will be tested from pre-vaccination, post-vaccination/pre-challenge, and post-challenge bleeds.*

In conclusion, we have designed a robust LT-neutralization assay. Our assay is capable of both rapid and mass testing, using a cell line that doubles in approximately 21 hours and 96-well plates that can run 9 different sera per plate. All reagents are commercially available except for LT toxin (which we can provide). This type of serum antibody assay can improve evaluations of clinical responses to ETEC vaccines with candidate vaccine formulations and promote rational vaccine design.
ADJ086  PGT new generation of cross-protective human vaccines

Anna Obolensky

Pacific GeneTech, Hong Kong, Hong Kong

Pacific GeneTech is an established developer of animal and food safety vaccines. PGT is undertaking preclinical development of its platform of vectored recombinant, cross-protective, live attenuated and inactivated vaccines that are low cost, orally administered and easily deployed. Robust humoral and cell mediated immune responses against the inherently low immunogenicity of highly conserved antigens with homology across multiple strains of target pathogens are induced by co-expression with a recombinant immunostimulant. Mucosal immunity (IgA) is crucial for prevention of infection, but IgA responses to parenterally administered inactivated vaccines are often minimal favoring the mucosal route of administration. The vaccines were primarily developed as orally administered food safety vaccines in Arkansas for protection of public health against and Salmonella spp and other enteric diseases originating in poultry.

PGT’s vaccines can be administered by multiple routes including mucosally (oral and intranasal) using mannosylated chitosan (MCA/Hercules) a patented PGT delivery system with potential for use with multiple different vaccines. Studies to date have shown that MCA is a safe and effective adjuvant/delivery system that compares favorably with other formulated chitosan adjuvants and that is suitable for oral administration with a variety of different vaccines.

PGT currently has two main vaccine candidates with cross protective activity against (i) Apicomplexa species (Eimeria, Toxoplasma, Cryptosporidium and potentially Plasmodium vivax) and (ii) enteric bacteria (Salmonella, E. coli (Enterotoxigenic E. coli) and potentially Shigella and Campylobacter). Ongoing studies across the world (UK, US, EU, Taiwan and SE Asia) are investigating these vaccines in multiple trials.

The Apicomplexa vaccine originally developed for Eimeria infection in poultry will also be investigated against Toxoplasma gondii, causing abortion in sheep and interruption of the sexual cycle in cats. Studies are ongoing against cryptosporidiosis and vivax malaria. Toxoplasmosis and cryptosporidiosis also cause disease in humans and vivax malaria is a significant neglected tropical disease.
Preclinical evaluation of vaccine candidates and models of enteric disease

ORAL PRESENTATIONS

PRE012  ST secreted conjugates are non-toxic and induce ST-neutralizing antibodies in immunized mice

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Enterotoxigenic *Escherichia coli* (ETEC) continues to be a significant cause of morbidity and mortality. In 2010, annual mortality from illness due to ETEC was estimated at 157,000 deaths (9 percent of all deaths attributed to diarrhea) and approximately 1 percent of all deaths in children 28 days to 5 years of age. ETEC cause disease by colonizing the small intestine through production of colonization factors and through elaboration of heat-labile (LT) and/or heat-stable (ST) enterotoxins. LT is highly immunogenic and induces antibodies to itself when given to human volunteers. On the other hand, ST is a small non-immunogenic secreted peptide. Previous attempts to make ST immunogenic require the use of genetic fusions or conjugation to specific carrier proteins. We have recently begun to investigate the ST+ETEC secreted conjugates (STSC) as a technology capable of producing inexpensive and effective ST immunogens using spent culture medium.

Our preliminary data shows that STSC preparations contain epitopes recognized by Protein A purified anti-ST IgG. Moreover, our preliminary data demonstrates that STSC are safe and non-toxic when applied to T84 cells. Our preliminary data also show that STSC immunization with mucosal adjuvant dmLT induces serum anti-ST IgG and anti-LT IgG. Moreover, pooled and diluted serum from immunized animals can neutralize ST in vitro. In addition, we have begun proteomic analysis to determine ST conjugation partners in the ST+ETEC secretome. Identification of preferential ST conjugation partners could speed the development of an antigen bearing coverage to ST for a potential ETEC subunit vaccine.

PRE051  Evaluation of Class 5a fimbrial adhesin-pilin fusion vaccines in *Aotus nancymae* against diarrhea caused by CS14 STh+ expressing ETEC

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Enterotoxigenic *Escherichia coli* (ETEC) is a leading cause of diarrhea among children and travelers in developing countries. ETEC pathogenesis is facilitated by adherence to the small intestine via fimbrial colonization factors (CFs) and the elaboration of enterotoxins. Vaccine strategies against ETEC have targeted the CF tip-localized adhesin and pilin subunits in order to mount appropriate immune responses to prevent bacterial adherence. Over 25 CFs have been identified that are grouped into related classes. Within Class 5 fimbriae there are three subclasses containing eight serologically distinct CFs: 5a (CFA/I, CS4, CS14), 5b (CS1, CS17, CS19, PCF071) and 5c (CS2). In an effort to identify an antigen that would potentially provide pan-5a coverage, two recombinant vaccine candidates, adjuvanted with dmLT, were tested in the *Aotus nancymae* non-human primate (NHP) challenge model. CfaEB, the CFA/I adhesin-pilin fusion previously shown to provide homologous protection in NHPs, was chosen to determine the ability of the antigen to
provide protection against challenge from a CS14 expressing strain. CfaEB-CsuA2-CsfA (CfaEp3), a fusion of CfaEB to the pilins of fellow class 5a CFs, CS14 (CsuA2) and CS4 (CsfA), was constructed to determine the ability of a vaccine containing antigens for all three fimbriae of class 5a to provide protection against challenge from either CS14 or CFA/I expressing strains.

Groups of 9-10 A. nancymaae were vaccinated intradermally in a four-dose regimen followed by challenge 14 days after the final dose. Robust functional hemagglutination inhibition (HAI) titers against the CFA/I expressing ETEC strain, H10407 (LT+ STh+ STp+), were detected in groups vaccinated with either CfaEp3 or CfaEB. This was in contrast to negligible HAI titers observed for both antigens against the CS14 (WS3294A) or CS4,CS6 (Bang-10) expressing ETEC strains. Following challenge, vaccination with CfaEp3+dmLT conferred significant protection (PE= 84.4%, p=0.02) against CS14-ETEC mediated diarrhea when compared to historical unvaccinated controls. Interestingly, despite robust HAI titers against H10407, CfaEp3+dmLT did not protect against CFA/I-ETEC H10407 challenge. However, CfaEB+dmLT vaccination resulted in significant heterologous protection against CS14-ETEC (PE=75%, p=0.04) challenge. Overall, these data demonstrate for the first time that CfaEB is able to provide heterologous Class 5a protection against challenge in the A. nancymaae model and is a viable candidate for clinical advancement. Additionally, these data indicate that protection against an ETEC strain expressing only the enterotoxin STh is achievable with a recombinant adhesin-based vaccine.

**PRE052 A synthetic conjugate toxoid vaccine candidate targeting heat-stable toxin-producing *Escherichia coli***

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Enterotoxigenic *Escherichia coli* (ETEC) are an important cause of diarrheal disease, malnutrition and death in children under five years of age. ETEC that express the heat-stable toxin (ST), with or without the heat-labile toxin, are among the four most important diarrhea-causing pathogens. This makes the ST toxin an attractive target for an ETEC vaccine. However, several challenges must be overcome in order to design a safe and efficacious ST vaccine. First, the 19-amino acid peptides must be made immunogenic by coupling it to a carrier. Second, ST must be altered by mutation(s) to make it non-toxic and to avoid eliciting an immune response that cross-reacts with the endogenous peptides uroguanylin or guanylin. We have developed two lead candidate human ST (STh) toxoids, guided by our screen of all possible 361 single amino acid substitutions in STh for effects on toxicity and antigenicity. The single mutant STh candidate has >1000-fold reduced toxicity and the double mutant toxoid is non-toxic, but importantly, key neutralizing epitopes are intact. Both toxoids have mutations in amino acids that are shared with the endogenous peptides, and thus have the potential to also reduce the risk of immunological cross-reaction. We have generated vaccine candidates based on these two toxoids by chemical synthesis of the mutants, and conjugated them to the B subunit of the ETEC heat-labile toxin (LTB) to make them immunogenic. Mice vaccinated with these candidates conjugate vaccines show strong anti-STh immune responses. We will also present data on the ability of the mouse sera to neutralize the ST toxin, as well as the degree of immunological cross-reactions to the endogenous peptides.
PRE064  ETEC strain TW10722 may be suitable for use in vaccine challenge studies for testing heat-stable toxoid based vaccine candidates

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Background: Infection with enterotoxigenic Escherichia coli (ETEC) producing the human version of the heat-stable enterotoxin (STh) is an important cause of moderate and severe diarrhea in young children. ETEC diarrhea is directly linked to the activities of ST, which has therefore become a desirable target for vaccine development. A controlled human infection model (CHIM) based on an ST-producing ETEC strain that does not produce the heat-labile toxin is needed to be able to test the protective efficacy of ST-based vaccine candidates. To identify a suitable strain and dose for use in this model, we experimentally infected human volunteers with wild-type ST-only ETEC strain TW10722. We here present results from 15 healthy volunteers that have been experimentally infected so far.

Methods: We recruited volunteers with no recent travels to low- and middle-income countries for experimental infection with TW10722 (O115:H5, STh-CS5 CS6, ETEC5 family). They ingested 1×10⁶ (n = 3), 1×10⁷ (n = 3), 1×10⁸ (n = 3) or 1×10¹⁰ (n=6) colony-forming units (CFU) of the bacterium in bicarbonate buffer. They were closely monitored for development of diarrhea and symptoms of enteric infection, and blood samples were collected at fixed time points. To evaluate the T cell responses to ETEC infection, we stimulated peripheral whole blood collected on days 0, 10, and 28 with purified ETEC Coli Surface Antigen 5 (CS5) and E. coli YghJ mucinase, and counted the resulting antigen-specific CD4+ T cells by flow cytometry.

Results: Among the six volunteers receiving the highest dose (1×10¹⁰ CFU) of ETEC, one developed moderate and four developed severe diarrhea, yielding a diarrhea attack risk of 83%. Among the nine volunteers receiving lower doses, two volunteers developed diarrhea (1×10⁶ CFU; mild, and 1×10⁸ CFU; severe, respectively). Other symptoms included malaise (47%), abdominal cramps (40%), nausea (40%) and headache (47%). These symptoms were mostly mild or moderate and seemed to become more common among the volunteers receiving higher doses. One volunteer developed a fever (1×10⁸ CFU) and one vomited (1×10¹⁰ CFU). None of the volunteers needed oral or intravenous rehydration therapy. A mean 11.0-fold (p = 0.001) increase in CSS-specific CD4+ T cells was found 10 days after experimental infection, and a mean 9.3-fold (p = 0.001) increase at day 28. For YghJ, the corresponding increases were a mean 1.9-fold (p = 0.002) at day 10 and mean 2.3-fold (p = 0.001) at day 28. While the CD4+ T cell responses to YghJ were fairly comparable across the different doses of inoculum, the responses to CS5 appeared to be dose dependent.

Conclusion: Our results indicate that by using relatively large doses, the ST-only ETEC strain TW10722 would be safe and efficient for testing ST-based vaccine candidates.
Human experimental infection with ST-only enterotoxigenic *Escherichia coli* wild-type strain TW11681

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**Background:** Infection with enterotoxigenic *Escherichia coli* (ETEC) producing the human version of the heat-stable enterotoxin (STh) is an important cause of moderate and severe diarrhea in young children. ETEC diarrhea is directly linked to the activities of ST, which has therefore become a desirable target for vaccine development. A controlled human infection model (CHIM) based on an ST-producing ETEC strain that does not produce the heat-labile toxin is needed to be able to test the protective efficacy of ST-based vaccine candidates. We here present results from 9 healthy volunteers experimentally infected with wild-type ETEC strain TW11681 to evaluate its suitability for use in CHIMs.

**Methods:** We recruited volunteers with no recent travels to low- and middle-income countries for experimental infection with TW11681 (O19:H45, STh-CFA/I CS21, ETEC8 family). They ingested 1×10⁶ (n = 3), 1×10⁷ (n = 3) and 1×10⁸ (n = 3) colony-forming units (CFU) of the bacterium in bicarbonate buffer. They were closely monitored for development of diarrhea and symptoms of enteric infection, and blood samples were collected at fixed time points. To evaluate the T cell responses to ETEC infection, we stimulated peripheral whole blood collected on days 0, 10, and 28 with recombinant Colonization Factor Antigen I (CFA/I) major fimbrial subunit (CfaB) and *E. coli* YghJ mucinase, and counted the resulting antigen-specific CD4+ T cells by flow cytometry. Using a multiplex bead assay, we also measured IgA and IgG/IgM antibody responses to the two proteins in serum and in lymphocyte supernatants (ALS) from these blood samples as well as from samples collected after 3, 6, and 12 months.

**Results:** Infection with TW11681 elicited mild diarrhea in two (22%) of the volunteers (both 1×10⁶ CFU). Other symptoms included abdominal pain (56%), abdominal cramping (44%) and nausea (11%). No volunteers experienced fever or vomiting. Averaged across the nine volunteers, we found no significant change in the number of CfaB-specific CD4+ T cells on day 10, while YghJ-specific CD4+ T cells had increased 2.3-fold (p = 0.011). All but one of the volunteers (89%) developed CFA/I-specific IgG/IgM antibodies in serum with a peak 8.5-fold increase at three months. YghJ-specific IgG/IgM antibodies in serum were more modest with a peak 1.3-fold increase on day 10. Compared to YghJ, the antibody response to CFA/I seemed to have a later onset, but it remained elevated until 12 months after experimental infection. In ALS, all but one volunteer (89%) developed YghJ-specific IgG/IgM antibodies, while the response to CFA/I was not pronounced.

**Conclusion:** TW11681 elicited strong antibody and CD4+ T cell responses to relevant ETEC virulence factors. However, only two volunteers out of nine (22%) developed mild diarrhea, and the resulting attack risk is too low for the strain to be useful in CHIMs. The relatively high frequency of undesirable symptoms like abdominal pain and cramping decided against any attempts to further increase the dose.
Towards bacterial glycoprotein antigens for vaccine development

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Enterotoxigenic Escherichia coli (ETEC) strains contribute significantly to diarrheal illness and mortality in third world countries. ETEC particularly affects children and is associated with millions of infections and hundreds of thousands of deaths each year and is the most frequent cause of diarrhea among deployed military personnel and travelers visiting the endemic areas. Despite significant efforts, no vaccine is currently available.

Much attention has been devoted to the understanding of how ETEC and other mucosa-associated pathogens interact with host tissue during infection. A number of studies have revealed that bacterial protein glycosylation plays an important role in mediating adhesion, colonization and invasion of host tissue. The bacterial glycan building blocks are distinct and therefore foreign to the human immune system. Thus, glycoproteins constitute an immunologically distinguishing feature, which can provide attractive targets for antimicrobial vaccine intervention. While the intimate coupling between protein glycosylation and bacterial pathophysiology has become apparent, the discovery of novel glycoproteins is advancing slowly. This is attributed to the inherent challenges associated with glycoproteomics.

To overcome this problem, we have developed a novel mass spectrometry-based technique, termed BEMAP, which can be employed to map O-linked glycoproteins from theoretically any biological source. Using BEMAP, we have identified a significant amount of previously unexplored glycoproteins in ETEC.

From our list of modified proteins, we have picked a novel glycosylated vaccine candidate against ETEC. Our lead molecule, YghJ, is a secreted metalloprotease that degrades the protective mucin layer of the human intestine. We have purified glycosylated YghJ as well as the non-glycosylated variant and used both proteins to immunize rats. Using antibody affinity techniques, we demonstrate that rats raised high affinity antibodies towards the glycan-peptide moiety of glycosylated YghJ, thus showing immunogenicity of this particular epitope. When analyzing serum samples from the H10407 refinement challenge study conducted by PATH and the Johns Hopkins Bloomberg School of Public Health, we can demonstrate that YghJ is recognized by antibodies withdrawn from pre- and post-challenged individuals. Our serum analysis also shows that the end point titer for both the glycosylated and non-glycosylated YghJ is similar in all tested individuals prior to exposure to ETEC. However, 28 days post exposure the titer for glycosylated YghJ has increased significantly more, in all tested samples, compared to the non-glycosylated protein variant. Based on our data, we speculate that O-linked bacterial glycoproteins may constitute an important reservoir of novel vaccine candidates with superior immunogenicity.

MACE, multi-antigen combination enteric, vaccine for broad protection against ETEC and Shigella infections

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Enteric bacterial infections have high rates of morbidity and mortality throughout the world with Shigella spp. and Enterotoxigenic E. coli (ETEC) being leading causes of bacillary diarrhea in children within developing regions of the
world. In addition to this, these pathogens are the leading causes of travelers’ diarrhea for individuals from industrialized nations who visit these developing countries. While significant resources have been expended, viable vaccines having broad (serotype-independent) coverage have yet to be produced and licensed.

Rather than developing a vaccine formulation that possesses components that would convey serotype-specific protection, we have developed broadly protective protein antigens to prevent ETEC and *Shigella* spp. infections. The ETEC antigen, the TX-tip MEFA fusion, is a fusion of an LT-ST toxoid (TX) genetically fused to the adhesin tip multi-epitope fusion antigen (tip-MEFA) which carries epitopes from the minor adhesive tip subunits of nine of the most prevalent ETEC adhesins. The LT-ST toxoid moiety of this TX-tip MEFA fusion elicits the production of antibodies that neutralize both ST and LT in cell cytotoxicity assays. Moreover, this toxoid fusion induces strong antibody responses in pigs and protects piglets against ETEC diarrhea. Mice immunized with the tip-MEFA moiety developed serum IgG antibodies specific to each adhesion, which significantly inhibited adherence to Caco-2 cells for ETEC expressing these nine adhesins. The TX-tip MEFA fusion protected at efficacy rates between 80-100%.

The *Shigella* antigen, DBF, is a fusion of the type III secretion system apparatus (T3SA) tip and first translocator proteins, IpaD and IpaB, respectively. Initially, we demonstrated that when administered intranasally (IN) IpaB and IpaD, admixed with the mucosal adjuvant dmLT, double mutant labile toxic from ETEC, induce systemic and mucosal immune responses that protect against homologous and heterologous challenge using the mouse pulmonary infection model. To reduce vaccine production costs, we genetically fused IpaD and IpaB to produce DBF which exhibits the same or even improved protective efficacy as IpaB+IpaD. We have demonstrated protection of mice against a lethal pulmonary challenge with three of the four *Shigella* species using the intranasal, intradermal and intramuscular (IM) routes. Furthermore, Dr. John Clements at Tulane University, School of Medicine, immunized Rhesus macaques IM with DBF+dmLT and demonstrated that 5 of 6 monkeys were protected against severe diarrhea.

We are now initiating proof of concept studies to combine these two fusion antigens into a single vaccine formulation to ensure their compatibility when co-administered. Biophysical analysis has indicated no non-specific aggregation upon combining the two proteins. Initially, groups of mice were vaccinated with each antigen alone or together. Serum was used to assess neutralization of both ST and LT as well as prevention of adherence of ETEC to Caco-2 cells. Additionally, the mice were challenged with *Shigella flexneri* to assess protective efficacy of the formulation. The results of these studies will be presented as well as the next steps forward.

**POSTER PRESENTATIONS**

**PRE013 Enhancing in Silico peptide-based vaccine discovery for enterotoxigenic Escherichia coli using molecular docking and molecular dynamics simulation approach**

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Enterotoxigenic *E.coli* is an important cause of diarrheal illness in infants and young children with the majority of the cases occurring in developing countries. The development of a safe and effective vaccine against these enteric pathogens can be the best alternative to control and reduce the number of cases. To contribute towards vaccine development, this work has applied in silico strategies in search of high potential peptide epitopes able to bind to different Major Histocompatibility Complex (MHC) Class I molecules from different human populations. The analysis led to the identification of highly promiscuous 9 antigens derived from 4,915 proteins conserved among ETEC strain E24377A. Furthermore, presented to OrthoMCL database, all the predicted 9 antigens have shown orthologs in *Shigella flexneri* and *Salmonella enterica*. Assessment of different immunological parameters including antigenicity, allergenicity, stability, localization, molecular weight and toxicity of the predicted epitopes essential for good vaccine candidate were distinguished by several algorithms. To validate the research, we included two known antigenic epitopes as control proteins, epitope 141STLAsia, India, Northeast Asia, South Asia and North America. The results depicted that the designed epitopes could manifest vigorous enduring defensive immunity against diarrhea.
PRE018 A study of serotypic differences in innate and adaptive immune responses in Shigellosis in humans.

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Shigella is a leading cause of morbidity due to diarrheal diseases in developing nations. Colonic mucosal invasion and resulting host inflammatory responses are thought to be key contributing factors to dysenteric form of this disease. Severity of shigellosis varies with serotype involved; S. dysenteriae (SD) producing the severest infections and complications and S. sonnei (SS) being at the other end of the spectrum usually causing mild self-limiting diarrhea. We studied the serotypic differences in innate and adaptive immune responses in shigellosis using various models. Explants (invitro organ culture, IVOC of human intestinal biopsies) were inoculated with 10^9 colony forming units (CFU) of Shigella flexneri 2a, SD-1 and SS. Both cell invasion assay and confocal microscopy conclusively demonstrated SD-1 to be more invasive as compared to SF and SS. S. sonnei was the least invasive. We also performed gentamicin protection assay in CACO 2 cells which revealed the same gradient of invasiveness. Shigella caused acute destructive inflammation of colonic mucosa in histology. We studied expression of various cytokines both by real time PCR assays and measured the cytokine levels in tissues by cytokine bead assay (CBA) using flow cytometry (Becton Dickinson FACS ARIA III). We found variable cytokine responses providing evidence of variability in host responses. Overall, real time PCR assay showed enhanced expression of IL-8, IL-12, IL-17, IL22, IL23, IFN-γ & TNF-α and downregulation of IL-1β and IL-6 at 24 hr. post-infection. Both IL-1β and IL-6 are secreted quite early in the infection at 3-4 hours. So in essence what we observed was a down regulation of IL-1β and IL-6 at 24 hrs post infection and up regulation of IL-12, IL-17, IL-8, IL-22, IL-23, IFN-γ & TNF-α which are parts of further activation of cytokine network. We also studied cytokine responses in dendritic cells as they are key regulators of immune response with ability to affect both innate and adaptive immune responses and are abundant in the gut mucosa. We demonstrated that SD caused maximum release of IL-8. Similarly SD also caused highest release of IL-17A and IL-22A. It was the only serotype which increased IL-23. IL-8 is the primary cytokine which induces neutrophil chemotaxis and a major molecule orchestrating mucosal inflammation in shigellosis. In another set of experiments, we purified CD4+CD45RO+ T cells and cultured them with Shigella-infected DCs supernatants, performed real time PCR assay which showed upregulation of all cytokines. CBA showed IL-17 to be the most abundant cytokine, though other cytokines were also found in higher concentrations as compared to controls. ELISA of β defensins (hβD2 and hβD3) showed increased levels after infection. We demonstrated that SS dampened HBD-2 responses whereas there was augmentation by SD and modest but nonsignificant increase by SF. Modest increase in HBD-3 by SS and SF was observed but was not statistically significant. There was no increase or decrease in LL-37. Our study sheds new light on serotype differences in innate and adaptive immune responses.

PRE021 Immunogenicity and characterization of enterotoxigenic Escherichia coli (ETEC) toxoid fusion and adhesin MEFA antigens in intradermally or intramuscularly immunized mice

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Enterotoxigenic Escherichia coli (ETEC) are the most common bacterial cause of diarrhea. ETEC bacterial adherence to the small intestinal epithelial cells and delivery of enterotoxins cause diarrhea in children and international travelers. Currently, there are no vaccines licensed for ETEC associated children’s diarrhea and travelers’ diarrhea. Recently, toxoid fusion 3xStaN125-mnLTR192G/L211A, adhesin MEFA CFA/I/II/IV, and toxoid-adhesin MEFA CFA-3xStaN125-mnLTR192G/L211A are demonstrated to induce neutralizing antitoxin and/or anti-adhesin antibodies in intraperitoneally (IP) or subcutaneously (SC) immunized mice, or intramuscularly (IM) immunized pigs, suggesting these antigens potential candidates for ETEC subunit vaccines. However, these antigens have not been examined in intradermal (ID) or intramuscular (IM) route in mice, which perhaps are more suitable for human vaccine administration. In this study we ID or IM immunized mice with toxoid fusion 3xStaN125-mnLTR192G/L211A, the CFA MEFA, alone or combined, toxoid-adhesin MEFA CFA-3xStaN125-mnLTR192G/L211A, and characterized antigen-specific antibody responses.
Data showed that mice ID or IM immunized with the toxoid fusion antigen developed anti-LT and anti-STa antibodies, and mice immunized with the CFA MEFA developed antibody responses to all seven adhesins (CFA/I, CS1-CS6). In addition, mice ID or IM co-administered with the toxoid fusion and the CFA MEFA, or with toxoid-adhesin MEFA CFA-3xStaN125-mILTR192G/L211A developed antibodies to both toxins and all seven adhesins. Antibody neutralization studies of the serum samples of the immunized mice showed induced antibodies neutralized enterotoxicity of LT and STa and/or inhibited adherence of ETEC or E. coli bacteria producing any of these seven adhesins. These data confirmed immunogenicity of these ETEC subunit vaccine target antigens and provide useful information for vaccine development against ETEC diarrhea.

PRE022 Computational characterization and functional annotation of hypothetical proteins of Shigella using in-silico tools: A bioinformatics study

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Belonging to the family Enterobacteriaceae, Shigella dysenteriae is a gram-negative bacterium which is mainly responsible for Shigellosis, an acute form of gastroenteritis and also for increasing mortality rate in children. Till now one strain of Shigella (Sd197) which has been analyzed and sequenced completely and reported to contain one circular chromosome which is having 4,369,232 nt (4,664 genes). In this study our group has focused to annotate the proteins which are otherwise known as “hypothetical” and to characterize the same computationally with the help of various bioinformatics tools and databases which are available particularly for the genome of Shigella dysenteriae strain Sd197. We have employed different online servers to predict the function for annotation of these proteins that remain still uncharacterized before in the genome S.dysenteriae. Sequences for total 828 hypothetical proteins were retrieved from the public repositories like NCBI. Various physicochemical properties like Theoretical PI, Extinction Coefficient, Aliphatic Index, and Grand average of hydropathicity (GRAVY) were analyzed with the help of these protein sequences. Some more characterization was done for functional prediction, structural prediction and virulent prediction by using different online servers available. Out of 828 hypothetical proteins, 38 proteins were found to be enzymes, 11 proteins were containing virulence factor, 85 proteins were reported as hydrolase proteins, 5 proteins were found to be isomerases, 4 proteins were lyase, 8 proteins were ligase, 71 proteins were identified as transporter, 18 proteins were DNA binding, 6 proteins were binding proteins, 5 proteins were adhesions, 6 proteins were cellular process, 30 proteins were regulatory proteins, 15 proteins were analyzed with kinase activity. Based on this bioinformatics analyses, we are aiming further to identify putative drug targets for designing the potential vaccine candidates for S.dysenteriae and also to develop a computational pipeline for better understanding of the drug resistance and the pathogenesis mechanism in S.dysenteriae which will help to develop novel mechanisms for treatment of the disease.

PRE029 Immunogenicity and protective efficacy of inactivated Shigella multivalent vaccines

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Shigella and Enterotoxigenic Escherichia coli (ETEC) remain major causes of diarrhea among children in developing countries and travelers to these areas. However, currently there are no effective vaccines against these enteric pathogens. Previously we demonstrated the vaccine and vector potential of formalin inactivated Shigella by showing that they induced strong protective immune responses against Shigella antigens in mice¹. In addition, we showed that these Onactivated strains can serve as effective vaccine carriers for fimbrial antigens (CFA/I and CS3) of ETEC. However, we observed that the harsh formalin treatment negatively affected the immunogenicity of the ETEC fimbrial antigens. We therefore explored alternative methods of inactivating Shigella. Here we present our results examining the vaccine and vector potential of Shigella cells inactivated by conversion to ghost cells and by exposure to gamma irradiation. We found that mice immunized orally or intranasally with ghost cells of S. flexneri 2a expressing ETEC CFA/I and CS3 induced strong IgG titers to the homologous LPS and to the ETEC antigens. These immune responses were protective as 100% of the vaccinated animals can survived a lethal challenge with the live homologous Shigella strain compared to negligible
or no survival in mice given PBS. More recently, we have explored gamma-irradiation as an alternative method to inactivate *Shigella* cells. Preliminary mouse studies show that gamma-irradiated *Shigella* cells induce strong and protective immune responses in mice. Multivalent vaccine formulations composed of various gamma-irradiated *Shigella* strains induce protective immunity to each of the component strains and these immune responses do not appear to be affected by immunological interference. We are currently assessing whether a *Shigella* multivalent vaccine can provide cross-protection against other *Shigella* strains which are not components of the vaccine formulation. These studies demonstrate that inactivated *Shigella* whole cells are highly immunogenic and can serve as effective carriers for exogenous antigens.

**PRE039 Establishment of a human colonoid model to study *Shigella* pathogenesis and evaluate vaccine candidates**

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The enteric pathogen, *Shigella*, is one of the leading causes of moderate-severe diarrhea and death in young children in developing countries. The urgent need and effort to develop a protective vaccine against *Shigella* infection is hindered by the lack of good preclinical models to evaluate correlates of vaccine reactogenicity and protective efficacy. Transformed cell lines and animal models have been widely used to study *Shigella* pathogenesis. In addition to their altered physiology, transformed cell lines are composed of a single cell type that does not sufficiently represent the complex multi-cellular environment of the human colon. Most available animal models do not accurately mimic human disease. In order to use a system that is more physiologically relevant, we utilized the human colonoid model, derived from LGR5+ stem cell-containing colonic crypts from healthy subjects, and evaluated its usefulness as a novel preclinical model to test potential vaccine candidates against *Shigella* infection.

Human colonoids contain multiple cell types including goblet cells, entero-endocrine cells, Paneth cells and colonocytes, which mimics the in vivo colonic epithelium. Colonoid monolayers supported on Transwell filters polarize with appropriate transporter localization and allow access to both apical (representative of gut lumen) and basolateral surfaces. The human colonoid model was amenable to wild type *Shigella flexneri* 2457T infection and demonstrated >20-fold increased efficiency of invasion via the basolateral surface over apical infection. The presence of intracellular bacteria was confirmed using gentamicin protection assays and confocal microscopy. Cytokines secreted into the apical and basolateral media compartments were measured. This model allows quantification of RNA and protein expression using qRT-PCR and Western Blot analysis. Ongoing studies are aimed at establishing host cytokine and gene expression responses that can be used as correlates of immunogenicity and reactogenicity to enable more rapid advancement of the most promising vaccine candidates into clinical trials.

**PRE048 A comparative immunogenicity study of enterotoxigenic *Escherichia coli* heat-stable toxin (STa) toxoid genetic fusion versus chemical conjugates**

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Enterotoxigenic *Escherichia coli* (ETEC) strains producing heat-stable toxin (STa), alone or together with heat-labile toxin (LT), are a leading cause of moderate-to-severe diarrhea in children from developing countries and diarreah in international travelers known as travelers’ diarrhea. As the key virulence factors in ETEC diarrhea, STa and LT are commonly targeted in ETEC vaccine development. Two antigen preparation strategies, genetic fusion and chemical conjugation, are often explored. In this study, we prepared genetic fusion 3xSTaN125-mnLTR192G/L211A and chemical conjugates BSA-STaA14T and BSA-STa, immunized mice with the LT-STa toxoid fusion or each chemical conjugate, and examined comparatively for induction of anti-STa antibody responses as well and antibody neutralization against STa toxin. Additionally, anti-STa antibodies derived from the toxoid fusion or the conjugate were assessed for cross-reactivity.
with guanylin and uroguanylin. Data showed that mice subcutaneously immunized with toxoid fusion 3xSTA12S-mnLTR192G/L211A, conjugate BSA-STA14T or BSA-STA developed high titer of anti-STA antibodies. Moreover, the pooled or individual serum sample from mice immunized with the toxoid fusion or conjugate prevented STA from stimulation of intracellular cGMP in T-84 cells. These mouse serum samples showed little cross-reactivity with guanylin or uroguanylin. Furthermore, toxoid fusion 3xSTA12S-mnLTR192G/L211A and conjugate BSA-STA14T when IM immunized induced protective antibodies against STA+ ETEC diarrhea in a pig challenge model. These results indicated that genetic fusion and chemical conjugation are equally effective at the preparation of STA toxoid antigens to induce antibodies neutralizing STA toxin, suggesting the potential application of STA toxoid fusion or conjugate antigens in vaccine development against ETEC children’s diarrhea and travelers’ diarrhea.

PRE050  A human-associated colonization factor in enterotoxigenic E. coli recognizes mucosal receptors present in human and pig small intestine

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Enterotoxigenic Escherichia coli (ETEC) infections are highly prevalent in humans and livestock animals, foremost in neonatal pigs and calves. The ability of an enteric pathogen to infect a host correlates with its ability to adhere to cells of a specific host. There are more than 25 different colonization factors identified in ETEC infecting humans and additional colonization factors in animal-associated ETEC strains. The Class 1b of human ETEC colonization factors are all related to the colonization factor 987P(F6) found in ETEC strains infecting neonatal pigs.

The recently characterized colonization factor CS30 is related to the pig-associated CF 987P (F6) in terms of sequence identity and operon structure. CS30 can either be harbored alone or together with CS13, a colonization factor related to K88(F4) which is another highly prevalent CF in strains causing neonatal enteric infections in pigs.

Bacteria expressing the novel CS30 was shown to adhere to human intestinal cells. However, a mutant strain, E873 DcsmA, with a disrupted gene encoding the major subunit, did not adhere. The novel CF CS30 is located on a plasmid of ~59 kB together with LT and STp and may be mobile with the help of a conjugative plasmid.

Using thin-layer chromatography we have shown that wild-type CS30 expressing strains bind to acid glycosphingolipids of human and pig small intestine. Sub fractions of the glycosphingolipids from the human small intestine were tested to specify the binding of CS30 and sulfatide was identified. Binding of CS30 to sulfatide-related glycosphingolipids was negative. A recombinant E. coli strain expressing CS30 is under construction which will be used for CS30 protein purification and production of CS30-antisera. We plan to look at immunological cross-reactivity between CS30 and 987P(F6).

In summary, we have shown that a CS30 expressing strain recognizes the glycosphingolipid sulfatide from both human and porcine small intestine. This indicates that CS30 expressing strains could bind to intestines of different hosts and potentially cause disease in multiple hosts. The results and subsequent analysis will indicate if there could be a need for an ETEC vaccine which protects against infections from both human and animal associated ETEC strains.

PRE053  Purification and characterization of native and vaccine candidate mutant enterotoxigenic Escherichia coli heat-stable toxins

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Enterotoxigenic Escherichia coli (ETEC) which secretes the heat-stable toxin (ST) is among the four most important enteropathogens that cause moderate to severe diarrhea in children in impoverished countries. ST is a causative
agent of diarrhea and hence an attractive vaccine target. A non-toxic and safe ST vaccine should include one or more detoxifying mutations, and rigorous characterization of such mutants requires structurally intact peptides. To this end, we have established a system for purification of ST and ST mutants by fusing the sequence encoding the mature ST peptide to the disulfide isomerase DsbC. A Tobacco Etch Virus protease cleavage site facilitates the proteolytic release of free ST with no additional residues. The purified ST peptides have the expected molecular masses, the correct number of disulfide bridges, and have biological activities and antigenic properties comparable to ST isolated from ETEC. We also show that free DsbC can assist in refolding denatured and misfolded ST in vitro. Finally, we demonstrate that the purification system can be used to produce ST mutants with an intact neutralizing epitope, that two single mutations reduces toxicity more than 100-fold, and that a double mutant has no measurable residual toxicity.

PRE061  Immune assays to evaluate ShigETEC, a live, attenuated combination vaccine against shigellosis and ETEC diarrhea

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Shigella and Enterotoxigenic Escherichia coli (ETEC) remain the two leading bacterial causes of diarrheal disease in both endemic countries and travelers visiting these destinations. The protective immunity against these pathogens is not fully understood. The immunodominant antigen of Shigella is the LPS O-antigen, and most Shigella vaccine approaches rely on O-antigen immunity. Protection from ETEC is thought to be mainly mediated by neutralizing antibodies against the diarrhogenic toxins, LT and ST, but contribution from other antibodies binding to surface antigens is also suggested (e.g. colonization factors). We have developed a prototype live attenuated Shigella vaccine, ShigETEC that lacks O-antigen expression, rendered non-invasive by deleting the ipaB and ipaC genes and expresses the LT-B and detoxified ST (N12S) as a fusion protein. As a preparation for future clinical testing, immune assays for the characterization of vaccine response are being developed.

ShigETEC is protective in the murine lung shigellosis model in a species- and serotype-independent manner. This suggest a LPS O-antigen independent protective mechanism. Upon nasal or oral vaccination, the vaccine induces anti-Shigella and anti-ETEC IgG and IgA responses in mice measured by whole cell lysates in ELISA. Importantly, the vaccine-induced antibodies bind to wild-type Shigella based on surface staining of live cell detected by flow cytometry. The protein antigens of the two pathogens detected by immunoblotting with immune sera have identical migration pattern. Previous work identified these immunoreactive proteins as highly conserved surface proteins (by mass spectrometry). LT- and ST-specific IgG and IgA are also detected in body fluids, and were functional based on in vitro toxin neutralization assays. To translate these immune assays to the human setting, we have initiated the analysis of > 400 serum samples collected from adults in disease endemic country (Bangladesh) and investigated whether the vaccine and its components were detected by naturally induced antibodies. These comparative studies with sera from vaccinated animals and Bangladeshi adults revealed interesting similarities and differences in the antibody repertoires. We are using these data to inform us about the most appropriate immune assays for clinical evaluation of ShigETEC.

PRE070  Evaluation of inactivated derivatives of the bi-valent Shigella-ETEC vaccine candidate CVD 1208S-122

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Shigella and ETEC are two of the most important pathogens causing diarrhea in children under five years of age in endemic regions. A combined vaccine that targets these two bacteria could provide substantial benefit to populations in need of interventions. We previously reported the development of a live attenuated derivative of S. flexneri 2a that contains deletions in the guaBA operon as well as set and sen genes, CVD 1208S. This strain was demonstrated to be safe and immunogenic in clinical trials. We utilized CVD 1208S to build a Shigella-ETEC multivalent vaccine by engineering the ETEC CFA/I-encoding operon as well as the heat labile toxin (LT) A2 and B subunits into the chromosome to form CVD 1208S-122. This live attenuated Shigella-ETEC candidate was safe and immunogenic
in the guinea pig model where it protected against wild type challenge. While CVD 1208S-122 advances through cGMP production on a path towards clinical trials we pursued a parallel strategy to render this strain inactivated. Inactivated bacterial vaccines are attractive for several reasons including a potential safety advantage in certain populations, manufacture, and vaccination scheduling. Inactivation procedures included the use of formalin, heat or β-propiolactone (BPL). Each of these treatments has been successfully utilized to produce other inactivated vaccines. Optimal conditions resulting in complete killing of the organism were identified and the expression of critical antigens including *Shigella* LPS, CFA/I fimbriae and LT expression were confirmed. The inactivated vaccines were evaluated for immunogenicity and protective efficacy against wild type *Shigella* using the guinea pig model. All inactivated derivatives induced robust serum and mucosal anti-*S. flexneri* LPS serum IgG and mucosal IgA responses. Formalin, and heat inactivated vaccines conferred 100% protection against *Shigella* challenge, whereas, the BPL-inactivated vaccine conferred only partial protection. All vaccines induced strong serum and mucosal antibody responses against the ETEC antigens albeit and levels that were generally lower than responses elicited by the live attenuated vaccine. The advantages of each inactivation strategy are discussed.

**PRE071 Characterization of the Invaplex vaccine using dynamic light scattering**

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The *Shigella* invasion complex (InvaplexNAT) is an anion-exchanged purified extract from virulent *Shigella* that has been developed as a vaccine to prevent bacillary dysentery. InvaplexNAT contains LPS and a collection of proteins including invasion plasmid antigens (Ipas) which are effectors of the type III secretion system responsible for bacterial invasion of host cells. Further purification of the InvaplexNAT vaccine elucidated the stoichiometry of key antigenic components that induced a protective immune response after vaccination in animal models. A second generation Invaplex product has been developed by assembling the complex using LPS extracted from *Shigella* in combination with recombinant forms of IpaB and IpaC (InvaplexAR). The complexing involved in the assembly process relies on the biophysical properties of the reactants potentially allowing variability in overall molecular size that are thermodynamically controlled.

The size of the Invaplex complexes is an important attribute since this parameter can dictate uptake by antigen-presenting cells (APCss) responsible for generation of an immune response. For example, dendritic cells preferentially uptake small particles through macropinocytosis which can lead to stronger T-cell responses, while larger particles may rely on receptor-mediated endocytosis and drive a humoral response. Although the optimal size required to induce a protective immune response against shigellosis is largely unknown, customizing the Invaplex complex size may be an additional parameter than can be used to generate more robust immunity.

InvaplexAR has increased concentrations of IpaB, IpaC and LPS contained within the complex, as compared to InvaplexNAT. The impact of increased concentration on the complex’s overall molecular structure was investigated using dynamic light scattering (DLS). Using DLS, the hydrodynamic radius (Rh) of InvaplexAR was determined to be approximately 2 times larger than the Rh of InvaplexNAT. Transmission electron microscopy of both the InvaplexNAT and InvaplexAR is consistent with the sizes obtained by DLS for these vaccines.

The InvaplexAR size did not vary over a wide range of concentration (27 µg/ml to 2700 µg/ml) indicating that overall complex size was not concentration dependent. Data from these experiments indicate that the dominant Rh of InvaplexAR is 12 nm and is a stable thermodynamic product. Examining the derived count rate of from the experiments revealed a linear relationship between count rate and the concentration of InvaplexAR.

Monitoring the Rh of Invaplex over time and under different conditions confirmed the homogeneity of InvaplexAR solution, which remained constant over time suggesting a stable, uniform distribution of the product in solution. The aggregation point, the temperature at which the individual complexes lose integrity and combine into larger units, of the product was monitored by slowly increasing the temperature of the sample. The complex was found to be stable, with no signs of aggregation up to a temperature of 80 °C. Thus, the structure of the InvaplexAR product in solution, while not directly controlled during complex formation, appears to remain constant over time, temperature, and total
concentration. Future work will be conducted to determine methods to influence the size of InvaplexAR allowing further customization of a *Shigella* vaccine to induce robust immunity.

**PRE084** An integrated mathematical and immunological approach to optimizing the functional immune responses in an adjuvanted subunit vaccine for ETEC

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Subunit vaccines provide a safe and flexible platform for targeting multiple pathogens and/or serotypes of the same pathogen. However, recombinant subunit antigens are often insufficiently immunogenic and may not program a protective immune response. We have developed a number of combination vaccine adjuvants that pair synthetic TLR4 agonists with formulations including nano-emulsions, liposomes, and alum. Each of these combination adjuvants has different effects on the quality and magnitude of the immune response to vaccine antigens.

To develop a prototype adjuvanted subunit vaccine against Enterotoxigenic *E. coli* (ETEC) we undertook a mathematical approach to explore the vaccine image space of the antigen and adjuvant dose, as well as different formulation matrices. We applied Design of Experiment (DoE) methodologies to select representative immunization groups that would enable calculation of predicted response equations for each immune parameter as functions of the vaccine component doses and identity. We then developed a desirability function that imputed the ideal vaccine image to produce optimal titers of functional antibodies from the intestinal mucosa and serum. We found that antigen and adjuvant dose and formulation choice were important for optimizing vaccine immunogenicity. These results highlight the potential benefit of adjuvant and formulation choices for a subunit vaccine against ETEC and the benefits of the DoE approach for modern vaccine development.

**PRE091** Heat-killed multi-serotype *Shigella* immunogens induce cell mediated adaptive immunity and protective efficacy in animal model

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Recently we have shown the humoral immunity of heat killed multi serotype *Shigella* (HKMS) immunogen and average 100% homologous and 80% heterologous protection efficacy in different animal models. In our present work, we investigated humoral immune response compared to cell mediated adaptive immune responses by intraperitoneal challenge with recent circulating different subtypes of *Shigellae* in mice model. After three doses of oral HKMS immunization, we demonstrated the ability of the HKMS immunogen to prevent bacteria-induced lethality and systemic inflammatory response. Peritoneal macrophages, bone marrow derived dendritic cell (BMDC) and CD4⁺ T-cells were isolated from immunized and non-immunized mice on different time interval after immunization. Production of nitric oxide(NO) from peritoneal macrophages of control and immunized mice and IL-12p70, IL-1β, IL-6 and IL-23 from BMDC of immunized mice upon stimulation with HKMS immunogens confirmed that HKMS is able to induce innate immune responses. Furthermore, incubation with HKMS immunogens with HKMS-primed splenic CD4⁺ T cells enhances the production of IFN-γ, IL-10 and IL-17 represented HKMS immunogens, which indicates HKMS immunization, induce Th1 and Th17 cell mediated immune responses. Our findings reveal, HKMS immunization effectively protects against bacteria-induced lethality and systemic inflammatory response primarily via Th1 and Th17 cell responses and promotes long term
protection against shigellosis. This study therefore provides a new perspective on the immunological detail regarding HKMS immunization. HKMS immunogen could be an effective non-living vaccine candidate in future.

**PRE096 Expression, characterization, and immunogenicity of CS21 subunit-based enterotoxigenic E. coli vaccine candidates**

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**Introduction:** The estimated total mortality attributed to Enterotoxigenic E. coli (ETEC) in 2015 was 74,000 deaths with about one-third happening to children under five years-old. Moreover, ETEC is a leading cause of diarrhea for travelers as well as the US military in endemic areas. The U.S. Navy is working on the development of ETEC vaccines by generating subunit vaccine candidates based on the adhesin domains of the most prevalent colonization factors (CFs). Among CFs, CS21 (a.k.a longus) is highly represented in ETEC isolates obtained from infected travelers, hence an interesting target to be part of a multivalent ETEC vaccine. The CS21 structure consists of two protein subunits, LngA, a repeating major structural unit, and LngB, a minor component thought to be located at the tip of the CF.

**Methodology:** Recombinant expression plasmids were engineered to express stable truncates of LngA (derived from B7A or B2C ETEC strains), LngB (from B2C), and of LngA fusions, containing both the B7A and B2C alleles. The proteins were characterized for purity and identity, and tested for immunogenicity by intradermal (ID) immunization using BALB/c mice. Animals were immunized intradermally with dmLT as adjuvant twice, three weeks apart, and serological responses determined two weeks after the final immunization by antigen-specific IgG ELISA with CS21(B7A or B2C), LngA(B7A or B2C) and LngBB2C proteins.

**Results:** All proteins showed the expected reactivity by western blot with anti-LngA and/or anti-CS21 antibodies (Abs). Purity, assessed by densitometry of SDS-PAGE gels, showed that the recombinant LngA and LngB proteins (LngAB7A, LngAB2C, LngBB2C) were all >95% pure, while the two versions of LngA fusions [LngAB2C-LngAB7A (herein called fusions 1 and 2; F1, F2)] were 92% and 97% pure, respectively. Monomers of LngA, from either ETEC strain (B7A or B2C), failed to promote serum anti-CS21 or anti-LngA IgG responses, although when given combined (LngAB7A + LngAB2C), a modest response was observed. Both LngA fusions, F1 and F2, elicited high levels of anti-CS21(B7A/B2C) and anti-LngA(B7A/B2C) IgG Abs, comparable the levels promoted by immunization with CS21 proteins from B7A or B2C ETEC strains. Interestingly, anti-CS21 and anti-LngA IgG responses were significantly higher when CS21 or LngA proteins from the B7A ETEC strain were used for the assays. Immunization with the LngBB2C monomer led to high anti-LngBB2C IgG Ab titers, while LngA monomers or fusions, as well as CS21(B7A or B2C) proteins did not elicit any anti-LngBB2C response.

**Conclusions:** LngA monomers from either strain were not immunogenic under the conditions tested, but became immunogenic when expressed as bi-valent fusion proteins that incorporated sequences from the two CS21 strains, i.e. B7A and B2C. The skewed higher responses against CS21B7A observed after the immunization with CS21 proteins or fusions suggest that only the B7A sequence might be sufficient for a vaccine candidate. Meanwhile, LngB was immunogenic by itself. Further studies will focus on the evaluation of functional neutralizing responses promoted by LngA and LngB fusion proteins.
**PRE098  Identification and characterization of human monoclonal antibodies for oral immunoprophylaxis against enterotoxigenic Escherichia coli infection**

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**Background:** Enterotoxigenic Escherichia coli (ETEC) cause significant diarrheal illness in infants in developing world and travelers to endemic countries including military personnel. Infection of the host involves colonization of the small intestinal epithelium and toxin secretion leading to watery diarrhea. There is currently no vaccine licensed to prevent ETEC. A number of colonizing factor antigens (CFAs) are expressed on the surface of ETEC isolates, with CFA/I being the most common and a prototype for studying ETEC adhesion process. The CFA/I adhesion subunit, CfaE, is required for ETEC binding to human intestinal cells. Human monoclonal antibodies against the binding domain of CfaE have potential to block ETEC colonization and serve as a potent immunoprophylactic therapeutic for ETEC-related diarrhea.

**Methods:** Mice transgenic for human immunoglobulin genes were immunized with the N-terminal binding domain of CfaE to generate a panel of human monoclonal IgG1 antibodies (HuMabs). The seven most potent IgG1 identified in the in vitro functional assays were selected and isotype switched to secretory IgA (sIgA), and both IgG1 and sIgA were tested in ETEC colonization assays in animals via oral administration.

**Results:** Over 300 unique anti-CfaE IgG1 HuMabs were selected and characterized. The lead seven IgG1 anti-CfaE HuMabs completely inhibited hemagglutination at concentrations of less than 0.3 ug/ml and blocked adhesion of ETEC to CaCo2 cells at an IC50 ranging from 0.3 to 1.3 ug/ml. Epitope mapping studies revealed that HuMabs recognized epitopes in the N-terminal domain of CfaE near the putative receptor binding site. Oral administration of anti-CfaE antibodies in either IgG or sIgA isotype inhibited intestinal colonization in mice challenged with ETEC strain H10407. Two to four log decrease of colony forming units was observed as compared to irrelevant isotype controls. Studies are underway to further assess the efficacy of HuMabs in non-human primate diarrhea model challenged with ETEC strains.

**Conclusions:** We have identified a panel of fully human monoclonal antibodies against CfaE adhesion domain that can be potentially employed as an oral immunoprophylaxis to prevent ETEC infection.

**PRE100  Microbiological surveillance of Shigella spp. and the enterotoxigenic Escherichia coli pathotype in Cuba**

Adalberto Aguila

We have in Cuba a National Program of Integrated Surveillance of enteropathogens that produce diarrhea, which annually taxes between 200 isolates of Shigella spp. and 300 of enterotoxigenic Escherichia coli (ETEC). The use of conventional diagnosis, phenotypic markers in clinical microbiology laboratories and confirmation by PCR in the National Reference Laboratory are the key to our work.

The ETEC pathotype ranks second among isolates in children under 5 years of age. In them predominate the producers of thermostable enterotoxin (ST), then the thermostable (LT) and finally isolates producers of both. There is currently a significant increase in resistance and antimicrobial multi-resistance patterns in diarrheagenic E. coli.

The serogroups of Shigella sonnei phase 1 and S. flexneri 2 are those that are isolated most frequently in the whole country, then S. boydii and S. dysenteriae appear, recovering from outbreaks of diarrhea and also from sporadic cases. It is considered that both Enterobacteria due to the incidence and antimicrobial resistance are tributaries of prevention and control policies, including immunization by vaccines.
Strategies for broader coverage of combination/co-administered vaccines

ORAL PRESENTATIONS

CMB083  Developing WHO preferred product characteristics for ETEC and Shigella vaccines

Birgitte Giersing, WHO ETEC and Shigella PPC Working Group

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According to current burden of disease estimates, Enterotoxigenic Escherichia coli (ETEC) and Shigella cause significant diarrheal-associated mortality and long-term, debilitating morbidity, particularly in infants in low- and middle-income countries (LMICs). In addition, both have developed high levels of antibiotic resistance. For these reasons, IVR’s Product Development for Vaccines Advisory Committee (PDVAC) has identified these pathogens to be a priority from a global public health perspective, and recommended the development of Preferred Product Characteristics (PPCs) for ETEC and Shigella vaccines.

Published by WHO, PPCs are intended to encourage innovation and development of vaccines for use in settings most relevant to the global unmet public health need. They describe parameters pertaining to vaccine indications, target populations, data required for safety and efficacy evaluation, research and development and immunization strategies, and are intended to provide early guidance to inform product specific target product profiles (TPPs). The primary target audience for WHO PPCs is any entity intending to eventually seek WHO policy recommendation and prequalification for their products.

In October 2017, WHO convened a global stakeholder consultation to discuss PPCs for ETEC and Shigella vaccines and the key considerations that would render these vaccines appropriate for licensure, recommendation and uptake in LMICs. The outcomes from this consultation will be presented.

POSTER PRESENTATIONS

CMB020  ETEC adhesin-toxoid MEFA CFA/I/II/IV-3xSTaN12S-mnLTG192G/L211A induces antibodies protecting against ETEC diarrhea

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Enterotoxigenic Escherichia coli (ETEC) are a leading cause of children’s diarrhea and travelers’ diarrhea. Vaccines inducing antibodies to broadly inhibit bacterial adherence and to neutralize toxin enterotoxicity are expected effective against ETEC-associated diarrhea. 6xHis-tagged adhesin-toxoid fusion proteins were shown to induce neutralizing antibodies to several adhesins and LT and STa toxins (X. Ruan, DA Sack, W. Zhang, PLoS ONE, 10:e0121623, 2015). However, antibodies derived from his-tagged CFA/I/II/IV-2xSTaA14Q-dmLT or CFA/I/II/IV-2xSTaN12S-dmLT protein were less effective in neutralizing STa enterotoxicity and were not evaluated in vivo for efficacy against ETEC diarrhea. Additionally, his-tagged proteins are considered less desirable for human vaccines. In this study, we produced a tag-less adhesin-toxoid MEFA (multiepitope fusion antigen) protein, enhanced anti-STa immunogenicity by including a third copy of STa toxoid STaN12S, and examined antigen immunogenicity in a murine model. Moreover, we immunized pregnant pigs with the tag-less adhesin-toxoid MEFA protein and evaluated passive antibody protection against STa+ or
LT+ ETEC infection in a pig challenge model. Results showed that tag-less adhesin-toxoid MEFA CFA/I/II/IV-3xSTaN12S-mnLTR192G/L211A induced broad anti-adhesin and antitoxin antibody responses in the intraperitoneally immunized mice and the intramuscularly immunized pigs. Mouse and pig serum antibodies significantly inhibited adherence of seven CFA adhesins (CFA/I, CS1 to CS6) and effectively neutralized both toxins. More importantly, suckling piglets born to the immunized mothers acquired antibodies and were protected against STa+ ETEC and LT+ ETEC diarrhea. These results indicated tag-less CFA/I/II/IV-3xSTaN12S-mnLTR192G/L211A induced broadly protective anti-adhesin and antitoxin antibodies and suggested this adhesin-toxoid MEFA a potential antigen for developing broadly protective ETEC vaccines.
Vaccine candidates in clinical trials and human challenge models

ORAL PRESENTATIONS

CL033  Functional antibodies and cytokine responses to live oral *Shigella sonnei* vaccine strain WRSS1 in Bangladeshi adults and children

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**Background:** WRSS1, a live attenuated *Shigella sonnei* vaccine strain, was given orally to healthy Bangladeshi adults and children in a placebo-controlled age descending study at icddr,b, Dhaka, Bangladesh. In this report functional antibodies and cytokine responses to WRSS1 in adults and children are evaluated.

**Methods:** In the first phase, 39 adults received either one dose of 3x10⁴ (cohort A1) or three doses of 3x10⁵ (A2) and 3x10⁶ (A3) CFU of WRSS1. In the second phase, 64 children, 5-9 years of age, received either one dose of 3x10³ (B1) or three doses of 3x10⁴ (B2), 3x10⁵ (B3) or 3x10⁶ (B4) CFU of WRSS1. The first, second and third vaccination of each dose level was given on days 0, 28 and 56, while follow-up visits were conducted on day 7, 28, 35, 63 and 84. Blood and stool samples were collected before and at various intervals after vaccination. Functional antibody response was assessed by serum bactericidal antibody (SBA) assay and concentration of cytokines were measured in lymphocyte supernatant (ALS) and stool samples by multiplex assay.

**Results:** Among A2 and A3 adult cohorts there was no increase in SBA activity against wild type *Shigella sonnei* after any doses of WRSS1 vaccine. In children, both B3 (p=0.02) and B4 cohorts (p=0.007) showed significantly higher SBA response compared to placebo after the 3rd dose of WRSS1. When cytokine responses were assessed in ALS samples, concentrations of IL-7 in both A2 and A3 cohorts and IL-5 and IL-10 in A3 increased after vaccination. In children, significant increases in cytokine concentrations were obtained after the 3rd dose in B2 (IL-1β, MCP-1 and G-CSF), and B4 cohorts (TNF-α, IL-17, G-CSF and MIP-1b) and after the 1st and 2nd doses in B3 (TNF-α and MIP-1b). After 2-3 doses of WRSS1, increased stool concentrations of MIP-1b in A3 and IL-8, IL-10 and TNF-α in B4 cohort were found.

When cytokine responses were compared between responders (4-fold increase in antigen-specific antibody titers) and non-responders, B4 cohort showed markedly higher concentrations of TNF-α and IL-12p70 in ALS and IL-1β, IL-8 and GM-CSF in stool among responders after multiple doses. None of the other cohorts showed such differences between responders and non-responders.

**Conclusion:** The lack of increase in functional antibody responses in adults to WRSS1 as opposed to children could be that adults have reached the threshold level of functional antibodies while growing children still require boosting to improve functional antibodies. Adults and children exhibited differential cytokine and chemokine responses to the WRSS1 vaccine in both ALS and stool.

CL035  Immune response characterization after controlled infection with a lyophilized *Shigella sonnei* 53G, (cGMP Lot 1794)

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**Background:** *Shigella* is a major cause of bacillary dysentery causing moderate to severe diarrhea in travelers and
children in low- and middle-income countries. Although several vaccine candidates are under investigation, there is currently no licensed vaccine to prevent shigellosis. An important step in vaccine development is the efficacy assessment in a controlled human infection model (CHIM). A lyophilized strain of S. sonnei 53G was produced using current good manufacturing processes (cGMP) and evaluated in a Phase 1 study to determine a dose that safely and reproducibly yielded in a 60-75% attack rate (VED, 2017).

Appreciating that prior Shigella infection can result in the generation of an immune response capable of protecting against re-infection in a serotype-specific manner, characterization of the immune response post-infection in the S. sonnei CHIM was undertaken. Systemic and mucosal immune responses were examined in the context of challenge dose delivered, diarrhea severity as well as shigellosis disease severity score.

**Methods:** Healthy, adults were enrolled in an open label, dose finding study that followed an adaptive design with five cohorts being dosed with either 500, 1000 or 1500 colony forming units (cfu). Samples were collected throughout both the inpatient and outpatient study phases for immunological analyses. Blood was collected to determine IgG and IgA titers directed to LPS, Invaplex, IpaB and IpaC. Serum was also utilized to evaluate functional antibody responses (bactericidal titers) specific for S. sonnei and S. flexneri 2a. Fecal and saliva samples were collected to examine mucosal responses induced post challenge. Peripheral blood mononuclear cells (PBMCs) were also separated into α4β7 positive and negative populations and cultured in vitro to collect antibody lymphocyte supernatant (ALS) for analysis by ELISA to determine LPS and Invaplex-specific IgG and IgA titers from cells homing to the intestine.

**Results:** Oral inoculation with S. sonnei 53G induced Shigella-specific serum IgG and IgA antibodies with peak responses observed on day 14 post-challenge. In addition to serum IgG and IgA, challenge induced robust S. sonnei-specific bactericidal activity with peak responses also on day 14. Interestingly, there were no significant differences either in serum responses or bactericidal activity between the S dose cohorts or based on disease post infection. In addition to systemic responses, antigen-specific fecal IgA responses, normalized to total IgA, demonstrated increases in anti-Invaplex and LPS IgA specific activity post-challenge. Additional immunological parameters to be presented will include fecal IgG titers, α4β7 positive and negative ALS ELISA results and quantification of the fecal inflammation markers calprotectin and myeloperoxidase.

**Conclusions:** The establishment of a lyophilized strain of Shigella sonnei allows for standardization and therefore comparisons across CHIM studies performed at different institutions. Preliminary results show robust functional and mucosal immune responses. It is expected that subjects will have an increase in their α4β7 positive LPS-specific IgA ALS titers indicating that infection with S. sonnei 53G induces a strong mucosal gut-homing response. Comprehensive immune response evaluation post-infection may lead to the establishment of immune profiles after infection that can guide development of an efficacious vaccine.
Immune response profiles following vaccination with a *Shigella* bioconjugate vaccine that correlate with a reduction in *shigellosis* severity

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**Background:** Vaccine development against *shigellosis* continues to be a priority for the World Health Organization, numerous pharmaceutical companies, non-governmental organizations as well as the US Department of Defense. *shigellosis* disease severity score.

LimmaTech Biologics has developed Flexyn2a, a bioconjugate vaccine that conjugates the O antigen of *Shigella flexneri* 2a (Sf2a) to the exotoxin A of *Pseudomonas aeruginosa* (EPA) using a unique, reproducible and greatly simplified production process. The bioconjugate has been shown, in the controlled human infection model, to protect individuals from the most severe illness after challenge with *S. flexneri* 2a strain 2457T (Talaat et al, VED 2017, manuscript in preparation). Immune responses following vaccination were now assessed for their association with an ordinal *shigellosis* disease severity score.

**Methods:** Humoral, mucosal and cellular responses induced by vaccination included *S. flexneri* 2a LPS specific serum IgG (including IgG subtypes), IgA, IgM, fecal IgA, antibodies in lymphocyte supernatants (ALS) and serum bactericidal responses. A *shigellosis* disease severity score was calculated for each subject as described by Porter et al (Porter et al, VED 2017). Correlations between immune response at pre-challenge time points and *shigellosis* disease score were assessed using Spearman correlations. Additionally, ordinal logistic regression models were developed using the *Shigella* disease score as the dependent variable and each immune parameter as the independent variable. Multivariate models were developed to determine the immune parameters most strongly associated with a reduction in the *Shigella* disease score.

**Results:** *Shigella* disease scores ranged from 0 to 8 across placebo and vaccine recipients. Among vaccinees, the median disease score was 1.5 (Quartile 1, 3: 0.0, 4.3) significantly lower (p=0.04) than for placebo recipients (median: 4.0; Q1, Q3: 0.5, 6.0). Among vaccinees, a lower *Shigella* disease score was significantly correlated with higher serum IgG (spearman rho: -0.55; p=0.002) and IgA (spearman rho: -0.36; p=0.047) pre-challenge titers (on the day of challenge). Among IgG subclasses, lower disease score in vaccine recipients was correlated with higher IgG1 (spearman rho: -0.47; p=0.009) and higher IgG2 (spearman rho: -0.43; p=0.02) pre-challenge titers. Higher Fecal IgA responses 4 weeks prior to challenge, but not on the day of challenge were similarly correlated with a lower disease score (spearman rho: -0.40; p=0.03). IgG ALS responses from α4β7 positive (spearman rho: -0.50; p=0.01) and negative (spearman rho: -0.47; p=0.02) cells also demonstrated significant correlation with a lower disease score. Neither IgG3, IgG4, IgM, nor serum bactericidal responses correlated with the *Shigella* disease score in vaccinees. There were no significant correlations with pre-challenge immune titers and *Shigella* disease score among placebo recipients. Additional multivariate data will be presented to demonstrate how the entire immune response profile varied across disease score and study group.

**Conclusions:** Subjects vaccinated with Flexyn2a had a significantly lower *Shigella* disease score than placebo recipients. Lower disease score among vaccinees was significantly correlated with serum IgG, and in particular IgG1 and IgG2 as well as serum and fecal IgA and ALS IgG. Future studies in more subjects will further enable the identification of immune surrogates (or correlates) of protection with this bioconjugate *Shigella* vaccine.
The global diarrheal disease burden remains high with approximately four billion cases estimated to occur annually in all age groups, with the highest incidence among infants and young children under five years of age. In this age group, enteric infections result in nearly 525,000 deaths each year, comprising approximately 9 percent of global child deaths and extracting an enormous physical and economic toll in low-resource countries. In developing countries like Bangladesh, enterotoxigenic Escherichia coli (ETEC) is a leading cause of pediatric and adult diarrhea accounting for 11–20% of all diarrhea cases (children <3 years of age are at the highest risk for ETEC diarrhea, with annual incidence of 0.5 episodes/child/year). Because of the importance of this disease, development of a safe and effective ETEC vaccine is a major goal and a high priority for the WHO. Against this background, we initiated a Phase I/II randomized double-blind, placebo-controlled, dose-escalation trial (VAC 014/OEV-122) of the second generation oral ETEC vaccine, ETVAX. ETVAX consists of inactivated E. coli bacteria overexpressing the most prevalent ETEC colonization factors (CFs) in combination with an LT toxoid (LCTBA) and dmLT, a new mucosal adjuvant based on two key amino acid substitutions in the LT enterotoxin which reduces it enterotoxicity but allows adjuvant activity to be retained. The trial purpose was to assess the safety and tolerability of ETVAX with and without dmLT, as well as its ability to induce mucosal and systemic immune responses against protective vaccine antigens when tested in the following age groups of Bangladeshi adults (18-45 years; n=45), and children (24-59 months; n=130; 12-23 months, n=100; 6-11 months, n=200). This study also provided a unique opportunity to test the safety profile of dmLT and assess its ability to enhance the mucosal and systemic immunogenicity of the ETVAX vaccine in developing country infants that have historically proven refractory to oral immunization with enteric vaccines. The study also allowed for assessment of the potential dose-sparing effect of dmLT. Finally, this clinical trial is a prerequisite for an ETVAX Phase 2b/3 efficacy trials in an ETEC endemic area. Enrollment and vaccination of all cohorts took place between October 2015-January 2017. As anticipated based on experience with the first-generation vaccine, moderate vomiting was observed in children 24-59 months using the full dose of ETVAX. Subsequent planned fractional dosing (1/8, ¼ and ½ dose) resulted in an acceptable tolerability profile in the youngest age groups. Antibody responses in plasma were determined against the colonization factors (CFA/I, CS3, CS5, CS6) and toxoid antigen present in the vaccine. In adults, we observed significantly increased ETEC antigen responses after vaccination (P<0.001) compared to in the placebo group. Plasma IgA responses against CFs and LTB were observed in 67%-93% and 100% of subjects, respectively. Addition of dmLT had no apparent effect on plasma antibody responses in adults. More in-depth analyses are ongoing in younger age-groups and will be summarized in the subsequent presentation.
Mucosal immune responses to an oral inactivated ETEC vaccine (ETVAX) among descending age groups in Bangladesh

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We have evaluated the capacity of the oral inactivated multivalent Escherichia coli (ETEC) vaccine (ETVAX) to induce mucosal immune responses in a large Phase I/II trial in Bangladesh (495 subjects). The following age groups received two vaccine doses (in different dosages) alone or with different amounts of dMLT adjuvant or placebo (buffer only) two weeks apart: adults 18-45 years, and children 24-59 months, 12-23 months, and 6-11 months. Blood and feces were collected from all subjects before the first dose and then 7, 19 days and 28 days (feces only) after vaccination.

Mucosal immune responses against all key vaccine antigens (i.e. CFA/I, CS3, CS5, CS6 and LTB) were studied in all age groups using the IgA antibody in lymphocyte secretions (ALS) assay and in younger infants also by measuring secretory IgA (SIgA) antibody responses in extracted stool specimens. Since only small volumes of ALS samples could be collected, especially from the younger cohorts, we established an electrochemiluminescence assay for antibody determinations in ALS specimens based on Meso Scale Discovery (MSD) technology. ALS IgA responses analyzed by the MSD assay (a single dilution of 5 ul of specimen) were highly correlated (p < 0.0001 for all antigens) when responses were compared to conventional ELISA titrations using larger specimen volumes.

ALS responses in the different age groups revealed that 100% of all vaccinated adults responded to all five key vaccine antigens, whereas only a few placebo recipients responded to a single antigen. Responses in the Bangladeshi adults were higher and more frequent than previously recorded in adult Swedes similarly vaccinated with ETVAX. Encouragingly, ALS responses in Bangladeshi children (2-5 years old) were similar in frequency and magnitude to those seen in Bangladeshi adults. In addition, the majority of children in the 1-2-year-old age group mounted strong responses to the vaccine. ALS responses against the CFs were less frequent and of lower magnitudes in infants 6-11 months than in older children and adults whereas anti-LTB responses were more comparable across the different age groups. For the composite endpoint of responses by ALS IgA and/or SIgA in stool extracts, the frequencies of mucosal anti-CF immune responses increased in 6-11-month-old infants. However, at variance with responses in the older pediatric and adults age groups, a higher percentage of placebo recipients in the infant cohort also had increased immune responses to key antigens, which may suggest a high incidence of asymptomatic infections in infants. No significant differences in response rates were noted among children given different doses of vaccine (1/8; ¼; ½ versus full adult dose) or vaccine +/- dMLT adjuvant. The studies indicate a clear age dependency of mucosal immune responses against ETVAX in this endemic setting with strong mucosal responses in children ≥1 year of age, and that reduced doses of vaccine may be used for vaccination of children.

Safety and immunogenicity study of SF2a-TT15, a synthetic carbohydrate-based conjugate vaccine against S. flexneri 2a in healthy adult volunteers

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No significant differences in response rates were noted among children given different doses of vaccine (1/8; ¼; ½ versus full adult dose) or vaccine +/- dMLT adjuvant. The studies indicate a clear age dependency of mucosal immune responses against ETVAX in this endemic setting with strong mucosal responses in children ≥1 year of age, and that reduced doses of vaccine may be used for vaccination of children.
**Background:** Glycoconjugates incorporating detoxified LPS from *Shigella flexneri* 2a or *Shigella sonnei* have been shown to be safe and immunogenic in healthy volunteers. Phase III trials demonstrated the protective capacity of a *S. sonnei* detoxified LPS-conjugate against *S. sonnei* infection in young adults and children older than 3 years of age. In the search for a highly immunogenic *S. flexneri* 2a vaccine able to generate protective immunity in children below 3 years of age, we have rationally designed SF2a-TT15, a tetanus toxoid (TT) conjugate encompassing a synthetic pentadecasaccharide hapten corresponding to three repeating units of the O-antigen from *S. flexneri* 2a. In preclinical studies, SF2a-TT15 has been shown to induce anti-LPS bactericidal antibodies.

**Objective:** We report the preliminary results of a phase I clinical trial of safety and immunogenicity of the SF2a-TT15 conjugate vaccine candidate.

**Methods:** We conducted a first-in-human, single-blinded, observer-masked randomized, dose escalation (2 different doses of the sugar component), placebo-controlled study in healthy Israeli volunteers. Volunteers were selected from those who were pre-screened for *S. flexneri* 2a LPS IgG serum antibody levels below 80 percentile and for HLA-B27 negativity. Sixty-four eligible subjects were assigned to one of two cohorts and randomized to receive the lower dose of 2 μg (with or without alum adjuvant) or matching placebo (cohort 1) and the higher dose of 10 μg (with or without alum) or matching placebo (cohort 2). The study agents were administered by 3 intramuscular injections on days 0, 28 and 56. There were 9 follow-up visits with the last visit 3 month after injection 3.

**Results:** At both doses, the SF2a-TT15 conjugate was well tolerated.

**At 2 μg:** The first vaccine injection induced a significant rise in serum IgG anti-*S. flexneri* 2a LPS GMTs of approximate 5-fold as compared with pre-vaccination or placebo recipients' levels. Alum enhanced the response after the second and third injection to 8.5 and 12-fold increases in GMT, respectively.

**At 10 μg:** The first injection of SF2a-TT15 elicited IgG anti-*S. flexneri* 2a LPS rises in GMTs of 25 and 27-fold with or without alum, respectively, as compared with pre-vaccination levels, with no further increase after the second and third dose. All the vaccinees were responders after the first dose, with 4-fold or greater rise in titer. A parallel significant response was also found for IgA and IgG ASC and ALS to *S. flexneri* 2a LPS, 7 days after the first injection. The vaccine induced an average of 15-fold increase in serum bactericidal antibody GMTs following immunization (range of post-vaccination GMTs: 5918-14669) with a 4-fold or greater rise in serum bactericidal titers in 88% of the volunteers after the first injection. Noteworthy, all vaccinees had a significant increase in the percent of IgG memory B cells specific to *S. flexneri* 2a LPS.

**Conclusions:** The results of the phase I study of the SF2a-TT15 conjugate in naive adult volunteers are very promising and support further evaluation of this vaccine candidate for safety, immunogenicity and protective efficacy in additional settings.
**CL079  Shigella-specific serum bactericidal and opsonophagocytic killing antibodies induced by oral S. flexneri 2a whole cell killed and live attenuated vaccines.**

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Various strategies have been pursued to develop safe and efficacious vaccines to prevent shigellosis, considering children the major target group. One of these approaches is oral delivery of intact organisms, either inactivated (killed) or live attenuated. Several oral vaccine candidates have been tested in humans and evaluated for safety and immunogenicity. Except for a few early candidates for which efficacy data in field settings is available, the protective capacity of newer vaccines remains to be determined. Through previous work in our laboratory, we have demonstrated that elevated levels of functional serum bactericidal (SBA) and opsonophagocytic killing antibodies (OPKA) were associated with clinical protection. Serum bactericidal activity has also been described in adult individuals living in endemic regions who acquire immunity following natural exposure.

We have applied qualified, high throughput SBA and OPKA assays to determine functional, anti-microbial antibody activity produced by oral immunization with the killed whole cell vaccine candidate Sf2aWC. SBA and OPKA titers were measured in serum from adult volunteers who participated in a multi-cohort dose escalation Phase I clinical study conducted at the Johns Hopkins University. Cohort 1 received 108 vaccine particles of on day 0 while Cohorts 2-4 (n=60) received 109, 1010 and 1011 particles, respectively, on days 0, 28 and 56. SBA and OPKA responses were detected in Cohorts 2-4. SBA and OPKA titers peaked 7 days after vaccination, with seroconversion rates being higher than 28 days post-vaccination (a typical time point for serological analysis). Shigella-specific antibodies were also detected in lymphocyte supernatant (ALS) and fecal samples, suggesting a potential mucosal origin for these antibodies. Functional antibody responses were also investigated in individuals who received a single immunization with live attenuated vaccines 1204, 1208 and 1208S in escalating doses (107, 108, and 109 CFU) in multiple clinical studies conducted at the University of Maryland, Baltimore. Rises in SBA and OPKA titers were observed 28 days after vaccination (the earliest time point available). For both the Sf2aWC and the CVD live attenuated vaccine series, the magnitude of responses was strongest in the highest dose cohorts. SBA and OPKA titers induced by oral vaccination were comparable with those of adults living in endemic regions and experimentally challenged with Shigella organisms. These results highlight the importance of evaluating kinetics of vaccine-induced serological responses, and suggest a possible mucosal origin for functional antibodies detected systemically following oral immunization, which we are currently investigating. There is precedent for the measurement of vaccine-induced serum bactericidal and/or opsonophagocytic killing antibodies and their use as immune correlates of protection against other mucosal pathogens (S. pneumoniae, Neisseria meningitides, Haemophilus influenzae and V. cholerae). Shigella SBA and OPKA measurements are useful in the evaluation of vaccine induced responses and might be relevant indicators of protective immunity.

**CL099  Experimental oral challenge with B7A ETEC induces proliferative CD4+ T cell responses to CS6 and LT, which are associated with enhanced systemic and mucosal B cell responses**

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**Introduction:** An important function of T cells responding to infection or vaccination is proliferation of clones bearing receptors specific for the pathogen or vaccine antigens. Detection of Ki67, a nuclear protein expressed during cell division, is a sensitive method to identify cells undergoing proliferation. In a study of experimental oral challenge with ETEC strain
B7A (CS6+LT+ST+, O148:H28; NCT02773446), we assessed the capacity of volunteers’ peripheral CD4+ T cells to express Ki67 following in vitro stimulation with ETEC colonization factor CS6 and heat-labile toxin, LT, demonstrating functional T cell responses to these antigens.

**Methods:** Twenty-nine healthy volunteers inoculated with B7A ETEC (ranging from 108-1010 CFU), for whom peripheral blood mononuclear cells (PBMCs) were available from study days (d) -1 and 7 or 28, were assessed. Seventeen volunteers (58.6%) reached the primary clinical endpoint of moderate-severe diarrhea (MSD; >400 mL or ≥4 episodes of loose/liquid stool in 24h).

Cryopreserved PBMCs were stimulated with overlapping 15mer peptides spanning CS6 subunits CssA and CssB (CS6pep), LTA and LTB (LTpep), or unstimulated. Following 72h culture, PBMCs were stained with LIVE/DEAD®, anti-CD3, -CD4, -CD8, and -α4β7, fixed and permeabilized, and stained intracellularly with anti-Ki67. Ki67 response levels were calculated as net increase in percent of Ki67+ cells above baseline on d7 or d28, with responders defined by a net increase above zero. B cell responses (serology, antibodies in lymphocyte supernatant-ALS, memory B cells-MBC, fecal antibodies) were compared between Ki67 responders and non-responders.

**Results:** At peak, on d7 or d28 following challenge, CD4+α4β7+ Ki67 responses were detected in 85.2% of volunteers to CS6pep and 84.6% to LTpep. While responder rates were similar on d7 and d28 for CS6pep, LTpep responders increased from d7 to d28, exclusively within MSDPos volunteers (41.1% to 76.5%). MSDNeg volunteers had significantly higher response rates and percentages of CD4+Ki67+ cells to LTpep than MSDPos on d7 (90% vs. 41.7% responders, p=0.03, Fisher’s; p=0.02, Wilcoxon signed-rank test).

CS6pep and LTpep CD4+α4β7+Ki67 responders on d7 had higher levels of serum anti-CS6 and –LT IgA, respectively, than non-responders, while only LTpep responders had significantly higher serum anti-LT IgG (p=0.01, Wilcoxon). Only LTpep Ki67 responders generated serum anti-LT IgG, demonstrating the role of CD4 proliferation in the development of IgG responses. ALS IgG and fecal IgA responses for both CS6 and LT tended to be higher for Ki67 responders. In addition, CS6pep Ki67 responders more frequently generated anti-CS6 IgG MBC (p=0.004, Fisher’s).

**Conclusions:** Oral challenge with B7A led to CD4+ T cell proliferation against CS6 and LT, two ETEC virulent factors and vaccine candidates. Higher levels of CD4+ T cell responses against the toxin were observed in MSDNeg volunteers. CD4+α4β7+Ki67 responders had significantly higher serum antigen-specific antibody levels, and tended to have higher ALS, MBC and fecal IgA. These data illustrate the connection between proliferation of gut-homing CD4+ T cells and systemic and mucosal antigen-specific B cell responses, which we and others have demonstrated to be associated with protection against ETEC. Therefore, further understanding of functional CD4+ responses may be fundamental for ETEC vaccine development.
Poster Presentations

CL040  Serotype and antigen specificity of serum bactericidal activity after intranasal immunization with *S. flexneri* 2a artificial Invaplex.

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**Background:** *Shigella* is a major diarrheal pathogen of global importance and despite several vaccine candidates in development, there is no licensed vaccine. One important step in the vaccine evaluation is developing standardized and qualified immunological assays capable of accurately and reproducibly measuring various immunological parameters. A simple and reproducible *Shigella*-specific serum bactericidal assay (SBA) has been recently developed and deployed to evaluate the functionality of antibodies induced after vaccination, natural infection or in CHIM evaluations. One application of the SBA has been to examine the target and specificity of these bactericidal antibodies using serum from subjects immunized with a subunit *Shigella* vaccine (*S. flexneri* 2a, InvaplexAR). Serum samples were used to determine bactericidal activity against homologous and heterologous *Shigella* serotypes. Additionally, serum competed with serotype-specific LPS and the broadly conserved Ipa proteins (IpaB and IpaC) to evaluate the contribution of antibodies with these antigen-specificities to the bactericidal activity.

**Methods:** To evaluate potential cross-reactivity against *Shigella* serotypes, serum was collected from subjects enrolled in the Phase 1 *S. flexneri* 2a artificial Invaplex study, heat inactivated, and SBA activity determined using an optimized SBA protocol specific for *S. flexneri* 2a, *S. flexneri* 3a and *S. sonnei*. A subset of samples with low, moderate and high bactericidal activity against *S. flexneri* 2a were used in the analysis. In separate experiments, the bactericidal antibody specificity was evaluated by pre-incubating serum samples with different *Shigella* antigens including IpaB, IpaC, and purified LPS extracted from *S. flexneri* 2a, *S. flexneri* 3a, and *S. sonnei*. Serum samples competed with purified antigens were then used in the optimized SBA to determine the effect of antigen-specificity on the bactericidal activity.

**Results and Conclusions:** Intranasal immunization with *S. flexneri* 2a InvaplexAR induced functional antibodies capable of killing *S. flexneri* 2a, 2457T (SBA titer range: 165 to 106,776; median 10,411) and *S. flexneri* 3a, J17B (SBA titer range: 1201 to 174,960; median 13,041). Bactericidal activity directed to *S. sonnei*, Moseley were low to undetectable. Pre-incubation of samples with *S. flexneri* 2a LPS significantly decreased (p=0.0034) the anti-*S. flexneri* 2a SBA titers in all samples, whereas pre-incubation with IpaB or IpaC resulted in a nominal decrease in *S. flexneri* 2a SBA titer. Antibody competition with *S. flexneri* 3a LPS also significantly (p=0.0217) reduced the bactericidal activity directed to *S. flexneri* 2a, further demonstrating the generation of cross-reactive antibodies.

Collectively, these data demonstrate that immunization with Invaplex induces functional antibodies directed to homologous and heterologous serotypes within the same *Shigella* species. LPS is the major antigenic target associated with bactericidal responses, but the contribution of Ipa-specific antibodies warrants further investigation. Finally, these investigations highlight the utility and application of the recently developed and optimized SBA and the potential of the assay to significantly contribute to the understanding of vaccine-induced immune responses.
**CL042**  Parenteral immunization with the *Shigella flexneri* 2a bioconjugate vaccine induces LPS-specific memory B cell responses.

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*Shigella* is a major cause of bacillary dysentery causing moderate to severe diarrhea in children under 5 years of age in low and middle-income countries, and in travelers to those areas. Although several vaccine candidates are under investigation, there is currently no licensed vaccine to prevent shigellosis. A novel *Shigella flexneri* 2a (Sfi2a) bioconjugate vaccine, Flexyn2a, carrying the Sfi2a O-antigen bioconjugated to EPA (exotoxin A of *P. aeruginosa*), has been shown to induce robust serum IgG and IgA responses as well as mucosal responses after intramuscular immunization. The Flexyn2a bioconjugate has also been shown to protect volunteers from severe disease after oral challenge with *S. flexneri* 2a 2457T and serum IgGs against Sfi2a-LPS, induced following vaccination, were shown to correlate with protection against shigellosis (K. Talaat et al, VED 2017).

Immunological memory, achieved after vaccination, is an important parameter responsible for protecting individuals from future infections. Memory B cells are one aspect of immunological memory that has been previously correlated with protection after immunization with live-attenuated *Shigella* vaccines. Therefore, memory B cell responses after intramuscular immunization with Flexyn2a in the context of a Phase 2b vaccination/challenge study was investigated to determine if the bioconjugate also induced memory responses potentially capable of protecting an immunized individual. Memory B cell responses induced after vaccination and at several time points post-challenge was assessed to evaluate the induction and duration of immunological memory.

Volunteers enrolled in the Phase 2b study were injected intramuscularly on days 0 and 28 with either 10 µg Flexyn2a or saline and were subsequently challenged one-month post vaccination with approximately 1500 cfu *Shigella flexneri* 2a 2457T. PBMCs collected at baseline, 28 days after the second vaccination, and 7, 28 days and 10-16 months post challenge were analyzed for Sfi2a LPS-specific memory B cell responses. Frozen PBMCs were thawed and stimulated in vitro with a B cell mitogen cocktail (R848 and IL-2) for 4 days, washed and then incubated without mitogen stimulation to obtain antibodies in lymphocyte supernatant (ALS). ALS samples were assayed by ELISA to determine Sfi2a LPS-specific IgG titers. Tetanus toxoid-specific IgG titers served as a positive control for successful memory B cell expansion. Flow cytometry was used to assess CD19, CD3, CD20 and CD27 expression in cell populations pre- and post-expansion to ensure an increase in the memory B cell population (CD19+, CD3−, CD20+, CD27+) post-expansion.

Significant increases in Sfi2a LPS-specific IgG ALS titers were specifically observed post-vaccination with Flexyn2a. The immunological memory response was detected at multiple time points through the 84-day study period and persisted 10-16 months post-challenge. Results from these studies not only demonstrate that intramuscular immunization with the candidate vaccine Flexyn2a is able to induce protective systemic and mucosal immunity, but also indicates the generation of vaccine-induced memory B cells specific for Sfi2a LPS.
**CL057  Immune responses to oral vaccination: Reduced dosages of a late booster dose**

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Though our research group focuses on the development of an oral killed whole-cell ETEC diarrheal vaccine, ETVAX, in the present study, we have used the related cholera vaccine Dukoral as a model to study memory immune responses to oral enteric vaccines.

In this study, we have compared antibody responses against cholera B subunit (CTB) and vibriocidal antibody responses to a late booster with reduced dosages of vaccine, as compared to a booster with a full dose, in subjects previously primed with two doses of Dukoral. To this end, we have immunized 52 adult Swedish volunteers with two full oral doses of vaccine at a 14-day interval, and approximately 4 months later we administered a late booster dose with either full, 1/5th or 1/25th dose of the vaccine. Immune responses were assessed on days 0, 19 and 44 after the first dose and day 0 and 5 after the second dose in all subjects. The immune responses studied included vaccine-specific serum IgA and IgG and vibriocidal antibody responses as well as IgA Antibodies in Lymphocyte Supernatant (ALS) and antibody avidity using KSCN elution.

Our preliminary results show that a late booster dose at a 1/25th dosage is sufficient to stimulate a mucosal ALS IgA antibody response with similar antibody avidity against CTB as a booster with full dose of vaccine. The 1/25th dosage late booster dose was however not sufficient to stimulate further increases in serum IgA or IgG titers against CTB. Further analyses are ongoing and will be presented at the conference.

**CL093  Establishment the dot blot for the identity of polysaccharide vaccines**

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Immunochemical methods constitute one of the fundamental pillars in the quality control of vaccines, either to evaluate the minimum quality requirements of the IFAs or the final batch of a vaccine. However, the use of cheaper, easy, quick and simple immunochemical techniques displaces those traditionally used. For this reason, the purpose of this work was to establish the Dot-Blot technique for identity determination, which was standardized for the Vax-TyVi vaccine, its IFA and the native Vi-TT vaccine candidate, in addition to being validated for Vax- MEN ACW and its IFAs. Finally, optimal concentrations of immobilization, application volume for PsVi, Vax-TyVi and native Vi-TT and determination of the optimal concentration detection of AcMαPsVi were obtained and its specificity for PsVi was demonstrated. It was also demonstrated that the AcMαPsA, AcMαPsC, AcMαPsW are specific and that the Dot-Blot technique in addition to presenting numerous advantages is a method that can be used to monitor stability in vaccine samples such as Vax-MEN ACW. We concluded that the Dot-Blot as a reliable technique to be used in determining identity in polysaccharide vaccines.
CL094  Development and validation of the method for determining Vi polysaccharide molecular integrity by HPLC in Salmonella typhi conjugated vaccine

Aida Yaima Merchan Milia, Yalaydis Plutin Ocaña, Mario Landys Chovel, Iliana Sánchez Cala

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Introduction: Nowadays, international regulations for the licensing and usage of vaccines are becoming more demanding, causing a real challenge for producers and forcing them to generate novel technologies in order to obtain increasingly sophisticated products, as well as to develop new techniques able to evaluate in a reliable way their quality characteristics, a necessary condition to demonstrate manufacturing consistency, significant requirement for regulatory acceptance.

Materials and methods: The development of a new High Resolution Liquid Chromatography (HPLC) method for evaluating the molecular integrity of the Vi polysaccharide from S. typhi present in the conjugate vaccine was carried out at the Physical Chemistry Laboratory of Finlay Institute. The new methodology required the validation of the method to offer greater reliability and safety to the product, using a pre-column-column system TSK-G5000PW-TSK-G6000PWXL (Tosohaas) in series, coupled to an HPLC-Shimadzu system with two detectors IR and DAD and autoinjector, data acquisition and control of the system was performed with a controller and software Lab Solutions.

Results: Once the method was established, distribution constant results were obtained with a mean of 0.23 which are in agreement with the established by the different regulatory agencies (Kd ≤ 0.5).

Conclusions: The HPLC method for assessing the molecular integrity of the VI polysaccharide in Salmonella Typhi conjugated vaccine is accurate and specific, so it can be used for routine analysis.

CL095  Phase I clinical studies of a bivalent, LPS-based conjugate vaccine against Shigella flexneri 2a and S. Sonnei in China

Lin Du, Weihua Zhu

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The epidemiology surveys in recent years indicated that Shigella infections remain to be a common enteric problem in rural China. The major serotypes among disease isolates were S. Flexneri 2a and S. Sonnei, similar to the global trend. We have prepared a bivalent conjugate vaccine against S. Flexneri 2a and S. Sonnei, which consists of 10μg of detoxified lipopolysaccharide (LPS) of each serotype and conjugated to tetanus toxoid. The bivalent vaccine was approved by Chinese FDA for clinical trials. In the phase I study, a total of 180 healthy volunteers were recruited and evenly distributed in 9 groups: adults (>17 yr), school age children (full or half dose, 6 to 17yr), preschool children (full or half dose, 1 to 5 yr), and infants (full or half dose, 6 to 11 mo and 3 to 5 mo). Those older than 1 year old received 1 injection. Infants received booster doses at 1 month apart: 2 injections for 6 to 11 months old and 3 for 3 to 5 months old. Active safety surveillance was conducted for 1 month after each injection. The vaccine was administered in age descending schedule after safety reports reviewed in the older groups. The study is completed; safety reports are under analysis.
A Phase 2b clinical trial of ETVAX, an oral whole-cell inactivated vaccine against enterotoxigenic Escherichia coli, in Finnish travelers to Benin

Nils Carlin¹, Anu Kantele², Ann-Mari Svennerholm³

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In developing countries, diarrheal diseases rank the second most common cause of death among children under five years of age (¹,²). Although ETEC is the most common causes of bacterial diarrhea in children in developing countries and in travelers to these areas there is no licensed vaccine. ETEC is also the most frequent cause of traveler’s diarrhea.

We have previously developed an oral ETEC vaccine consisting of a combination of recombinantly produced CTB (rCTB) and formalin-inactivated ETEC bacteria expressing major CFs³. The rCTB-CF vaccine provided significant protective efficacy (PE 77%, p=0.039) against moderate-severe ETEC disease in American travelers to Mexico and Guatemala⁴, however there was no significant protection (PE 20%) against ETEC diarrhea in Egyptian infants with mostly mild disease ⁵.

Based on the experience with the rCTB CF ETEC vaccine we have developed a modified second-generation oral inactivated ETEC vaccine with the aim to improve the immunogenicity without increasing the dosage of bacteria allowing administration of reduced doses to infants. This multivalent ETEC vaccine (ETVAX) contains four different inactivated E. coli strains expressing substantially higher levels of CFA/I, CS3, CS5 and CS6 than the first-generation vaccine, plus a CTB/LTB hybrid protein (LCTBA), which induces stronger anti-LT responses than CTB in mice and humans ⁶. In addition, we have further enhanced the immunogenicity of the vaccine by co-administration with the mucosal dmLT adjuvant ⁷, which significantly improved both the anti-CF and anti-LT responses following oral immunization with ETVAX in mice⁸, and increased the CS6 response as well as resulted in significantly better toxin neutralization as compared to the first generation vaccine (N Carlin unpublished) The vaccine with the dmLT adjuvant is administered orally as a drink in a glass of bicarbonate solution.

The OEV 123 study will encompass 800 travelers from Finland going to Grand Popo, Benin, West-Africa for a 12 days on-site visit. The study period is the 12 days in Benin plus the first six days on returning back to Finland.

The study is a double-blind, placebo-controlled study of the ETVAX vaccine using buffer alone as placebo. The participants are given 2 doses of vaccine/placebo 14±7 days apart and travel to the African trial site within 7-30 days after the last dose.

The primary objectives are safety and immunogenicity of the vaccine as well as to compare diagnostic methods, i.e. classical culture of fecal samples versus molecular based methods; Mobidiag and Tacman, (⁸,⁹). As a secondary objective the protective efficacy of the vaccine against moderate to severe, incapacitating diarrhea caused by an ETEC considered to be a Vaccine Preventable Outcome (VPO). A VPO is defined as an ETEC expressing LT or LT/ST or ST alone in combination with one of the following colonization factor antigens :CFA/I, CS3, CS5 and CS6. In all approximately 400 persons have completed the journey by end of April 2018.

The incidence of TD has been relatively high, 50-70%. A significant number of travelers have recorded disease first when they have returned to Finland. Our aim is to have the last participants travelling in the end of December 2018.


INDEX OF AUTHORS

<table>
<thead>
<tr>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>Adegbola, Richard A. 19</td>
</tr>
<tr>
<td>Adeyemi, Mitchell 19</td>
</tr>
<tr>
<td>Aguila, Adalberto 53</td>
</tr>
<tr>
<td>Ahmed, Tahmeed 9, 10</td>
</tr>
<tr>
<td>Ahmed, Tasnuva 59, 60</td>
</tr>
<tr>
<td>Ahmen, Tasnuva 60</td>
</tr>
<tr>
<td>Akhtar, Marjahan 59, 60</td>
</tr>
<tr>
<td>Alaimo, C. 65</td>
</tr>
<tr>
<td>Alam, Masud 23</td>
</tr>
<tr>
<td>Alam, Munirul 8</td>
</tr>
<tr>
<td>Albino-Flores, Ivan 46</td>
</tr>
<tr>
<td>Amaya, Mirna 8</td>
</tr>
<tr>
<td>Anderson, John 8, 24</td>
</tr>
<tr>
<td>Antonio, Martin 11, 19</td>
</tr>
<tr>
<td>Araujo Maciel, Irene 9</td>
</tr>
<tr>
<td>Ariel-Cohen, Ortal 60</td>
</tr>
<tr>
<td>Armah, George 12</td>
</tr>
<tr>
<td>Artaud, Cecile 60</td>
</tr>
<tr>
<td>Asafo-Adjei, Edward 50</td>
</tr>
<tr>
<td>Asato, Valeria 60</td>
</tr>
<tr>
<td>Ashkenazi, Shai 60</td>
</tr>
<tr>
<td>Ateudjieu, Jerome 25</td>
</tr>
<tr>
<td>Atsmon, Jacob 60</td>
</tr>
<tr>
<td>Aziz, Fatima 9, 10</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>Bagamian, Karoun 8, 24</td>
</tr>
<tr>
<td>Baleux, Françoise 40</td>
</tr>
<tr>
<td>Bano-Zaidi, Mussaret 16, 22</td>
</tr>
<tr>
<td>Baral, Ranju 24</td>
</tr>
<tr>
<td>Barman, Anaxee 34, 40</td>
</tr>
<tr>
<td>Barman, Soumik 51</td>
</tr>
<tr>
<td>Barnard, B.A. 56</td>
</tr>
<tr>
<td>Barry, Eileen M. 17, 27, 41, 42, 47, 49, 53</td>
</tr>
<tr>
<td>Bauer, David 39</td>
</tr>
<tr>
<td>Behar, Adi 12</td>
</tr>
<tr>
<td>Berit Guttormsen, Anne 41</td>
</tr>
<tr>
<td>Bessong, Pascal 9, 10</td>
</tr>
<tr>
<td>Bhogal, Vishal 45</td>
</tr>
<tr>
<td>Bhuivyan, Taufiqur Rahman 29</td>
</tr>
<tr>
<td>Bhutta, Zulfiqar 9, 10</td>
</tr>
<tr>
<td>Bialik, Anya 12, 60</td>
</tr>
<tr>
<td>Bitoun, Jacob 17, 39</td>
</tr>
<tr>
<td>Blackwelder, William 11</td>
</tr>
<tr>
<td>Bodhidatta, Ladaporn 9, 10</td>
</tr>
<tr>
<td>Boedeker, Edgar 30</td>
</tr>
<tr>
<td>Bosomprah, Samuel 21, 34</td>
</tr>
<tr>
<td>Bourgeois, A. Louis 8, 25, 26, 56, 58, 59, 60</td>
</tr>
<tr>
<td>Boysen, Anders 43</td>
</tr>
<tr>
<td>Brubaker, Jessica 58</td>
</tr>
<tr>
<td>Bruno, Laura 36</td>
</tr>
<tr>
<td>Bucardo, Filemon 16</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>Campos-Freddy, Daniel 16</td>
</tr>
<tr>
<td>Carlin, Nils 68</td>
</tr>
<tr>
<td>Carmolli, Marya 23</td>
</tr>
<tr>
<td>Cassataro, Juliana 36</td>
</tr>
<tr>
<td>Cavacini, Lisa A. 53</td>
</tr>
<tr>
<td>Cavailler, Philippe 14, 15</td>
</tr>
<tr>
<td>Cerna-Cortes, Jorge Francisco 18, 22</td>
</tr>
<tr>
<td>Chakraborty, Subhra 8, 26, 27, 43, 58, 62</td>
</tr>
<tr>
<td>Chandrasekaran, Lakshmi 29, 56</td>
</tr>
<tr>
<td>Chavan, Sonali 28</td>
</tr>
<tr>
<td>Chen, Wilbur 17</td>
</tr>
<tr>
<td>Chilengi, Roma 21, 34</td>
</tr>
<tr>
<td>Chisenga, Caroline Cleopatra 21, 34</td>
</tr>
<tr>
<td>Chowdhury, Fahima 29</td>
</tr>
<tr>
<td>Chowdhury, Mohiul Islam 59, 60</td>
</tr>
<tr>
<td>Clarkson, K.A. 56, 65</td>
</tr>
<tr>
<td>Clarkson, Kristen 58</td>
</tr>
<tr>
<td>Clements, John D. 39, 40</td>
</tr>
<tr>
<td>Cohen, Dani 12, 60</td>
</tr>
<tr>
<td>Coic, Yves-Marie 40</td>
</tr>
<tr>
<td>Colgate, E. Ross 23</td>
</tr>
<tr>
<td>Coria, Mirta Lorena 36</td>
</tr>
<tr>
<td>Cravioto, Alejandro 20</td>
</tr>
<tr>
<td>Crawford, John M. 30</td>
</tr>
<tr>
<td>Cerna-Cortes, Jorge Francisco 18, 22</td>
</tr>
<tr>
<td>Chisenga, Caroline Cleopatra 21, 34</td>
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<td>Chowdhury, Fahima 29</td>
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<td>Chowdhury, Mohiul Islam 59, 60</td>
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<td>Clarkson, K.A. 56, 65</td>
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<td>Clarkson, Kristen 58</td>
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<td>Clements, John D. 39, 40</td>
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<td>Coria, Mirta Lorena 36</td>
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<td>Crawford, John M. 30</td>
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<td>Cerna-Cortes, Jorge Francisco 18, 22</td>
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<td>Chisenga, Caroline Cleopatra 21, 34</td>
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<td>Fatima Aziz</td>
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<td>Fiskerstrand, Torunn</td>
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<td>Fix, Alan</td>
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<td>Fonk, Veronica</td>
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<td>Frenc Jr, R.W.</td>
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<td>Garcia, Carolina</td>
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<td>Garcia Suarez-Villamil, Adina</td>
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<td>Garduño-Guadarrama, Hécto</td>
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<td>Gautheron, Sylviane</td>
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<td>Gessner, Bradford D.</td>
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<td>Giersing, Birgitte</td>
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<td>Giraldi, Petra</td>
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<td>Giuntini, Serena</td>
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<td>Gómez-Duarte, Oscar G.</td>
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<td>Gonzalez, Fredman</td>
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<td>Goren, Sophy</td>
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<td>Gottlieb, Michael</td>
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<td>Gougeon, Marie-Lise</td>
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<td>Govasli, Morten L.</td>
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<td>Grassel, Christen</td>
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<td>Gratz, Jean</td>
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<td>Guenou, Etienne</td>
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<td>Guerra, Julio</td>
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<td>Guerry, Patricia</td>
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<td>Halvor Sommerfelt</td>
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<td>Hanevik, Kurt</td>
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<td>Haque, Rashidul</td>
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<td>Harald Skutlaberg, Dag</td>
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<td>Hardwidge, Philip</td>
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<td>Harriett, Amanda</td>
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<td>Harro, Clayton</td>
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<td>Harutyunyan, Shushan</td>
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<td>Havr, Alexandre</td>
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<td>Hays, Michael</td>
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<td>Hazen, Tracy</td>
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<td>Heien, Astrid</td>
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<td>Heine, Shannon J</td>
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<td>Heinrichs, Jon</td>
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<td>Henics, Tamás</td>
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<td>Hessler, Catherine</td>
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<td>Holton, Carla</td>
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<td>Holgersson, Jan</td>
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<td>Hoq, Rubel</td>
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<td>Hossain, Jahangir</td>
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<td>Hossain, Nazima</td>
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<td>Hossain, M. Jahangir</td>
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<td>Houben, Diane</td>
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<td>Houpt, Eric</td>
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<td>Huang, Jiachen</td>
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<td>Huang, Xiao Xian</td>
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<td>Huerta-Campillo, Jazmin</td>
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<tr>
<td>Hyesuk Seo</td>
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<td>Hzen, Tracy</td>
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<td>Ikumapayi, Usman N.</td>
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<td>Illboudo, Patrick G.</td>
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<td>Imerbsin, Rawiwan</td>
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<td>Iqbal, Najeeha</td>
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<tr>
<td>Islam, Dilara</td>
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<tr>
<td>Islam, Md. Obedul</td>
</tr>
<tr>
<td>Jasseh, Momodou</td>
</tr>
<tr>
<td>Joseph, Sabrina</td>
</tr>
<tr>
<td>Kabir, Furqan</td>
</tr>
<tr>
<td>Kaim, Joanna</td>
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<tr>
<td>Kalam, Adil</td>
</tr>
<tr>
<td>Kaminski, Robert W.</td>
</tr>
<tr>
<td>Kanellos, Jerry</td>
</tr>
<tr>
<td>Kang, Gagandeep</td>
</tr>
<tr>
<td>Kania, Dane</td>
</tr>
<tr>
<td>Kantele, Anu</td>
</tr>
<tr>
<td>Kant, Ravi</td>
</tr>
<tr>
<td>Kasumba, Irene</td>
</tr>
<tr>
<td>Khanam, Farhana</td>
</tr>
<tr>
<td>Khan, Ashraful Islam</td>
</tr>
<tr>
<td>Khan, Fariya</td>
</tr>
<tr>
<td>Kim, Aaron</td>
</tr>
<tr>
<td>Kirkpatrick, Beth</td>
</tr>
<tr>
<td>Kiwelu, Ireen</td>
</tr>
<tr>
<td>Klemper, Mark S.</td>
</tr>
<tr>
<td>Kleppa, Elisabeth</td>
</tr>
<tr>
<td>Kole, Hemanta</td>
</tr>
<tr>
<td>Kosek, Margaret</td>
</tr>
<tr>
<td>Kotloff, Karen</td>
</tr>
<tr>
<td>Kotloff, Karen K</td>
</tr>
<tr>
<td>Kotloff, Karen L</td>
</tr>
<tr>
<td>Kumar, Ajay</td>
</tr>
<tr>
<td>Kuroiwa, Janelle</td>
</tr>
<tr>
<td>Laban, Natasha</td>
</tr>
<tr>
<td>Laban, Natasha Makabilo</td>
</tr>
<tr>
<td>Landys Chovel, Mario</td>
</tr>
<tr>
<td>Lang, Dennis</td>
</tr>
</tbody>
</table>
INDEX OF AUTHORS

Langeland, Nina 41
Larsen, Martin R. 43
Laytnr, Lindsey 8
Leach, Susannah 66
Lee, Tida 32
Le Gargasson, Jean-Bernard 14, 15
Leite, Jose Paulo 10
Leon Alamilla, Luis Antonio 20
Lerner, K.T. 56
Lertsethtakarn, Paphavee 10
Levine, Myron M 19
Liang, Xiaowu 26, 27
Liang, Yuanyuan 11
Licona-Moreno, Delia 13, 20
Liete, Jose Paulo 9
Lillebo, Kristine 41
Lima, Aldo 9, 10
Liu, Jie 9, 10
Liu, Jining 28
Long, Kurt Z 18, 22
Lopez Salas, Karla Andrea 20
Lopez-Saucedo, Catalina 16, 18, 22
Luirink, Joen 36
Lundgren, Anna 29, 59, 60, 66
Lu, Ti 54

M
Maciel, Irene 10
Maciel, Irene Araujo 9
Maciel Jr, Milton 39, 51
Maciel, Milton 62
Maier, N. 56
Maier, Nicole 59, 60
Maiti, Suhrid 51
Maldonado-Puga, Samantha 16
Mani, S. 56
Mani, Sachin 26, 27, 29, 43, 62
Marianne Sævik 42
Martinez-Becerra, Francisco 43
Martinez, Franco Luis 36
Martin, P. 65
Martin, Patricia 58
Mason, Carl 9, 10
Masud Alam 23
McCoy, Andrea 39
McMurry, Timothy 10
McNeal, M. 56
Mduma, Estomih 9, 10
Menchel, Micayla 46
Mengel, Martin A. 14, 15
Meron-Sudai, Shiri 60
Meza-Segura, Mario 16
Meza-Segura, Mario A 22
Mily, Akhirunnesa 56
Mishra, Vishwas 34, 40
Mogasale, Vittal 15
Moller-Jensen, Jakob 43
Moon, Jonathan 49
Moran-Garcia, Nadia Elisa 18, 22
Mottram, Lynda 28, 66
Motyka, Natalya 39
Muhib, Farzana 8, 24
Mujaga, Buliga 9, 10
Mukherjee, Priyadarshini 51
Mulard, Laurence 40, 60
Murei, Arinao 10
Mwaba, John 21, 84
Mwanyungwe, Abel 15
Mwila-Kazimbaya, Katayi 21

N
Nag, Dhrubajyoti 51
Nagy, Eszter 49
Nagy, Gábor 49
Nahm, Moon 29, 64
Nandi, Somesh 34
Nandre, Rahul 54
Nandre, Rahul M 47
Nasrin, Dilruba 11, 19
Navarrete, Karla 46
Navarro, Armando 13
Navarro, Fernando 16
Ndungo, Esther 27
Neuhauser, Irene 49
Ngwira, Bagrey 14, 15
Nishat, Naoshin Sarmin 29
Nordgreen, Johann 16
Norton, Elizabeth 37
Nshama, Rosemary 9, 10
Nunez, Gladys 39

O
Obolensky, Anna 38
Ocaña, Armando Navarro 20
Ochieng, John B. 11
Ochoa, Theresa J. 14
Ofori, Michael 12
Okamoto, Keinosuke 51
Omore, Richard 11
Operario, Darwin 10
Orr, Mark 39, 51
Osorio, Manuel 46
Ou, Bingming 45

P
Pacheco-Gil, Leova 22
## INDEX OF AUTHORS

<table>
<thead>
<tr>
<th>V</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valli, Eduardo 37</td>
<td>Yadav, Apeksha 46</td>
</tr>
<tr>
<td>Vedøy, Oda 33</td>
<td>Yaima Merchan Milia, Aida 67</td>
</tr>
<tr>
<td>Velazquez, F Raul 22</td>
<td>Yang, Yang 36</td>
</tr>
<tr>
<td>Venkatesan, M. 56</td>
<td>Yori, Pablo 10</td>
</tr>
<tr>
<td>Venkatesan, Malabi 56</td>
<td>Yu, Jigui 29</td>
</tr>
<tr>
<td>Vilchez, Samuel 16</td>
<td></td>
</tr>
<tr>
<td>Villar, Zuzana 62</td>
<td></td>
</tr>
<tr>
<td>Visheswariah, Sandhya 34</td>
<td></td>
</tr>
<tr>
<td>Visheswariah, Sandhya S. 40</td>
<td></td>
</tr>
<tr>
<td>Vitved, Lars 43</td>
<td></td>
</tr>
<tr>
<td>Voeglein, Joseph 8</td>
<td></td>
</tr>
<tr>
<td>Volokhov, Inna 60</td>
<td></td>
</tr>
<tr>
<td>von Mentzer, Astrid 48</td>
<td></td>
</tr>
<tr>
<td>Vortherms, Anthony 50</td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>Z</td>
</tr>
<tr>
<td>Walker, Richard 56, 59</td>
<td></td>
</tr>
<tr>
<td>Wang, Yang 53</td>
<td>Zachos, Nicholas 47</td>
</tr>
<tr>
<td>Ward, Elizabeth 62</td>
<td>Zegeye, Ephrem D. 40, 48</td>
</tr>
<tr>
<td>Weerts, Hailey 29, 50, 64</td>
<td></td>
</tr>
<tr>
<td>Weerts, H.P. 56</td>
<td>Zhang, Qi 43</td>
</tr>
<tr>
<td>Weiping Zhang 47</td>
<td>Zhang, Weiping 43, 45, 54</td>
</tr>
<tr>
<td>Wenzel, Heather 29, 51</td>
<td></td>
</tr>
<tr>
<td>WHO ETEC and Shigella PPC Working Group 54</td>
<td></td>
</tr>
<tr>
<td>Wierzba, Thomas 8, 39, 51, 56, 59, 60</td>
<td></td>
</tr>
<tr>
<td>Wikland, Gudrun 60</td>
<td>Zhou, Fan 35</td>
</tr>
<tr>
<td>Wu, Tao 49</td>
<td>Zhu, Weihua 67</td>
</tr>
<tr>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Xiao, Nan 45</td>
<td></td>
</tr>
<tr>
<td>Xicohtencatl-Cortes, Juan 14</td>
<td></td>
</tr>
</tbody>
</table>