Abstracts
1st International VASE Conference 2016

PATH
VASEConference.org
# Table of Contents

Global burden of disease and epidemiology ...................................................................................................................... 5

## Oral Presentations

GB05  The Global Burden of *Shigella* and Enterotoxigenic *E. coli* .................................................. 5

GB06  A Systematic Review of Shigella Epidemiology in Travelers .................................................. 6

## Poster Presentations

GB01  Burden and risk factors of *S. sonnei* shigellosis in hyperendemic communities in Israel..... 7

GB03  Epidemiology of Culture-confirmed Shigellosis – United States 2003–2013......................... 8

GB04  Colonization Factors of Enterotoxigenic *Escherichia coli* strains isolated from Nicaraguan children with Diarrhea ............................................................................................................................ 9

GB07  Epidemiology of Rotavirus Infection of Children Below Five Years in Momo Division of the North West Region – Cameroon .......................................................................................................... 10


GB09  Updates on studies on ETEC epidemiology and vaccine efforts in Bangladesh................... 12

GB11  Lack of association of symptomatic Enterotoxigenic *Escherichia coli* infections and Lewis phenotype. ........................................................................................................................................... 13

GB12  Investigating the role of climate in increasing ETEC and Shigella Burden in East Africa through Spatial Modeling ........................................................................................................................................ 14

GB13  Call to Action: Impact of Diarrhea Mortality and Morbidity: Case Study of Nyahururu County Hospital - Kenya ...................................................................................................................... 15

## Oral Presentations

IMM02  Human ETEC Challenge: Differences in the Symptomatic and Asymptomatic Host Response 16

IMM06  Development and application of proteome microarrays to characterize immune responses in human volunteers following ETEC experimental challenge ........................................................................................ 17

IMM09  Characterization of the humoral immune response to *Shigella* towards identification of correlates of protection .................................................................................................................................... 18

## Poster Presentations

IMM04  Modulation of antibody responses against enterotoxigenic *Escherichia coli* (ETEC) by T cells in humans ................................................................. 19

IMM05  *Shigella* Outer Membrane Vesicles: A Novel Particles of Next Generation Vaccine ........ 20

IMM07  Low-endotoxin lipopolysaccharides from *Escherichia coli* O157:H7 and O104:H4 induce long-term specific immune response in mice and protect them against enterohemorrhagic *Escherichia coli* (EHEC) infection ........................................................................................................ 21

IMM08  Monoclonal antibodies to Shigella LPS that are useful for vaccine production ................. 22

IMM10  Diarrhoea caused by enterotoxigenic *Escherichia coli* induces immune responses in the circulation 23

IMM11  Usefulness of the Shigella bactericidal assay for investigating immunological correlates of immunity in clinical trials and with non-human primate samples ...................................................................... 24

## Impact of the microbiome on pathogenesis and vaccination

Oral Presentations ................................................................................................................................................................. 25

---

Cover page photo credits: Top: PATH/Aaron Joel Santos; Central three photos: PATH/Doune Porter; Central bottom: PATH/Aaron Joel Santos; Bottom far left: PATH/Molly Mort; Bottom far right: PATH/Gabe Bienczycki
MB01 Diversity, dynamics and diarrhea: how ETEC infection impacts resident E coli .......... 25
MB02 Individual-specific changes in the human gut microbiota after challenge with enterotoxigenic Escherichia coli and subsequent ciprofloxacin treatment ................................................. 26
Poster Presentations ............................................................................................................................... 27
MB04 Human enteroid model of Enterotoxigenic E. coli (ETEC) diarrhea and demonstration of MRP-related epithelial cell secretion of cGMP .................................................................................... 27
New Antigen Discoveries ............................................................................................................................... 28
Oral Presentations .................................................................................................................................. 28
ANT02 Studying enterotoxigenic Escherichia coli (ETEC) using whole genome sequencing........... 28
ANT03 Towards rational design of a toxoid vaccine against the heat-stable toxin-producing Escherichia coli .......................................................... .......................................................... 29
ANT04 Blood Group A Antigen Expression Influences Pathogen-Host Interactions and Clinical Outcome Following Infection with Enterotoxigenic Escherichia coli .......................................................... .......................................................... 30
Poster Presentations ............................................................................................................................... 31
ANT01 A broadly protective ETEC vaccine candidate .................................................................... 31
ANT05 Finding novel common immunogenic antigens common to Shigella spp. through reverse vaccinology .......................................................................................................................... 32
Novel adjuvants and immunization strategies for vaccination .......................................................... 33
Poster Presentations ............................................................................................................................... 33
ADJ01 Evaluation of antacid buffers for use with oral enteric vaccines ................................................. 33
ADJ02 Low-cost multivalent ETEC vaccine to prevent childhood diarrhoea ................................................. 34
ADJ03 Nanoparticles formulated from protein food-born polymers in the development of a mucosal complex vaccine against ETEC .................................................................................................................. 35
ADJ04 Self-adjuvant multicomponent vaccine against Shigella .......................................................... 36
Preclinical evaluation of vaccine candidates and animal models of enteric disease .... 37
Oral Presentations .................................................................................................................................. 37
PRE01 An adhesin tip MEFA (multiepitope fusion antigen) of enterotoxigenic Escherichia coli (ETEC) induced antibodies against adherence of nine ETEC adhesins: CFA/I, CS1, CS2, CS3, CS4, CS5, CS6, CS21 and EtpA .................................................................................................................. 37
PRE03 A live attenuated vaccine against Shigella and ETEC: characteristics and potency of the ShigETEC prototype strain .................................................................................................................. 38
PRE04 Preclinical Evaluation of Combined Shigella-ETEC Vaccine Candidates .......................................................................................................................... 39
PRE06 From a monovalent to a multivalent synthetic carbohydrate-based vaccine candidate against Shigella flexneri .......................................................................................................................... 40
PRE08 Adhesin tips vs. major structural subunits for anti-adhesin vaccines: a comparative study of enterotoxigenic Escherichia coli (ETEC) adhesin tip MEFA (multiepitope fusion antigen) and CFA MEFA for antibodies against adherence of ETEC adhesins (CFA/I, CS1-CS6, CS21 and EtpA) ...... 41
PRE16 Comparison of sublingual and intradermal delivery routes with ETEC vaccine candidates delivered with LT-based adjuvants .......................................................................................................................... 42
Poster Presentations ............................................................................................................................... 43
PRE02 Moving toward a clinical trial of a live attenuated oral vaccine against shigellosis and typhoid fever .......................................................................................................................... 43
PRE05 Evaluation of a Tri-valent Shigella flexneri Vaccine .......................................................................................................................... 44
PRE07 Evaluation of Serotype-independent Immunity Elicited by a T3SS-Based Shigella Subunit Vaccine

PRE10 Refinement of a Guinea Pig Intrarectal Shigella Challenge Model and use in Vaccine Efficacy Studies

PRE11 Evaluation of Shigella flexneri 2a Artificial Invaplex Formulated for Parenteral Immunization with Deacylated LPS

PRE12 Expanded Development and Use of the Aotus nancymaae Shigella Immunogenicity and Efficacy Model

PRE13 Functional complement-fixing antibodies are generated against a Campylobacter jejuni capsule conjugate vaccine in non-human primates

PRE14 The Universal Shigella Vaccine Development

PRE15 Protection of Aotus nancymaae non-human primates from enterotoxigenic Escherichia coli (ETEC) diarrhea by ID or IM immunization with a fimbrial subunit prototype vaccine

PRE17 Evaluation of local skin reactogenicity compared to serum and mucosal antibodies after intradermal ETEC immunization with CfaEB and E. coli heat-labile toxin-derived proteins

Strategies for broader coverage through combination vaccines

Oral Presentations

CB01 A conjugate vaccine approach to provide protection against Campylobacter jejuni, Enterotoxigenic Escherichia coli, and Shigella sp.

Systems biology and genomics

Oral Presentations

SB02 A systems approach to the study of ETEC H10407 challenge

Poster Presentations

SB01 Host genetics factors influence altered susceptibility of Enterotoxigenic E. coli: determining the association between FUT2, the Lewis antigens, ABH blood groups and susceptibility to infection

SB03 Serological cross-reaction between Shigella dysenteriae type 4 and Escherichia albertii DM104 is caused by inter-species transfer of the O-antigen gene cluster

Technical, manufacturing, and regulatory challenges in vaccine development and implementation

Poster Presentations

TM01 Development of in vitro assays to document neutralization of LT-mediated toxicity based on its mechanism of action

Vaccine candidates in clinical trials and human challenge models

Oral Presentations

CL01 A Randomized, Double-Blinded, Placebo-controlled, Dose-Escalation, Age-Descending Study to Assess the Safety and Tolerability of Live, Attenuated, Oral Shigella WRSS1 Vaccine candidate in Bangladeshi Adults and Children

CL02 A Phase 1 Open-label, Dose Escalating Study of Artificial Shigella flexneri 2a Invaplex administered intranasally to healthy, adult volunteers

CL03 Safety and Immunogenicity of a Candidate Bioconjugate Vaccine against Shigella flexneri 2a Administered to Healthy Adults: a Single Blind, Randomized Phase I Study

CL04 Memory B cell responses of volunteers intradermally immunized with the ETEC fimbrial tip adhesin CfaE plus LT(R192G) followed by oral challenge with CFA/I+ ETEC H10407
CL05  One step forward towards a phase 1 clinical trial with the first semi-synthetic glycoconjugate vaccine against *Shigella flexneri* 2a, SF2a-TT15 ........................................................................................................ 62

CL06  Clinical trials of an oral inactivated ETEC vaccine (ETVAX) .................................................................................. 63

Vaccine health economics, investment case for vaccines, and impact assessments ... 64

Oral Presentations .................................................................................................................................. 64

HE01  Enteric Vaccines, Ethical Imperatives for Health and the SDGs ........................................... 64

Index of Authors ..................................................................................................................................... 65
Global burden of disease and epidemiology
Oral Presentations

GB05   The Global Burden of Shigella and Enterotoxigenic E. coli
Christopher Troeger¹, Danny V. Colombara¹, Puja Rao¹, Ibrahim Khalil¹, Alexandria Brown¹, Ali H. Mokdad¹, Mohammad H. Forouzanfar¹
¹ Institute for Health Metrics and Evaluation, 2301 5th Ave, Suite 600, Seattle, WA, USA

Background: More than 1,200,000 deaths were attributable to diarrhea in 2013, with more than 99% of this burden being borne by those in developing countries. The Global Burden of Disease Study (GBD) seeks to regularly update and refine estimates of diarrheal disease burden attributable to Shigella spp., enterotoxigenic Escherichia coli (ETEC), and other enteric pathogens. We are currently updating burden estimates for these pathogens, as well as Campylobacter, enteropathogenic Escherichia coli (EPEC), and Cryptosporidium, for the GBD 2015.

Methods: Beginning with GBD 2013, we used a counter-factual approach to estimate years of life lost (YLLs), years living with disability (YLDs), and total disability adjusted life years (DALYs) attributable to diarrhea and its etiologies, including Shigella and ETEC. To estimate the burden of diarrhea etiologies, we conducted a systematic review of the proportion of diarrheal cases positive for each assessed pathogen and analyzed this data using a Bayesian meta-regression tool called DisMod-MR. This tool generates estimates of the pathogen distribution for national and some subnational geographies, all age groups, and for both sexes from 1990 to 2015. We used these estimates, in conjunction with odds ratios for diarrhea given pathogen detection from the Global Enteric Multicenter Study, to calculate the population attributable fraction for each pathogen.

Results: In 2013, shigellosis (73,908, 95% UI: 58,919-93,757) and ETEC infection (59,212, UI: 44,181-77,685) ranked second and fourth with regard to pathogen contributions to global diarrheal deaths. Shigella contributed more than four million YLLs (4,114,685, 95% UI: 3,193,381 - 5,223,964) and 212,081 (95% UI: 141,084-294,803) YLDs. Nearly three million YLLs were attributable to ETEC (269,796, 95% UI: 172,164-393,904) as were 212,081 YLDs (95% UI: 141,084-294,803). DALYs per 100,000 attributable to Shigella and ETEC were greatest in Chad (1,966, 95% UI: 1,022-3,292) and Somalia (1,089, 95% UI: 612-1,788), respectively. Among children under 5 years of age, Shigella and ETEC ranked fourth and fifth, respectively, with regard to attributable pathogen specific global diarrheal deaths.

Conclusion: The global burden of disease attributable to Shigella and ETEC is substantial. Our GBD2013 and soon to be released GBD2015 estimates will enable evidence-based decision making which will lead to optimal utilization of vaccines that are currently under development to reduce the burden.
GB06  A Systematic Review of Shigella Epidemiology in Travelers

Kayla M. Jaep¹, Giacomo Tomasello², Mark S. Riddle¹, Chad K. Porter¹

¹Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD, USA
²Tulane University School of Medicine, New Orleans, LA, USA

Background. Historically, shigellosis has been responsible for substantial morbidity in travelers from developed to developing countries. While shigellosis is treatable with appropriate antibiotics, increased resistance to frontline antibiotics highlights the need for primary prevention including vaccination. Current vaccine strategies are based on LPS-serotype specific antibodies; however, the serotype-specific etiology of Shigella infections in the adult travel population is poorly understood and may or may not overlap with the etiology in developing world pediatric populations. We sought to summarize the current literature regarding Shigella etiology in the adult traveler.

Methods. We conducted a systematic review of the published literature regarding Shigella etiology in the adult travel population. Studies were identified by searching electronic bibliographies (PubMed) using the following search terms: “shigell* travel*” and “shigell* travel* epidemiology”. Additionally, we performed manual searches of bibliographies, conference proceedings, book chapters and technical reports. Experts in the field of Shigella research were also utilized to identify eligible published and unpublished studies. All articles, publications and abstracts were reviewed, scored and underwent data abstraction by two independent investigators. Point estimates and confidence intervals were calculated using random effects models.

Results: We identified 20 eligible studies describing 8,798 Shigella isolates. The majority of studies (17, 85.0%) identified were of adult, civilian populations. Of the described isolates, S. sonnei [52.1% (47.8, 55.7)] and S. flexneri [38.3% (33.0, 42.6)] were most common with S. boydii [3.5% (2.6, 5.2)] and S. dysenteriae [2.6% (1.7, 4.3)] less common. Two studies reported S. flexneri serotypes with only 2a and 3a reported. The proportion of S. sonnei identified in travelers to Europe and North America (both 63.5%) was significantly higher (p<0.01) than in travelers to Sub-Saharan Africa (30.4%). In contrast, there were no regional differences in the prevalence of S. flexneri (p=0.10).

Discussion. These data highlight the variability in Shigella species in adult travelers and may help guide the target product profile for a future Shigella vaccine with a traveler indication. Recent studies in developing world pediatric populations have reported comparable estimates in similar regions. Furthermore, those studies have provided S. flexneri serotype-specific data that may inform vaccine development in that population. While S. flexneri serotype data in adult travelers may be similar to endemic pediatric populations, these data are lacking in this population. Variability in pathogen etiology seen with other enteropathogens when comparing endemic and travel populations and the lack of robust serotype data in the adult traveler demonstrate the need for additional epidemiologic studies to direct and refine future vaccine efforts.
Background: Country-wide propagated epidemics of S. sonnei shigellosis occur in Israel every 2-3 years for the last two decades. Jewish ultraorthodox towns and communities with good sanitary infrastructure but with living conditions of crowding have been the epicenter of these outbreaks.

Objective: We determined the burden of S. sonnei shigellosis and identified specific host and environment-related risk factors of the disease in children less than 5 years of age in a high-risk town in Israel.

Methods: We determined incidence proportions of culture-proven S. sonnei shigellosis in the years 2000-2012 using data on all S. sonnei isolated at the laboratories serving the population of the high risk town (E) as numerator. Data from the Central Bureau of Statistics were used for denominator estimates and calculation of crowding levels.

We carried out a case-control study on incident cases of culture-proven S. sonnei shigellosis identified through a sentinel laboratory based surveillance system. Data were collected by phone interviews conducted with the parents of cases and of controls (children without diarrhea during the last two weeks). Cases and controls were matched by age, sex and neighborhood. Multivariate analyses using logistic regression models were performed to assess the independent effect of each variable on the risk of shigellosis while controlling for other independent variables in the model. Odds ratio and 95% confidence intervals were calculated.

Results: The mean incidence proportions of S. sonnei shigellosis in children less than 5 in the high-risk town were 413 and 1930 per 100,000 in non-epidemic and epidemic years, respectively as compared to 278 and 662 per 100,000 in the rest of the district.

The high risk town was characterized by a significantly higher proportion of children less than 5 as compared with the district (27% versus 10%) and a higher level of crowding. The main risk factors of S. sonnei shigellosis in the high risk town were diarrheal disease among another person in the household (OR = 5.77, CI = 2.22 to 15.32) and using pacifiers very often (OR = 6.18, CI = 2.07 to 19.17). Certain characteristics of the kindergarten were also related to increased risk of S. sonnei shigellosis such as presence of multiple sinks in the kindergarten (OR = 4.67, CI = 1.07-32.13), the same caretaker being responsible for both food handling and also of changing diapers (OR = 4.0, CI = 1.4-11.65).

Conclusions: Enhanced control measures to ensure an adequate level of hygiene and reduce the risk of Shigella transmission in both households and kindergartens are needed. S. sonnei candidate vaccines can be tested and safe and efficacious ones routinely used in these settings when available.
GB03 Epidemiology of Culture-confirmed Shigellosis – United States 2003–2013

Hurd J*1, Collier S1, Judd M1, Bowen A1
1. Division of Foodborne, Waterborne & Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, GA

Abstract text:

Background: Shigellosis is the third most common enteric bacterial infection in the United States, with an estimated 500,000 infections per year. Large, protracted outbreaks of S. sonnei have occurred in childcare settings and communities; more recently, outbreaks of antimicrobial-resistant shigellosis have been reported among men who have sex with men. Although shigellosis is a nationally notifiable condition, recent epidemiologic patterns in the United States have not been well described. We aimed to describe the epidemiology of shigellosis in the United States from 2003–2013, which may help identify a target vaccine strategy when a vaccine is developed.

Methods: We analyzed all cases of culture-confirmed shigellosis reported to CDC’s Laboratory-based Enteric Disease Surveillance (LEDS) system from Jan 2003–Dec 2013. National and regional incidence rates were calculated per 100,000 population per year by region, sex, age group, and Shigella species, using census data. We defined adults as persons ≥18 years old and children as persons <18 years old. We compared rates by age group and region using Poisson regression.

Results: From 2003–2013, 112,581 Shigella isolates were reported to LEDS. The distribution of isolates by species was: S. sonnei, 74.5%; S. flexneri, 12.0%; S. boydii, 0.9%; S. dysenteriae, 0.3%; 12.3% were reported without species data. Shigellosis case counts were greatest in 2003 (15,951 cases) and decreased to a low of 5,983 cases in 2013, except for a spike of 14,805 cases in 2008. The median annual shigellosis incidence nationally was 3.3 (range, 1.9–5.5) overall, 2.5 (range, 1.5–3.7) for S. sonnei, and 0.4 (range, 0.3–0.6) for S. flexneri. Shigellosis rates by county were highest in the South (4.7; CI: 1.9–11.6; p<0.001), specifically in Texas (8.9) and Georgia (9.0). The highest rates of shigellosis were reported among children aged <10 years old (15.4; CI: 9.4–25.4; p<0.001). Incidence rates were similar among adult males and females overall, but significantly differed by species. Adult male-to-female incidence ratios were 0.6 for S. sonnei and 2.6 for S. flexneri infections. S. flexneri infection rates declined among women and children but not among men from 2003–2013.

Conclusions: From 2003–2013, reports of culture-confirmed shigellosis declined in the United States, but rates among some populations remained high. Incidence rates were the highest in the South, and among children <10 years old. The proportion of S. flexneri infections among adult males appears to be increasing; efforts to understand risk factors for, detect illness clusters, and prevent such illnesses among men should be enhanced. National Shigella surveillance data may be used to target future vaccine efforts among high-risk populations.
GB04 Colonization Factors of Enterotoxigenic *Escherichia coli* strains isolated from Nicaraguan children with Diarrhea

Bayardo Samuel Vilchez-Rugama*, PhD, Sylvia Becker-Dreps¹, MD, MPH, Claudia Perez¹, MSc, Johann Perez¹, MSc, Margarita Paniagua¹, MSc, Daniel Reyes¹, MD, PhD, Felix Espinoza¹ PhD, Erick Amaya¹, PhD, and Andrej Weintraub³ PhD

¹Department of Microbiology and Parasitology, Faculty of Medical Sciences, National Autonomous University of Nicaragua (UNAN), León, Nicaragua

²Department of Family Medicine, University of North Carolina at Chapel Hill, 590 Manning Drive, Chapel Hill, North Carolina, USA

³Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital, Huddinge, S-141 86, Stockholm, Sweden

Abstract Text:

Enterotoxigenic *Escherichia coli* (ETEC) is one of the most common causes of diarrhea among young children in Nicaragua. ETEC vaccines offer promise in reducing the burden of ETEC disease in these settings. To best reflect the full spectrum of ETEC disease in León, Nicaragua, the aim of this study was to characterize ETEC strains isolated from children with diarrhea in various settings, in terms of their colonization factors, and to determine whether these factors varied with setting, age group and year of collection. ETEC isolates were obtained from children under the age of 60 months from 1) the regional public hospital (years: 2005-2006), 2) four public primary care clinics (years: 2005, 2006 and 2014), and 3) a population-based cohort (year: 2010). In all, 81 ETEC-positive samples underwent a multiplex PCR assay for the identification of colonization factors CS1, CS2, CS3, CS4, CS5, CS6, CS7, CS8, CS12, CS13, CS14, CS15, CS17, CS18, CS19, CS20, CS21, CS22, CS23 and CFA/I. The frequency of colonization factors were compared between the three settings and for different age groups, using the Fisher's exact test. At least one CF was detected among 65.4% of samples; CS19 was the most common CF detected, either alone or in combination with another CF. Among all CFs detected, 54.7% were members of the Class 5 fimbrial family. CFs were more commonly detected among infants and among cases captured in the health facility setting. Also, we observed a shift in terms of different CFs ETEC strains circulation during our study periods. In summary, to protect against diarrhoeal cases among infants and those requiring health facility visits, an ETEC vaccine which effectively targets the arqueotype CFA/I of the Class 5 fimbrial family would be the most effective in this setting.
GB07  Epidemiology of Rotavirus Infection of Children Below Five Years in Momo Division of the North West Region – Cameroon

Tah Fon Phillip MSc .Nursing, Hassan Ali BSc Nursing"
Bonadikombo Health Clinic and Founder , President-CARITAS FORUM, Mbengwi General Hospital"

Introduction: Diarrhoea can be caused by a broad spectrum of viral, parasitic and bacterial enteropathogens of which rotavirus is the leading cause of severe diarrheoa in children under five years of age.

Objectives: The fundamental objectives of this study was (1) To identify the prevalence of rotavirus group A (RVA) in children under the age of five in Momo division of the North west regional capital of Cameroon. (2) To introduce the use of rotavirus vaccines for the prevention and treatment of this infection. And (3)To boost our fight against poverty associated with the economic cost of diarrheoa.

Methodology: Stool samples were collected form five hundred and six children below five years old presenting with diarrheoa in the two renowned hospitals in momo division namely (a)the Mbengwi general hospital and (b)the Acha Tudig hospital run by the Presbyterian church in Cameroon. The study period ran from 2011-2012. The stool samples were collected in sterile stool containers following microbiologically approved method. The stool samples were screened for the presence of parasites.

Result: children below twenty-four months were most affected (44.7% where as children between 49-60months had the lowest prevalence 25%)

In Acha Tudig hospital the prevalence rate of RVA among the 256 children screened was 56.55 % while in the government hospital Mbengwi of the 250 children screened 33.35%had the RVA.

Conclusion:This study showed very vividly the high incidence of rotavirus A infection among hospitalized children in Momo division of Cameroon. Suggesting that it is a major cause of childhood morbidity and mortality in the area. It is relevant to note that before we embarked on this critical study no data on rotavirus epidemiology was available in Momo division in particular and North West region in general. Since 2006 two rotavirus vaccines have been licensed and are recommended for use in all countries by WHO with high diarrheoa mortality in children younger than five years.

Anna Bowen, Julian Grass, Amelia Bicknese, Davina Campbell, Jacqueline Hurd, Robert D. Kirkcaldy
Centers for Disease Control and Prevention

Background: Shigellosis causes approximately 500,000 illnesses in the United States annually. Resistance to ciprofloxacin, ceftriaxone, and azithromycin, the first-line treatments for shigellosis, is emerging, but whether the risk of antimicrobial resistance differs by demographic group is unknown.

Methods: We queried CDC’s enteric disease cluster management database for shigellosis clusters during Jan 2011 – Dec 2015 and linked clusters to CDC’s National Antimicrobial Resistance Monitoring System (NARMS), excluding clusters without documented antimicrobial susceptibility data or transmission route. We classified a cluster as ciprofloxacin-, ceftriaxone-, or azithromycin-resistant if NARMS reported such resistance among any isolate from the cluster. Using exact methods, we compared prevalence of resistance by transmission category.

Results: Among 32 clusters, transmission was reported to involve childcare centers, camps, or schools (10); MSM (7); other person-to-person transmission (7); food (6); or recreational water (2). Nine clusters were caused by Shigellae resistant to ciprofloxacin (3), ceftriaxone (2), or azithromycin (7); resistance to more than one of these antimicrobials was reported in three clusters. Among these nine antimicrobial-resistant clusters, eight were caused by S. sonnei and one by S. flexneri. We observed resistance to any of these antimicrobials in all 7 MSM-associated clusters, but in only 2 of the other 25 clusters (prevalence ratio 12.5, p<0.001). The prevalence of azithromycin resistance was approximately 20 times greater among MSM-associated clusters than among other clusters (p<0.001).

Conclusions: All U.S. shigellosis clusters identified among MSM from 2011 – 2015 were caused by strains resistant to first-line shigellosis treatments; the prevalence of such resistance appears markedly higher among MSM than among others. Shigella vaccines targeting S. sonnei and S. flexneri would substantially reduce diarrhea morbidity in the United States and elsewhere and prevent transmission of multidrug-resistant shigellae, particularly among MSM."
GB09 Updates on studies on ETEC epidemiology and vaccine efforts in Bangladesh

Firdausi Qadri on behalf of the ETEC Working Group

Mucosal Immunology and Vaccinology Unit, Infectious Disease Division, icddr,b, Bangladesh; Department of Immunology and Microbiology, University of Gothenburg, Gothenburg, Sweden; PATH Vaccine Solutions (PVS), Seattle, WA

Enterotoxigenic Escherichia coli (ETEC) is a noninvasive mucosal pathogens that cause acute watery diarrhea in children but also adults in Bangladesh as well as other developing countries. Protective immune response occurs after ETEC infection as evidenced from epidemiological studies which show that there is a decline in ETEC diarrheal incidence with increasing age. In addition, multiple infections with cross-reacting CFs produced by ETEC leads to broad spectrum protection against ETEC diarrhea. The epidemiology of ETEC infections and the growing evidence of the problems associated with both symptomatic and asymptomatic diarrhea is now being supplemented by a nationwide surveillance program in Bangladesh. Diarrheal stools from different sites distributed from all over Bangladesh are being screened for ETEC using both phenotypic and genotypic methods. Demographic and clinical data are being collected in order to better understand the impact of the infection in context to Bangladesh and in terms of the evaluation of the vaccine needed for this setting.

We are also currently carrying out a phase I/II clinical trial with the oral multivalent ETEC vaccine (ETVAX) in different age group of participants down to six months of age in Bangladesh where humoral and adaptive immune response are also being evaluated. This is an age-descending, dose escalating study in adults, toddlers and infants in an urban ETEC endemic site in Dhaka city.

We hope that by these studies an all rounded effort to better understand the epidemiology of ETEC diarrhea, the clinical and demographic features in patients as well as the mechanism of response to ETEC infection and an oral inactivated vaccine will be generated. Hopefully in the near future these efforts will lead to a better understanding the correlates of protection to ETEC as well as safety and immunogenic properties of an effective vaccine will be better identified.

Funding: EU, PATH and Swedish Sida
GB11  Lack of association of symptomatic Enterotoxigenic *Escherichia coli* infections and Lewis phenotype.

Johann Perez MSc, Fredman Gonzales MSc, Filemon Bucardo PhD Daniel Reyes MD, PhD, Samuel Vilchez PhD

Department of Microbiology, Faculty of Medical Sciences, National Autonomous University of Nicaragua, Leon

A recent study suggest a predisposition for infectious diarrhea caused by enterotoxigenic *Escherichia coli* expressing certain adhesion fimbria types in individuals with Lewis phenotype Lea+b-. The aim of the study was to investigate if exists any genetic predisposition associated with the Lewis histo-group with the symptomatic infections due to enterotoxigenic *Escherichia coli*. During the period September 2014-October 2015, a total of 127 stool and saliva samples were collected from children under 60 months of age (55 with and 72 without diarrhea) in León, Nicaragua. In order to detect different diarrhogenic *Escherichia coli* pathotypes a mixture of primer pairs were used in a multiplex PCR reaction. Primers for the following pathotypes were included: enterotoxigenic *E. coli* [ETEC], enteropathogenic *E. coli* [EPEC], enteroaggregative *E. coli* [EAEC], enteroinvasive *E. coli* [EIEC], and enterohemorrhagic *E. coli* [EHEC]. Additionally, we tested all ETEC isolates through a panel of multiplex PCRs for the following colonization factor (CF): CS1, CS2, CS3, CS4, CS5, CS6, CS7, CS8, CS12, CS13, CS14, CS15, CS18, CS20, CS21, CS22, CFA/I, CS17 and CS19. The saliva samples were analyzed for the Lewis phenotype through an ELISA based assay.

At least one diarrheogenic *E. coli* (DEC) was detected in 60.4% (78) of the samples. In the diarrhea group 41% (32) and 58.9% (46) in the non-diarrhea group. EPEC was the most prevalent DEC type both in the diarrhea and the non-diarrhea group with 41.2% (14) and 45.5% (25), respectively. All EPEC were atypical meaning eaeA positives only. The EAEC pathotype was found in 17.7% (6) of the diarrheal samples and 12.7% (7) in the non-diarrheal. EHEC was found mostly in the non-diarrhea group, 9.1% (5) as compared with the diarrhea group where only 1 sample (2.9%) tested positive for EHEC. The ETEC prevalence was 23.5% (8) in the diarrhea group and 27.3% (15) in the non-diarrhea group.

The distribution of Lewis phenotypes in the diarrhea group was Lea+b- 12.7% (7), Lea+b+ 74.5% (41) and Lea-b- 12.7% (7). Similarly, in the non-diarrhea group Lea+b- 15.2% (11), Lea-b+ 65.2% (35) and 19.4% (14).

We did not find any association between symptomatic ETEC infection and the Lewis phenotype. No specific fimbria type was detected with a higher prevalence in the diarrhea group compared to the non-diarrhea group. Although, CFA/I ETEC tended to be more frequent in positives isolates in the diarrhea group 25% (2) than the non-diarrhea group 6.7% (1), numbers are to small to draw any conclusion. Our results further suggests a variation in the virulence properties of the ETEC bacteria circulating during the study period and more research is needed to understand this infectious agent to best inform vaccine developers and reduce the diarrhea cases associated with it."
GB12 Investigating the role of climate in increasing ETEC and Shigella Burden in East Africa through Spatial Modeling

Karoun H. Bagamian*, Richard Rheingans

Dept. of Environmental and Global Health/Emerging Pathogens Institute, University of Florida

Diarrheal diseases are a major cause of childhood (<5 years) mortality worldwide, with nearly half of deaths occurring in Africa. Recent molecular epidemiological studies in developing nations have identified that enterotoxigenic *Escherichia coli* (ETEC) and *Shigella* spp. are responsible for the majority of moderate to severe diarrheal diseases (MSD) in children under 5. Unequal access to adequate nourishment, healthcare, and sanitation among socioeconomic groups contributes to MSD disease burden. Undernourished children are highly susceptible and experience higher morbidity and mortality from diarrhea. Although global mortality from diarrhea is declining, much of Africa experiences a disproportionate burden of these treatable diseases. In addition, climate change is considered to be especially consequential in Africa, and may lead to increases in diarrheal burden in vulnerable East African populations. Climate change is projected to increase climate variability and frequency of extreme events (e.g. floods and droughts). As agriculture in much of sub-Saharan Africa is heavily reliant on rainfall and weather patterns, precipitation alterations may influence agricultural production and availability of adequate nourishment for children. Here, we identify the ways that climate change can impact diarrheal burden in East Africa, and build spatial models to visualize and analyze the interplay of social and environmental factors influencing ETEC and *Shigella* occurrence. We are exploring spatially-informed regression methods, using diarrheal occurrence data and social variables from DHS surveys, population density estimates from GRUMP, and environmental and climate data from publicly available spatial repositories.
GB13  Call to Action: Impact of Diarrhea Mortality and Morbidity: Case Study of Nyahururu County Hospital - Kenya

Charles Munene Kabuga¹, ², ³, Josephine Muriithi¹, ² Mbugua Sammy¹, ²
1. Ministry of Health (moh)
2. Laikipia County
3. Mount Kenya university.(std)

INTRODUCTION  Globally there are nearly 1.7 billion cases of diarrhea disease every year. Diarrhea disease is the 2nd leading cause of death in children under 5 years. It is a leading cause of child mortality and morbidity in the world, it also contributes to childhood malnutrition. Diarrhea kills about 760,000 children under 5 years every year. In Kenya, diarrhea is among the top ten diseases for under 5 years children. In Nyahururu County Hospital diarrhea is the 2nd top ten diseases for children.

METHODOLOGY  This is a retrospective study. In the hospital we have tools for documentation, which are nationally and globally acceptable. Analysis of under 5 years children treated as an outpatient and also admitted with diarrhea diseases was done between January 2015 and December 2015. The death due to diarrhea during the same period was also captured and also analyzed.

RESULT  669 children were treated as outpatient during that period, 200 under 1 year children were admitted in the pediatric unit for management and care, 150 children between the ages of 1-4 years were also admitted. Among the admitted children 20 of under 1 year and 10 of between 1-5 years died due to diarrhea disease. The rest our discharged home in a stable condition.

CONCLUSION  10% of both under 1 year and children between 1-5 years during the period died. Majority of admission were children below 1 year of life, who also lead in the number of death.

RECOMMENDATION  Children under the age 5 years and especially those below the 1st year of life are at high risk of diarrhea disease due to delicate age and low immunity. Introduction to vaccine to target the pathological agent, and give immunity to children will reduce the mortality and morbidity of the under 5 years children, this will also reduce hospital hospitalization and attendance, thus improving health, economy and reduce poverty level especially the developing counties like Kenya.
Host immune responses and correlates of protection

Oral Presentations

IMM02 Human ETEC Challenge: Differences in the Symptomatic and Asymptomatic Host Response

William E. Yang1,2, Sunil Suchindran2, Bradley P. Nicholson2,3, Micah T. McClain2,3,4, Thomas Burke2, Geoffrey S. Ginsburg2, Clayton D. Harro5, Subhra Chakraborty6, David A. Sack5, Christopher W. Woods2,3,4, Ephraim L. Tsalik2,4,6

1Duke University School of Medicine, Durham, NC
2Center for Applied Genomics & Precision Medicine, Department of Medicine, Duke University, Durham, NC
3Internal Medicine Service, Durham VA Medical Center, Durham, NC
4Division of Infectious Diseases, Department of Medicine, Duke University Medical Center, Durham, NC
5Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
6Emergency Medicine Service, Durham VA Medical Center, Durham, NC

Introduction. Enterotoxigenic Escherichia coli (ETEC) is a globally prevalent cause of diarrheal disease. Little is known about the host transcriptional response to infection and its contribution to the clinical variability of ETEC infection. We report the first gene expression analysis of the host response to ETEC infection through an experimental challenge in a non-endemic population.

Methods. Thirty healthy adults without history of ETEC infection for two years prior were challenged with unattenuated ETEC H10407. Peripheral blood RNA samples were collected for transcriptomic analysis eight hours after challenge (baseline) and daily thereafter for approximately eight days. Analysis focused on subjects developing severe symptoms (n=6) and matched controls who shed ETEC but were asymptomatic (n=6). Gene expression was characterized using Affymetrix microarrays. Unsupervised Bayesian factor regression modeling was used for dimension reduction whereas limma was used for longitudinal analysis. Gene ontology enrichment and drug repositioning analyses were performed to gain functional insights into the transcriptomic findings.

Results. Symptomatic subjects demonstrated differential expression of 406 genes between baseline and the time of peak symptoms. Functional annotation revealed increased immune response, and decreased protein synthesis. When compared to time-matched asymptomatic subjects, those with symptomatic ETEC infection had 254 differentially expressed genes, mostly related to immune response. We also observed the differential expression of 29 genes at baseline that discriminated subjects who went on to develop symptoms compared to those who remained asymptomatic, suggesting host-factors conferring susceptibility/resilience to infection. These 29 genes were largely involved in immune function such as MHC class I and complement. Drug repositioning analysis was used to correlate ETEC-induced changes with known transcriptional responses induced by a catalogue of >6100 small, bioactive molecules. This revealed significant overlap between ETEC infection and Hsp90 inhibitors, NF-κB inhibitors, protein synthesis inhibitors, typical antipsychotics, and zinc-dependent histone deacetylase inhibitors. These similarities suggest novel mechanisms of pathogenesis and potential therapeutic candidates.

Conclusions. ETEC infection induces statistically significant and biologically plausible differences in host gene expression, detectable by microarray analysis of peripheral blood. These analyses offer novel insights into ETEC pathogenesis. Differential baseline expression of some genes identifies candidate host factors conferring susceptibility or resilience to ETEC infection.
Development and application of proteome microarrays to characterize immune responses in human volunteers following ETEC experimental challenge

Arlo Randall1, Subhra Chakraborty2, James M. Fleckenstein3, Doug Molina1, Clayton D. Harro2, Barbara DeNearing2, Jessica Brubaker2, David A. Sack2, A. Louis Bourgeois2, Philip L. Felgner1, Xiaowu Liang1, Sachin Mani4, Heather Wenzel4, and David A. Rasko5

1 Antigen Discovery, Inc. (ADI), Irvine, California; 2Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; 3Department of Medicine, Division of Infectious Diseases and the Molecular Microbiology and Microbial Pathogenesis Program, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri; 4PATH, Washington, DC; 5The Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA

The GEMS study has reaffirmed enterotoxigenic Escherichia coli (ETEC) as a major cause of childhood morbidity and mortality in the developing world. Profiling the breadth of antigens recognized after ETEC infection can identify antibody signatures associated with protective immunity, and provide additional targets to inform vaccine development. Mucosal and serum antibody responses in human subjects experimentally challenged with ETEC strain H10407 has recently been characterized by ELISA against canonical ETEC antigens, CFA/I, LTB and O78 LPS. To expand antigen discovery efforts, permit further refinement of immunological benchmarks of protection, and to develop an additional tool to assess vaccine performance, we developed prototype ETEC proteome microarray. Here we detail array development, and demonstrate the utility of this platform in testing antibody in lymphocyte supernatants (ALS) and sera from 20 subjects experimentally infected with H10407 and 10 subjects re-challenged with H10407 4-6 weeks after their initial challenge. Following cloning and expression via high-throughput in vitro transcription-translation (IVTT), selected ETEC proteins were printed directly onto nitrocellulose-coated microarrays. After sample probing of these microarrays bound antibodies were detected using anti-human antibody biotin conjugates followed by streptavidin-conjugated SensiLight PBXL-3. Finally, microarrays were scanned and fluorescence signals quantified. In general, reactivity to IVTT proteins was congruent with responses to recombinant antigens, and similar to earlier ELISA results for LTB, CFA/I. For ALS samples, the magnitude and breadth of responses was greatest at day 7 after initial challenge with H10407. Average increases from baseline (Day -1) to Day 7 were statistically significant for 7 of 9 recombinant proteins (paired t-test). The greatest responses were directed against CFA/I, LTB, EtpA, EatA, YghJ, EaeH and surprisingly CS3. Similarly, ALS responses to IVTT proteins also tended to peak on day 7 after challenge. Subjects mounted statistically significant responses to 15 of the 957 proteins after correction for false discovery. In addition, strong mucosal responses were also noted to flagellin (FlfC) (ETEC_2032), Antigen 43 (ETEC_4462), and a protein of uncertain immunological significance, outer membrane protein W (ETEC_1358). Most of the proteins showing the greatest ALS reactivity were also recognized by serum IgA and/or IgG. Mucosal and serum antibody response patterns on the arrays after re-challenge were similar in subjects challenged with H10407 a second time, with the exception that responses to both CFA/I and LTB appeared to be enhanced upon re-exposure. Based on these initial encouraging results, further development of these proteome arrays is warranted. Their utility in measuring serum and mucosal responses in subjects receiving candidate ETEC vaccines and in infants and young children experiencing natural ETEC infection in the endemic areas should be evaluated.
Characterization of the humoral immune response to *Shigella* towards identification of correlates of protection

Daniel Cohen¹, Shiri Meron-Sudai¹, Anya Bialik¹, Alexandra Dorman¹, Sophy Goren¹, Amit Hochberg², Uri Rubinstein³, Shai Ashkenazi⁴

¹School of Public Health, Sackler Faculty of Medicine, Tel Aviv University
²Department of Pediatrics, Hillel Yaffe Medical Center, Hadera
³Department of Pediatrics, Laniado Medical Center, Natanya
⁴Department of Pediatrics A, Schneider Children Medical Center and Sackler Faculty of Medicine

**Background:** Identification of correlates of protection is important in the process of vaccine development and evaluation. We have previously shown in sero-epidemiological studies and later on in efficacy studies of *Shigella* conjugate vaccines that high levels of serum IgG antibodies to *Shigella* LPS correlated with protection against shigellosis caused by the homologous serogroup.

**Objectives:** In frame of the STOPENTERICS program, we determined functional capabilities of the serum IgG anti-*Shigella* LPS and examined additional immune parameters induced by natural *Shigella* infection towards the development of a single or composite correlate of immunity.

**Results:** We found a diverse susceptibility of *S. sonnei* phase 1 strains to serum killing with a significant correlation with the level of *S. sonnei* LPS antibodies (r=0.495; p=0.014) in samples of children and young adults. Interestingly, sera obtained from adult convalescent cases of *S. sonnei* shigellosis showed more frequently bactericidal anti-*S. sonnei* activity as compared with sera of children (75% vs 39.1%, p=0.02). Using the thiocyanate elution assay, we found that sera with bactericidal activity (n=17) had a significantly higher avidity index than sera with no bactericidal activity (n=9) against *S. sonnei* (Avidity index: 2.267 vs 1.76, p=0.048).

The B memory cell response (ELISPOT method, Crotty et al. 2004), to *Shigella* homologous LPS was the highest at early convalescence, 18 days to 2.5 months after the onset of disease. Nine of 12 subjects examined (75% with a GMT of 76 BM/10⁶PBMC) had a significant IgA B memory cell response which was still persistent in 32% of children at late convalescence corroborating data on the limited length of immunity conferred by natural *Shigella* infection. We found significant correlations between the IgA B memory cell response and both IgA and IgG serum response to homologous LPS in children with *S. sonnei* and *S. flexneri 2a* shigellosis (n=84, Pearson corr.=0.723, P<0.01 for IgA and Pearson corr.=0.762, P< 0.01 for IgG).

**Conclusions:** Serum IgG antibodies to *Shigella* LPS emerge as a good correlate of protection with mechanistic capabilities. We continue to evaluate the possible added value of other immune parameters following exposure to natural infection and candidate vaccines.
Poster Presentations

**IMM04 Modulation of antibody responses against enterotoxigenic *Escherichia coli* (ETEC) by T cells in humans**

Monica A. McArthur, Wilbur H. Chen, Myron M. Levine and Marcelo B. Sztein

Center for Vaccine Development, University of Maryland, Baltimore Maryland, USA

Enterotoxigenic *Escherichia coli* (ETEC) is one of the most important pathogens contributing to moderate to severe diarrhea in children in low- and middle-income countries. Moreover, it is also the most common cause of diarrhea among travelers who visit developing countries. Despite the threat this organism poses to global health, there is currently no licensed vaccine available. Vaccine development has been hindered, in part, by lack of known immunological correlates of protection. Furthermore, there is no animal model that faithfully recapitulates human disease requiring human studies to assess immunogenicity and protective efficacy of vaccine candidates. In order to establish a controlled human infection model (CHIM) for ETEC, volunteers were challenged with wild-type ETEC at the University of Maryland. Peripheral blood mononuclear cells were collected prior to and at multiple time-points following challenge in order to study immune responses in volunteers who developed moderate to severe diarrhea and those who did not. Due to the non-invasive nature of ETEC, antibodies have been proposed as a major contributor to protection. Thus far, however, few studies have investigated the potential contribution of T cells and their role in protection against this organism. Peripheral T follicular helper cells (pTfh) are a circulating subset of CD4+ T cells characterized by the expression of CXCR5 which are involved in providing B cell “help”. Here we report the first study of modulation of ETEC-specific antibody responses by pTfh in humans. We used mass cytometry to characterize the T cell responses against ETEC-specific antigens in volunteers prior to and at multiple timepoints after challenge with wild-type ETEC. We identified higher levels of expression of the gut-homing molecule integrin α4β7 by pTfh at early time-points post-challenge in volunteers who did not develop moderate to severe diarrhea. Furthermore, integrin α4β7 expression by pTfh was inversely correlated with stool output in volunteers post-challenge. We also performed B memory (Bm) assays to identify ETEC-specific IgA and IgG producing Bm cells following challenge with wild-type ETEC. Of importance, there was a correlation between higher expression of integrin α4β7 by pTfh and higher ETEC-specific IgA BM responses; however, there was no correlation with ETEC-specific IgG BM responses. Taken together, our results indicate that the gut-homing potential of pTfh may be an important indicator of protection against ETEC and that the presence of pTfh with gut-homing potential soon after challenge may play a role in development of IgA Bm responses at later timepoints, suggesting that it might be an early indicator of long-term protection.
IMM05  *Shigella* Outer Membrane Vesicles: A Novel Particles of Next Generation Vaccine

Hemanta Koley*, Soma Mitra, Ritam Sinha, Dhrubajyoti Nag

Division of Bacteriology, National Institute of Cholera and Enteric Diseases, P-33, C.I.T. Road, Scheme XM, Beliaghata, Kolkata-700010, India. *

The gram-negative bacterium *Shigella* secretes outer membrane vesicles evidently during normal growth condition. We have observed under electron microscope a distinctive vesicle secretion of different serogroups. Among them, *Shigella boydii* type 4 BCH 612 strain releases outer membrane vesicles (OMVs) more vigorously during growth than the other strains, selected for this experiment. In this study, we immunized female adult mice by the intragastric route with purified single outer membrane vesicles (SOMVs, 32µg per 100µl), isolated from *Shigella boydii* type 4 BCH 612 strain. Immunized mice induced specific, high-titer immune responses against a variety of antigens present in the OMVs. We challenged the offspring of immunized female mice with *Shigella* sp. of four serogroups via the oral route in two consecutive periods, approximately 70th and 120th day after the 4th and last immunization. The offspring was protected against colonization with homologous strain, *Shigella boydii* and also heterologous strains *Shigella dysenteriae* 1, *Shigella flexneri* 2a and *Shigella sonnei* in challenge experiments. Our results showed that 100% homologous protection was quite satisfactory, 75% average heterologous protective efficacy bestowed by the OMVs of *S. boydii* was not at all promising from the purview of disease prevalence and severity. Then, we have advanced our research by formulating multi-serotype outer membrane vesicles (MOMVs), mixing the OMVs of *Shigella dysenteriae* 1 stx, *Shigella flexneri* 2a, 3a and 6, *S. boydii* type 4 and *Shigella sonnei* to achieve a broad spectrum protection against shigellosis. Adult mice were immunized orally with 50 g of MOMVs, four times at weekly intervals. Immunological parameters were observed at various time points, before, during and after immunization, in adult mice. Passive protection was examined in their offspring by measuring protective efficacy and studying intestinal colonization, after challenging with various *Shigella* strains. Immunized dams exhibited a consistent broad spectrum antibody response. 3–4 day-old offspring of immunized dams showed significant long term passive protection against wild type *S. flexneri* 2a, 3a, and 6, *S. boydii* type 2 and *S. dysenteriae* 1. Their stomach extracts, essentially containing mother’s milk, have also exhibited significant levels of anti-MOMVs immunoglobulins. In conclusion, MOMVs formulation represents an easy, safe immunization strategy that was found suitable to provide complete passive protection to the neonatal mice against all four serogroups of *Shigellae*. In this study, we observed the inefficient of *Shigella* to secrete significant amount of outer membrane vesicles naturally, during growth, making the study very time consuming and costly. To overcome this trouble, we disrupted tolA gene, necessary to maintain outer membrane integrity, from *Shigella boydii* type 4 BCH612 strain. 60% increase of OMVs secretion was noticed (80mg/500ml culture) in the tolA mutant than the wild type (50mg/500ml culture). This study has efficiently established a new technique for better cost effective production of OMVs in *Shigellae*. ΔtolA-OMVs was showing valuable role in the field of next-generation non-living vaccine against human shigellosis in near future.
Low-endotoxic lipopolysaccharides from *Escherichia coli* O157:H7 and O104:H4 induce long-term specific immune response in mice and protect them against enterohemorrhagic *Escherichia coli* (EHEC) infection

A.A. Markina¹, V.L. L’vov², M.E. Golovina², I.V. Ankudinov², V.V. Firstova³, I.A. Dyatlov³, P.G. Aparin¹,²

¹National Research Center Institute of Immunology Federal Medical-Biological Agency (FMBA of Russia), ²ATV D-TEAM Co., Ltd., Moscow, Russian Federation, ³State Research Center for Applied Microbiology and Biotechnology, Russian Federation

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 and O104:H4 are important causes of food-borne illness such as severe hemorrhagic colitis and the extraintestinal complication of hemolytic–uremic syndrome (HUS), which is the leading cause of kidney failure and deaths of children under 5 years as well as the elderly. HUS occurs in more than 20% of the identified cases of EHEC with a fatality rate of up to 30%. *E.coli* O157:H7 and O104:H4 are Shiga-toxin-producing strains, that’s why antibiotic treatment does not ameliorate the course of the enteritis caused by these two pathogens and may increase the incidence of HUS due to the release of Shiga-toxins.

There were detected high titers of serum IgG anti-O-specific polysaccharide domain of lipopolysaccharide (LPS) of *E.coli* O157:H7 and O104:H4 in patients who recover of HUS caused by the same EHEC strains, which indicates a high antigenic activity of LPS. However, the use of LPS is limited due to its high endotoxicity. The way to obtain clinically applicable preparations is associated with a rational reduction of the endotoxicity of LPS without reduction of immunogenicity, i.e. with conservation of the primary structure of O-specific polysaccharide domain of LPS. We first obtained low-endotoxic apyrogenic LPS (LET-LPS) from enterohemorrhagic *E.coli* O157:H7 and O104:H4. These LET-LPS are structural analogs of native pyrogenic *E.coli* O157:H7 and O104:H4 LPS (nLPS), with low-acetylated derivatives of lipid A. Endotoxicity of *E.coli* O157:H7 and O104:H4 LET-LPS was in 19 and 108 times less, respectively, of this index for nLPS of the same strains.

To study immunogenic properties of *E.coli* O157:H7 and O104:H4 LET-LPS mice (CBA×C57B1/6)F1 were intraperitoneally (i/p) immunized two times (0, 30 day) with sterile saline (control group) and LET-LPS at a dose of 1,25 mg/kg. Serum IgG anti-LPS levels were determined after 15 days after secondary immunization. LET-LPS of *E.coli* O157:H7 and O104:H4 induced a powerful immune response: the titers of IgG against nLPS *E.coli* O157:H7 and O104:H4 in immune sera were higher compared with controls at 36 and 32 times, respectively.

To reproduce the experimental lethal infection mice Balb/c were i/p immunized with sterile saline (control group) and LET-LPS of *E.coli* O157:H7 and O104:H4 at doses of 1,25 and 2,5 mg/kg according to the abovementioned scheme and after 15 days after secondary immunization they were i/p infected with the minimum lethal dose (~ 1x10⁸ microbial cells/mouse) of cells of *E.coli* O157:H7 or O104:H4, respectively. Immunization with *E.coli* O157:H7 LET-LPS at doses of 1,25 and 2,5 mg/kg provided 60 % and 70 % levels of survival, respectively, compared to 50% of survivors in the control group. The mortality was effectively suppressed in mice immunized with *E.coli* O104:H4 LET-LPS at doses of 1,25 and 2,5 mg/kg; the survival rates were 60% and 100%, respectively, compared to 90% death of the control animals.

Immunization with LET-LPS of *E.coli* O157:H7 and O104:H4 induce in mice the formation of long-term (≥ 45 days) specific immune response and serum IgG against LPS *E.coli* O157:H7 and O104:H4 may confer protective immunity to these enteric pathogens.
**IMM08  Monoclonal antibodies to Shigella LPS that are useful for vaccine production**

Jisheng Lin\(^a\), Mark A. Smith\(^b\), William H. Benjamin\(^a\), Robert W. Kaminski\(^b\), Heather Wenzel\(^c\), Jigui Yu\(^a\), Moon H. Nahm\(^a\)

a Department of Pathology, University of Alabama at Birmingham, USA;  
b Subunit Enteric Vaccines and Immunology, Walter Reed Army Institute of Research, USA;  
c PATH, Washington DC, USA.

**Background:** Shigellosis is a major public health problem and there is a need for an effective *Shigella* vaccine. As antibodies to LPS are protective, one vaccine approach is to produce multivalent LPS-protein conjugate vaccines. Development of such a vaccine would be facilitated by monoclonal antibodies (Mabs) to LPS from the *Shigella* species included in the multivalent conjugate vaccines.

**Method:** To produce hybridomas, BALB/c mice were intraperitoneally immunized four times with 10 µg of LPS purified from several *Shigella* isolates. Four days after the last immunization, the splenocytes of immunized mice were fused with SP2/0-Ag14 cells and desirable hybridomas were identified by testing culture supernatants for antibodies binding LPS-coated ELISA plates. Cells in positive wells were subcloned by limiting dilution.

Mabs from the hybridomas were studied by inhibition ELISA for quantifying LPS and by flow cytometry to identify the serotypes of bacteria in suspension. Mabs were also tested for activity in a bactericidal assay (BCA). Briefly, variously diluted Mabs supernatants were mixed with 100 cfu of target bacteria and baby rabbit serum (source of complement). After two hours at 37°C, reaction mixtures were spotted on agar plates. After an overnight incubation at 29°C, the plates were overlaid with agar containing 100 µg/mL triphenyltetrazolium chloride. After two hours, bacterial colonies were counted.

**Results:** We obtained 10 hybridomas against *S. flexneri* 2a, 18 against *S. flexneri* 3a and 8 against *S. sonnei*. We chose, for further studies, six hybridomas (two for each species) that were specific to the target serotype. Their supernatants agglutinated target bacteria and produced results identical to those obtained with the commercially available polyclonal serotyping rabbit sera. An inhibition ELISA for *Shigella* LPS could be developed with the mAbs, and it could be used to identify and quantitate LPS in samples. The mAbs were also useful for identifying the target bacteria in suspension by flow cytometry. All six mAbs killed *Shigella* of the homologous serotype only, except for Hflex2a4 that killed both *S. flexneri* 2a and 2b.

**Conclusion:** The Mabs are useful for quantifying the amount of LPS and the number of bacteria in vaccine components. They are also useful as positive controls and standards for BCAs. Discovery of Hflex2a4 suggests that *S. flexneri* 2a LPS may induce cross-protection against *S. flexneri* 2b.
IMM10  Diarrhoea caused by enterotoxigenic *Escherichia coli* induces immune responses in the circulation

Taufiqur Rahman Bhuiyan¹, Rasheduzzaman Rashu¹, Marjahan Akhtar, Naoshin Sarmin Nishat¹, Rubel Hoq¹, Lazina Hossain¹, Fahima Chowdhury¹, Ashraful Islam Khan¹, Anna Lundgren², Jason B Harris³, Edward T Ryan³, Stephen B Calderwood³, Ann-Mari Svennerholm², Firdausi Qadri¹

¹ Infectious Disease Division, icddr,b, Dhaka, Bangladesh; ² Department of Microbiology and Immunology, University of Gothenburg, Sweden; ³ Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts

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. We are currently carrying out a phase I/II clinical trial with the oral multivalent ETEC vaccine (ETVAX) in different age group of participants in Bangladesh. Humoral and adaptive immune response will be evaluated in plasma, ALS, mononuclear cells and mucosal fecal specimens. In a recent time kinetics experiment, we have shown the importance of selecting optimal time points for analysis of mucosal antibody responses in clinical trials of oral vaccines. In natural ETEC diarrhea, we showed a significant increase in toxin-specific gut homing ASCs at day 7 compared to day 2 and 30 after onset of illness, and in comparison to healthy controls. A similar elevation of responses to the ETEC colonization factors CS6 and CFA/I were observed in patients infected with CS6 and CFA/I positive ETEC strains. Meanwhile we have also reported that patients infected with ETEC expressing LT or LT+ heat stable toxin (ST) and CFA/I group or CS6 colonization factors developed LTB, CFA/I or CS6 specific memory B cell responses at day 30 after infection. Similarly, these patients developed high avidity IgA and IgG antibodies to LTB, CFA/I or CS6 at day 7 that remained significantly elevated at day 30 when compared to the avidity of these specific antibodies at the acute stage of infection (day 2). The memory B cell responses, antibody avidity and other immune responses to CFA/I not only developed in patients infected with ETEC expressing CFA/I but also in those infected with ETEC expressing CFs with CFA/I cross-reacting epitopes. We also detected a significant positive correlation of LTB, CFA/I and CS6 specific memory B cell responses with the corresponding increase in antibody avidity. In addition, we also 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 EatA in natural ETEC infection. We demonstrated that natural infection with ETEC induces ASC and memory B cells and high avidity antibodies to LTB and colonization factor CFA/I and CS6 antigens that could mediate anamnestic responses on re-exposure to ETEC; these findings may help in understanding the requirements to design effective vaccination strategies.
IMM11 Usefulness of the Shigella bactericidal assay for investigating immunological correlates of immunity in clinical trials and with non-human primate samples

Hailey Petersena, Robert Kaminskia, Moon Nahmb

a Subunit Enteric Vaccines and Immunology, Walter Reed Army Institute of Research, USA
b Department of Pathology, University of Alabama at Birmingham, USA

Background: A clear immune correlate of protection to guide the rational design of Shigella vaccines does not currently exist. However, measurements of functional antibody responses may provide an important step toward establishing an immune correlate of protection. Recently, a Shigella-specific serum bactericidal assay (SBA) has been developed to assess the functionality of antibodies generated after infection or vaccination. The SBA was used to determine bactericidal titers in several studies of high interest to the Shigella vaccine development field which were previously conducted in non-human primates (NHPs) and humans.

In a study previously described by Formal and colleagues, NHPs were orally challenged with S. flexneri 2a, 2457T, rested for 5 weeks, and then re-challenged with S. flexneri 2a, 2457T or challenged with S. sonnei, 53G. Veterans of the S. flexneri 2a, 2457T challenge were completely protected against subsequent challenge with S. flexneri 2a, 2457T (0/11 with illness) but not protected against challenge with S. sonnei, 53G (8/12 with illness) demonstrating that protection was serotype-specific. Blood collected prior to and after each infection was analyzed in the SBA for titers against S. flexneri 2a and S. sonnei. Significant S. flexneri 2a bactericidal antibodies were generated following primary challenge and were boosted upon re-challenged with S. flexneri 2a were significantly correlated with protection. Furthermore, SBA titers were serotype-specific and there was no significant cross reactivity in the bactericidal antibodies between Shigella strains used in the experiment.

In a second study previously described by Coster et al, human volunteers were immunized orally with 10^4 CFU live attenuated vaccine strain S. flexneri 2a, SC602 and challenged 8 weeks later with 2x10^3 virulent S. flexneri 2a, 2457T. Of the seven immunized volunteers, all were protected against shigellosis and several protected against diarrhea whereas all but one individual in the control group developed severe shigellosis following challenge. Sera collected prior to and after challenge were evaluated for SBA titers directed to S. flexneri 2a, 2457T. Following immunization, levels of bactericidal activity significantly increased in vaccinated individuals. Unimmunized volunteers saw an increase in bactericidal antibody following challenge, but their bactericidal antibody titers remained significantly lower than immunized volunteers. The SBA titers in the vaccinated group were significantly correlated with protection following subsequent challenge with virulent S. flexneri 2a 2457T.

The Shigella SBA offers a means to measure the capacity of vaccine induced antibodies to kill live, virulent bacteria and will contribute to the understanding and development of a protective immune responses. Furthermore, these results suggest that bactericidal antibody titers may serve as an immune correlate of protection. Additional studies are underway to validate the SBA as a reliable correlate of immunity and to elucidate the role of antibody isotypes and subclasses in bactericidal activity.
Impact of the microbiome on pathogenesis and vaccination
Oral Presentations

MB01 Diversity, dynamics and diarrhea: how ETEC infection impacts resident E coli
Taylor K.S. Richter (1), Jane Michalski (1), Wilbur H. Chen(2), and David A. Rasko(1,3)

(1) The Institute for Genome Sciences, (2) The Center for Vaccine Development, (3) Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, USA

Enterotoxigenic E. coli (ETEC) is the main cause of traveller’s diarrhea and a leading source of diarrhea in the developing world. While the physical and economic tolls of this disease are well documented, impacts of this pathogen, and its treatment, on the commensal gut microbiome are not well understood. Using samples from an ETEC challenge study, E. coli colonies were isolated from before, during, and after ETEC infection. Whole genomes obtained from the E.coli colonies were used in phylogenomic and gene content analyses to investigate the diversity of the resident E. coli, as well as the dynamics of the challenge strain, H10407. Overall, results demonstrate that there is greater diversity in commensal E. coli and in E. coli community response than previously expected. Phylogenomic analyses demonstrated that individual patients had a unique community of E. coli. Investigations in to gene content, focusing on virulence and resistance genes, demonstrated the genomic diversity among commensal E. coli not otherwise captured by phylogenomics. Several patterns emerged regarding the dynamics of the E. coli community throughout the course of the trial that may be linked to the observed difference in disease state of the individual patients and suggests resiliency of the resident community following antibiotic exposure. This study, the first to observe the dynamics of E.coli colonization before, during and after ETEC infection. This study provides valuable insights into the interactions of the gastrointestinal microbiome, its responses to infection and antibiotic exposure, the impact of the community of disease severity, as well as the alterations in the pathogen genome during the course of disease.
MB02  Individual-specific changes in the human gut microbiota after challenge with enterotoxigenic Escherichia coli and subsequent ciprofloxacin treatment

Mihai Pop1,2, Joseph N. Paulson1,3,4,5, Subhra Chakraborty6, Irina Astrovskaia1, Brianna R. Lindsay7,8, Shan Li7, Héctor Corrada Bravo1,2, Clayton Harro6, Julian Parkhill9, Alan W. Walker9,10, Richard I. Walker11, David A. Sack6, O. Colin Stine7.

1- Center for Bioinformatics and Computational Biology, University of Maryland, College Park, Maryland, USA; 2 – Department of Computer Science, University of Maryland, College Park, Maryland, USA; 3 – Graduate Program in Applied Mathematics & Scientific Computation, University of Maryland, College Park, Maryland, USA; 4 – Department of Biostatistics and Computational Biology, Dana Farber Cancer Institute, Boston, Massachusetts, USA; 5 – Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts, USA; 6 – John Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; 7- School of Medicine, University of Maryland, Baltimore, Maryland, USA; 8 - Merck & Co. Inc. North Wales, Pennsylvania, USA; 9 – Pathogen Genomics Group, Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, UK; 10 – Microbiology group, Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK; 11– PATH, Washington, DC, USA

Introduction. Enterotoxigenic E. coli (ETEC) is a major cause of diarrhea in inhabitants from low-income countries and travelers to these countries. The impact of the human commensal microbiota on the initiation and progression of ETEC diarrhea is not yet well understood.

Methods. We examined the changes in the gut microbiota as a component of a series of volunteer challenge studies in JHU to determine the dose response with ETEC strain H10407 (O78:H11; LT+ ST+ CFA/I+). In this study we used the low doses 1x10^5 and 1x10^6 CFU of ETEC H10407. All of the subjects received a three-day course of ciprofloxacin whether or not they exhibited symptoms and were followed up on days 28 and 84. Out of 30 patients who were challenged, we chose the 5 subjects with 54 specimens who developed moderate to severe diarrhea and 7 subjects with 78 specimens of the 25 who did not. We used 16S rRNA gene sequencing to study changes in the fecal microbiota of 12 volunteers before and after infection with ETEC (H10407) and subsequent treatment with ciprofloxacin. We developed and trained a model for predicting disease based on microbiota features. For feature set selection we only used samples prior to infection.

Results. The number of sequences assigned to the genus Escherichia varied substantially across time and was linked to the clinical observations. Subjects with ETEC diarrhea shed high concentrations of E. coli, which can be detected by quantitative culture, quantitative PCR, and 16S rRNA gene sequencing, while those with asymptomatic infections have concentration below detectable levels using 16S rRNA gene sequencing. The diversity of the stool microbiota as measured by the Shannon diversity index, was highly variable across all individuals, and neither the onset of symptoms nor administration of antibiotics produced the same consequences in all individuals. Both diarrhea and ciprofloxacin treatment led to a significant decrease in overall diversity (p<0.05, rank-sum Wilcoxon test), while the overall diversity of the gut microbiota was largely restored in all patients by follow-up visits 28 and 84 days after the initiation of the study. Co-existence of ciprofloxacin-susceptible and resistance strains was demonstrated. At the time of inoculation with ETEC, the presence of specific species predicted that the infection would remain asymptomatic.

Conclusions. We found that symptomatic ETEC infections, but not asymptomatic infections were associated with high concentration of E. coli. Both infection and ciprofloxacin treatment caused variable changes in other bacteria. Ciprofloxacin had a temporary effect, including induction of a surge in bacteria detected only while ciprofloxacin was administered, but not long-lasting effects on intestinal microbiota composition. Our analysis provide clear hypotheses to be tested in subsequent studies.
Poster Presentations

MB04 Human enteroid model of Enterotoxigenic E. coli (ETEC) diarrhea and demonstration of MRP-related epithelial cell secretion of cGMP

Huimin Yu1, James Fleckenstein2, Olga Kovbasnjuk1, Mark Donowitz1, and Jennifer Foulke-Abel1

1. Johns Hopkins University School of Medicine 2. Washington University School of Medicine

Background: According to the Global Enteric Multicenter Study (GEMS) study, Enterotoxigenic E. coli producing heat stable enterotoxin (STa-ETEC; with or without co-expression of heat-labile enterotoxin, LT) is one of the four leading pathogens world-wide that causes acute diarrhea and diarrheal deaths in developing countries. It is also the leading cause of traveler’s diarrhea. Previous studies on ETEC are mainly based on animal models and use of transformed cell lines. The pathophysiology of ETEC and host intestinal response is not well understood. Enteroids are an ex vivo primary cell culture model which propagates indefinitely without significant change. Enteroids from normal human intestine represent a powerful model to understand intestinal physiology and pathophysiology. An enteroid biobank from normal subjects has been developed and is being used to understand normal GI physiology and host-pathogen interactions for common human diarrheal diseases.

Methods: Human intestinal biopsies or surgical specimens were obtained through protocols approved by the Johns Hopkins University School of Medicine Institutional Review Board. Crypt isolation, media, and 3-D culture methods were derived from Sato et al, Gastroenterology 2011. 2-D human enteroid monolayers were developed by seeding enteroid fragments from 3-D cultures on Corning Transwells coated with collagen IV and grown to confluency, as judged by light microscopy and TER measurements. Enteroids were differentiated by exposure to medium lacking Wnt3a. In 2-D culture, the apical cell surface faces outward allowing interaction with ETEC. Elevated cGMP in apical, basolateral, and intracellular fractions was evaluated by ELISA.

Results: To study the role of STa, human enteroid monolayers were exposed to wild type ETEC. 1) Different exposure times and bacterial concentrations were tested. Wild type ETEC added to human jejunal enteroids at 10^9 cfu/ml for 6 hours released STa apically and increased intracellular cGMP. The detected cGMP amount was comparable to that seen with direct apical addition of 1 nM STa to human enteroids. Exposure to E. coli K12 or HS strains did not elevate cGMP. 2) cGMP was transported into the apical and basolateral compartments. The role of the secreted cGMP is not understood but it is speculated that the basolateral cGMP may modify gastrointestinal pain via modulating sensory fiber activity. 3) The basolateral secretion of cGMP was decreased by multidrug resistance-associated protein (MRP) inhibitors probenecid and MK571.

Summary: 1) In the human enteroid model, STa released by ETEC results in elevated epithelial cell cGMP. 2) The cGMP increase is comparable to a 1 nM STa luminal concentration. 3) Increased intracellular cGMP is associated with apical and basolateral secretion of the molecule. 4) Basolateral cGMP secretion is likely mediated by MRPs.

Conclusions: The human enteroid monolayer model of ETEC diarrhea provides an enhanced platform to mimic intestinal function, study the pathophysiology of the disease, and to dissect host-pathogen interactions, all of which can substantially reduce the burden of ETEC diarrhea world-wide.
New Antigen Discoveries
Oral Presentations

ANT02 Studying enterotoxigenic *Escherichia coli* (ETEC) using whole genome sequencing

Astrid von Mentzer¹,², Åsa Sjöling³, Gordon Dougan² and Ann-Mari Svennerholm¹

¹Department of Microbiology and Immunology, Gothenburg, Sweden; ²Wellcome Trust Sanger Insitute, Hinxton, United Kingdom; ³Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden

Enterotoxigenic *Escherichia coli* (ETEC) infections are common but a vaccine targeting ETEC in humans is not yet available. Based on results from both preclinical and clinical studies we have concluded that a successful ETEC vaccine should include both toxin and common colonization factor (CF) antigens.

Using whole genomes sequencing and epidemiological data of 362 ETEC isolates we study ETEC evolution on a global level. We have shown that major lineages comprising isolates with specific virulence profiles, i.e. CFs and LT and/or ST, are stable and spread worldwide. This indicates that the virulence genes have been acquired once and then spread through clonal expansion. It indicates that the development of a vaccine based on the most prevalent CFs could be protective against a large proportion of ETEC diarrhoea cases.

It is known that at least 30% of all clinical ETEC isolates lack a known CF, depending on geographical location. Several attempts have been made to identify novel CFs for future inclusion in an ETEC vaccine to broaden possible protective coverage.

In total, 94 isolates of the 362 sequenced ETEC isolates are “CF negative” (lack a known CF). These isolates have been studied in more detail with the aim of identifying novel CFs. Through comparative genomics we have identified a novel CF, CS30, which is related to the porcine CF 987P (F6). The major subunit of CS30 is 18.5 kD in size and fimbriae assembly is dependent on its expression. In addition, CS30 positive bacteria are heavily fimbriated, showed by electron microscopy, and also proven to be functional, i.e. this CF promotes binding to human intestinal (Caco-2) cells and is thermo-regulated. Furthermore, using phenotypical analysis (SDS-PAGE, thermoregulation, Caco-2 adhesion assays and electron microscopy) we have identified a number of isolates that may harbour additional putative fimbrial and afimbrial novel CFs. Comparative genomics have revealed one or two operons in the whole genome sequence data that are further analysed.
ANT03 Towards rational design of a toxoid vaccine against the heat-stable toxin-producing 
*Escherichia coli*

Pål Puntervoll\textsuperscript{a,b}, Yuleima Diaz\textsuperscript{a}, Arne M. Taxt\textsuperscript{b}, Ephrem D. Zegeye\textsuperscript{a}, Morten A. G. Larsen\textsuperscript{a}, James P. Nataro\textsuperscript{c}, John D. Clements\textsuperscript{d}, Halvor Sommerfelt\textsuperscript{b,e,f}

\textsuperscript{a}Centre for Applied Biotechnology, Uni Research Environment, Bergen, Norway; \textsuperscript{b}Centre for International Health, University of Bergen, Bergen, Norway; \textsuperscript{c}University of Virginia School of Medicine, Charlottesville, Virginia, USA; \textsuperscript{d}Tulane University School of Medicine, New Orleans, Louisiana, USA; \textsuperscript{e}Centre for Intervention Science in Maternal and Child Health, Centre for International Health, University of Bergen, Bergen, Norway; \textsuperscript{f}Department of International Public Health, Norwegian Institute of Public Health, Oslo, Norway

Enterotoxigenic *Escherichia coli* (ETEC) are an important cause of diarrheal disease 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 peptide must be made immunogenic by coupling it to a carrier. Second, ST must be altered by mutation to make it non-toxic and to avoid eliciting an immune response that cross-reacts with the endogenous peptides uroguanylin or guanylin. Due to its small size, the ST peptide has a limited repertoire of epitopes, and any alteration of the molecule may thus disrupt neutralizing epitopes. These challenges call for rational vaccine design. To identify non-toxic variants of ST with intact neutralizing epitopes, termed toxoid candidates, we have screened a library of all possible 361 single-amino acid mutants for effects on toxicity and the ability to bind to neutralizing antibodies. The screens identified residues A14, N12, and L9 as key residues to target for eliminating toxicity, and allowed us to rank the best toxoid candidates. The screens also allowed us to map the epitopes of three neutralizing monoclonal antibodies, of which one cross-reacts with the human ligand uroguanylin. Interestingly, all the non-cross-reacting antibodies had the ST-specific Y19 as their dominant epitope residue. These results form the foundation for our current efforts towards a rational design of ST toxoids that can elicit neutralizing immune responses against ST, with minimal risk of immunological cross-reactivity.
ANT04 Blood Group A Antigen Expression Influences Pathogen-Host Interactions and Clinical Outcome Following Infection with Enterotoxigenic Escherichia coli

Frederick M Kuhlmann¹, Subhra Chakraborty⁴, A. Louis Bourgeois⁴, David A Sack⁴, Barbara DeNearing⁴, Clayton D. Harro¹, Pardeep Kumar¹, Jeffrey C. Gildersleeve⁵, Matthew Ciorba², Srikanth Santhanam², Qingwei Luo¹, and James M. Fleckenstein¹,³,⁶

Department of Medicine, Divisions of Infectious Diseases¹, and Gastroenterology² and the Molecular Microbiology and Microbial Pathogenesis Program, Division of Biology and Biomedical Sciences³, Washington University School of Medicine, St. Louis, Missouri; Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland⁴; Center for Cancer Research, Chemical Biology Laboratory, National Cancer Institute, Frederick, Maryland⁵; Medicine Service, Veterans Affairs Medical Center, St. Louis, Missouri, USA⁶.

Previous studies in Bangladesh demonstrated that A blood group individuals are at increased risk of ETEC infection. To define a molecular basis for selection of blood group A (bgA) hosts, we first examined bgA glycan interactions with subcellular fractions of the extensively characterized H10407 strain, originally isolated from an individual with severe diarrheal illness. Interestingly, bgA bound exclusively to EtpA, a secreted high molecular weight adhesin. Conversely, the adhesin interacted almost exclusively with bgA on microarrays printed with more than 400 individual human glycans; and in biolayer interferometry studies, these interactions occurred at sub-micromolar affinity. We also found that EtpA specifically agglutinated bgA red blood cells, but not those from blood groups B, or O. Similarly, enzymatic removal of terminal N-acetylgalactosamine (GalNAc) residues from bgA glycans, or pre-incubation of rEtpA with GalNAc, reversed hemagglutination. These studies established the EtpA adhesin as a blood group A specific hemagglutinin/lectin. Because blood group antigens expressed on glycoproteins of the gastrointestinal mucosa can govern effective pathogen host interactions, we next investigated the impact of bgA expression on EtpA-mediated ETEC adhesion and toxin delivery. Compared to wild type enterocytes expressing bgA, isogenic CRISPR-engineered cells lacking the glycosyltransferase required for bgA expression exhibited significantly reduced EtpA protein binding, EtpA-mediated bacterial adhesion, and effective heat-labile toxin delivery by H10407. Furthermore, using polarized differentiated small intestinal monolayers from human intestinal stem cells obtained during endoscopic biopsy (enteroids) we observed that EtpA interacted exclusively with the surface of cells obtained from individuals with blood group A, but not those of blood group O. These studies suggest that this secreted adhesin, recently shown to be conserved across a broad range of ETEC phylogenies, could drive selection of susceptible blood group A hosts. Finally, we examined the impact of A blood group expression on clinical outcome in 63 ETEC naïve adult human subjects challenged with ETEC H10407 as part of earlier studies conducted at the Johns Hopkins University Center for Immunization Research. Consistent with prior reports, the overall attack rate in this population (challenged with 10⁷ cfu) was 68.3% (43 of 63 volunteers). Among volunteers with blood group A, 87.5% (14 of 16 volunteers) developed moderate-severe diarrhea (MSD), whereas only 50% (1 of 2) of group AB volunteers, 60% (6 of 10) of group B, and 63% (22 of 35) of group O volunteers developed MSD. Collectively, these studies reaffirm the importance of A blood group antigen expression as a determinant of clinical outcome following ETEC infection. They also illustrate the advantage of challenge studies conducted in immunologically naïve subjects in stratifying disease susceptibility. Finally, the determination of a molecular basis for host selection has important implications for both the design and testing of future iterations of ETEC vaccines.
Poster Presentations

ANT01 A broadly protective ETEC vaccine candidate
Ankur Mutreja¹, Nidhi Shukla¹, Abhishek Sen¹, Tarun Sharma¹, Derek Pickard², Gordon Dougan² and Davinder Gill¹

¹MSD-Wellcome Trust Hilleman Laboratories, New Delhi, India; ²Wellcome Trust Sanger Institute, Cambridge, United Kingdom

ETEC, the most common cause of bacterial diarrhea, causes more than 800,000 deaths annually in children under 5 years of age. In recently published Global Enterics Multi-centre Study (Kotloff et al, 2013), ETEC was among the top four most important diarrheal pathogens, surpassing (0-23 months) or matching (24-59 months) V. cholerae occurrence. Thus, the freshly highlighted need for an ETEC vaccine specific enough to distinguish between commensal *E. coli* and ETEC and sensitive enough to cover the diversity of ETEC is a crucial need that is still unmet. MSD Wellcome Trust Hilleman laboratories (HL), alongside two other major diarrheal vaccines (Rotavirus and Cholera), has started working on a new ETEC vaccine design. ETEC express a range of colonisation antigens (fimbria; colonization factors, CF; *coli* surface, CS), toxins (Heat-labile, LT; Heat-stable, ST) and O antigens that are candidate protective antigens, with some clinical validation (Holmgren et al, 2013). Recent whole genome sequence based phylogenetic analysis has indicated that some ETEC genetic lineages with distinct antigenic composition are globally distributed and may form the basis of a more rational vaccine design (Mentzer et al, 2014).

We used this most up to date epidemiological genomic data to select ETEC isolates of three most significant serogroups - O78, O25 and O6, to serve as founder strains for whole cell vaccine development. We have sourced these founder strains and are currently engineering them to express a range of CF/CS antigen combinations. LT and ST knockout clones of these strains are being constructed and the LT-B subunit, the equivalent of the CT-B subunit of cholera vaccine, producing *E. coli* strain is being separately engineered. CS and CF vaccine antigens of interest are either being pulled from the sourced strains or artificially synthesized. The two most promising vaccine candidates in the clinical pipeline are a) live attenuated ACE527 (Turner et al, 2011) and b) second generation whole cell inactivated ETVAX (Holmgren et al, 2013). Both these vaccines candidates consist of a cocktail of 3-4 ETEC strains of single serogroup, however our selection of different serogroups aims to provide the best possible coverage targeting even the multi drug resistant ST131 lineage. We aim to optimise the final strains to favour antigen expression within fermenters and optimal delivery in the final vaccine formulation.

References
ANT05 Finding novel common immunogenic antigens common to Shigella spp. through reverse vaccinology

Neelam Taneja, Sapna Pahil, Rehman HR, Raghava GPS, Sharma Meera

Department of Medical Microbiology, Post Graduate Institute of Medical Education and Research, Chandigarh 160012-India

Introduction: Shigellosis is an important cause of morbidity and mortality with high endemicity in India. In view of emergence and dissemination of multidrug-resistant strains of Shigella spp. WHO has emphasized the need for a safe and effective vaccine. The major challenges in vaccine development against Shigella include providing coverage for the numerous serotypes (52) of Shigella, antigenic complexity of serotypes and lack of cross-protective epitopes. For a Shigella vaccine to make an impact globally, it must protect at least against S. dysentariae type 1 (cause of epidemic and pandemic dysentery), S. sonnei (main serogroup in industrialized countries) and S. flexneri 2a (most common serotype in developing countries). Although several strategies have been used in the past, a successful vaccine is elusive yet. Reverse vaccinology has been successfully applied for many organisms. We used this approach to identify novel antigens common to multiple serotypes of Shigella.

Methods: The complete protein sequences (downloaded from www.ncbi.nlm.nih.gov) of three serotypes of Shigella (S. dysentariae type 1 strain sd197, S. sonnei strain ss046, S. flexneri 2a strain 301 and S. flexneri 2a strain 2457T) were used. A protein-protein BLAST was carried to identify proteins common to the above serotypes followed by localization prediction of the common proteins by various softwares to identify those proteins which were exposed on the surface or secreted out. B-cell, T-cell epitope and MHC (Major histocompatibility complex) class-I and Class-II binding predictions were carried out to find immunogenic epitopes by using multiple softwares. The humoral immune responses (IgG and IgA antibodies) were checked by in-house ELISA (enzyme linked immunosorbent assay) and cellular immune responses were assessed as cytokine levels of both Th1 and Th2 type responses i.e. IL-1β, IL-2, IL-4, IL-10, IFN-γ and TNF-α both in animal (3 different routes i.e. intraperitoneal, subcutaneous and intranasal) and human sera.

Results: Of 39,000 immunogenic peptides, 48 had nine or more amino acids. Five peptides with high MHC binding affinity (low IC50 value) namely putative lipoprotein (EL PGI I), putative heat shock protein (EL PGI II), Spa32 (EL PGI III), IcsB (EL PGI IV) and hypothetical protein (EL PGI V) were synthesized and tested in vivo. Among these, putative HSP and hypothetical protein showed very good humoral response whereas putative lipoprotein, spa32 and IcsB showed good T-cell response eliciting both INF-γ and TNF-α cytokines. Further testing with shigellosis patient’s sera to check occurrence of peptide specific antibodies demonstrated high levels of IgG and IgA antibodies against HSP and hypothetical proteins in significant number of patients.

Conclusions: The antigens identified are novel, common to multiple serotypes and have potential as vaccine candidates.
Novel adjuvants and immunization strategies for vaccination

Poster Presentations

ADJ01 Evaluation of antacid buffers for use with oral enteric vaccines

Jessica White, Marcus Estrada, Changcheng Zhu, Manjari Lal

PATH

Infections associated with enteric and diarrheal disease pathogens like enterotoxigenic *Escherichia coli* (ETEC), *Shigella*, and rotavirus continue to infect children, contributing to the estimated 760,000 diarrheal-related deaths affecting children under five each year. Although the oral route offers simplicity, acceptability, and safety for administration of vaccines against these pathogens, delivery through this route is complicated by the degradative effects of the gastrointestinal tract—especially the low pH of the stomach—resulting in the need to protect vaccines if they are to be effectively delivered by this route. Keeping the dose volume low is critical, especially for infants who have slow and uncoordinated swallowing abilities that can lead to reflux and vomiting of the vaccine. Additionally, adjuvants are needed for oral vaccines, not only to increase immunogenicity, but also to help overcome the natural immune tolerance to antigens introduced at mucosal portals of entry. A liquid formulation for rotavirus vaccine was developed that combines an antacid buffer to neutralize gastric acid in an administration dose volume of less than 2.5 ml for oral administration. The adjuvant double mutant heat liable toxin (dmLT), which is being investigated for use in several oral vaccine candidates, was evaluated in the current study for both compatibility with several antacid buffers and stomach acid neutralization capacity following the Baby Rossett-Rice method. The stability, dose volume, and administration approach for the dmLT adjuvant was determined based on an enzyme linked immunosorbent assay (ELISA) assay for dmLT. Results of the buffer evaluation with dmLT identified optimal antacid buffer and the minimal administration volume with sufficient buffering capacity to neutralize stomach acid in infants was determined.
ADJ02  Low-cost multivalent ETEC vaccine to prevent childhood diarrhoea

Diane Houben¹, Wouter Jong¹, Marien de Jonge², Else Marie Agger³, Peter Andersen³, Joen Luirink¹

¹ Abera Bioscience, Sweden; ² RUMC, The Netherlands; ³SSI, Denmark

The aim of Abera Bioscience is to design effective vaccines that can be given orally or nasally and can easily and quickly be produced at low cost. Our proprietary vaccine platform is based on outer membrane vesicles (OMVs) derived from attenuated Salmonella bacteria (Jong et al., Microb Cell Fact, 2014). OMVs are spherical nanoparticles that are naturally shed from Gram-negative bacteria. The surface of OMVs resembles the surface of its parental bacterium and can be used to display multiple different antigens for optimal exposure to the immune system. The OMVs are robust and safe vaccine carriers as they contain intrinsic adjuvant activity (they are rich in immune stimulatory molecules) and lack the ability to replicate (they do not contain infectious bacterial DNA). Thus, in contrast to the live-attenuated Gram-negative bacteria that are used as vaccine vectors, OMVs are safe to use. OMVs are suitable for direct administration onto mucosal surfaces (via oral or nasal administration), which is the natural route of the human body to develop immunity against infectious pathogens, such as ETEC. Moreover, needle-free vaccines are cheaper (no medical specialist is required) and less intrusive for young children compared to parentally administered vaccines.

An OMV-based vaccine candidate will thus offer several important advantages to prevent diarrhoea in children, as it:

- can provide broad protection by inducing immunity against multiple ETEC antigens at the same time;
- results in high immunogenicity by stimulating humoral and cellular immune responses;
- can be administered needle-free;
- is potentially suitable for storage at room-temperature, thereby minimizing the need for cold-chain supply;
- can be produced fast at very-low costs at a large scale due to the simplicity of the OMV production process;
- can be later exploited as scaffold to generate more complex polyvalent vaccines against a broader spectrum of diarrhoea causing pathogens.

In proof-of-concept studies, we demonstrate high-density display of multiple antigens from Mycobacterium tuberculosis and Streptococcus pneumoniae on the surface of Salmonella OMVs (Daleke-Schermerhorn et al., Appl Environ Microbiol, 2014). The resulting vaccine candidates confer protection in mouse models for tuberculosis and pneumococcal disease, respectively (Kuipers et al., Vaccine, 2015), in a manner that correlates with local and systemic antigen-specific production of IL-17A.
Enterotoxigenic \textit{Escherichia coli} (ETEC) infection is the most common enteric type of colibacillosis of young weanling pigs, where induce diarrhea, dehydration, anorexia and death, similar to clinical outcomes of human diarrheic patients. The similarity between porcine and human ETEC infections suggests that pigs would be a good model for vaccine development.

The only immune protection of the piglets comes from maternal colostrum since this species do not receive maternal antibodies through placenta. In the present study, antigenic complexes (60% proteins, 16% LPS) obtained from main ETEC serotypes involved in piglets infection (F4 and F18) were used to immunize pregnant sows, using protein food-born nanoparticles (NP) as oral adjuvant. The obtained loaded NP (11 µg antigenic complex/mg NP) were homogeneous and spherical in shape (240 nm; -36 mV). In vitro studies performed with RAW 264.7 macrophages indicate that NP were not cytotoxic, were efficiently captured and activated macrophages by inducing antigen presenting costimulatory molecules. A fluorescence study of interaction with intestinal mucus demonstrated that NP diffused efficiently through mucus in vitro. Taking all this into account, studies were performed in sows and mice in order to support it as a new vaccine candidate to face ETEC.

Eight weeks-pregnant sows were orally immunized with F4/F18 antigenic complexes either free or encapsulated into NP. The commercial vaccine Suiseng© was included as a control. Specific serum (IgG, IgA) and fecal (IgA) antibodies were detected in the sows vaccinated with the commercial vaccine or with the new F4/F18-NP vaccine until the day of delivery. Furthermore, colostrum analysis revealed the presence of specific antibodies (IgG) and, what is a most relevant, suckling piglet presented specific IgG in serum. These results indicate that NP was able to efficiently deliver the antigens through the mucosal barrier. As a consequence, vaccine-specific plasma cells maintained a sustained level of systemic and mucosal IgG susceptible to neutralize bacterial penetration and also neutralize the effect of toxins.

In addition, a single dose of this vaccine formulation administered orally into BALB/c mice, induced high levels of specific antibodies, both IgG1 (Th2) and IgG2a (Th1) and appreciable levels of IgA mucosal level (intestinal).

This NP formulation is also currently being tested as mucosal adjuvant in vaccination against \textit{Shigella flexneri}.

[supported by the Ministerio de Economía y Competitividad from Spain, RTC-2014-2004-2, and by the Plan Nacional de Investigación Científica, co-funded by the European regional development fund, under the grant PI12/01358].
ADJ04 Self-adjuvant multicomponent vaccine against Shigella

Yadira Pastor¹, Ana Camacho¹, Ana Gloria Gil², Rocío Ramos³, Adela López de Ceráin², Iván Peñuelas³, Juan M Irache, and Carlos Gamazo¹

¹Department of Microbiology; ²Department of Toxicology; ³Department of Nuclear Medicine, Clínica Universidad de Navarra; ⁴Department of Pharmaceutical Technology; (Institute of Tropical Health - University of Navarra 31008, Pamplona/ SPAIN

We show here that a simple and safe heat-treatment-based strategy to obtain an immunogenic complex (HT) is able to protect mice from experimental infection by *Shigella flexneri*. This process, directly from a batch culture, simultaneously achieve the complete bacterial inactivation, leading a safe working process, as well as the efficient release of main bacterial virulence factors involved in protection. The obtained HT vaccinal complex presents a similar morphology (electron microscopy) and chemical composition than the classical outer membrane vesicles (OMV), nonetheless, HT was enriched in Ipa virulence factors, more favorable for protection on an a priori basis.

HT formulation was not cytotoxic in Raw 267.4 macrophages cell line, and main differentiation markers of antigen presenting cells, such as CD40 and MHC-II, were highly expressed during incubation with HT.

Acute and chronic, oral or nasal, toxicity studies were performed in rats. Clinical, hematological and histopathological profiles of target organs indicate there were no adverse effects of the new HT formulation.

Biodistribution studies performed in mice demonstrated that this vaccinal product remained in the small intestine even after nasal administration, showing evidences of lymph node permanence.

Finally, one single dose of HT nasally administered was able to protect mice from a pulmonary challenge with *S. flexneri* 2a (1×10⁵ CFU/mice).

In conclusion, our findings support the use of HT as a new vaccine candidate to face shigellosis.

[Suported by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica of Spain, co-funded by the European regional development fund, under the grant PI12/01358]."
Preclinical evaluation of vaccine candidates and animal models of enteric disease

Oral Presentations

PRE01 An adhesin tip MEFA (multiepitope fusion antigen) of enterotoxigenic Escherichia coli (ETEC) induced antibodies against adherence of nine ETEC adhesins: CFA/I, CS1, CS2, CS3, CS4, CS5, CS6, CS21 and EtpA

Rahul M Nandre1, Xiaosai Ruan1, Qiangde Duan1, David A Sack2, Weiping Zhang1

1: Department of Diagnostic Medicine/Pathobiology, Kansas State University College of Veterinary Medicine, Manhattan, Kansas, USA. 2: Department of International Health, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA.

Diarrhea continues to be a leading cause of death in children younger than 5 years in developing countries. Enterotoxigenic Escherichia coli (ETEC) is a leading bacterial cause of children’s diarrhea and travelers’ diarrhea. ETEC bacteria initiate diarrheal disease by attaching to host receptors at epithelial cells and colonizing in small intestine. Therefore, preventing ETEC attachment has been considered the first defense line against ETEC diarrhea. However, developing vaccines broadly against ETEC bacterial attachment has encountered challenge because ETEC strains produce over 23 immunologically heterogeneous adhesins. In this study, we applied MEFA (multiepitope fusion antigen) approach to integrate epitopes from adhesin tips or adhesive subunits of CFA/I, CS1, CS2, CS3, CS4, CS5, CS6, CS21 and EtpA adhesins and to construct an adhesin tip MEFA peptide. We then examined antigenicity of this tip MEFA in mouse immunization, and assessed potential application of this tip MEFA for ETEC vaccine development. Data showed mice intraperitoneally immunized with this adhesin tip MEFA developed IgG antibody responses to all nine ETEC adhesins. Moreover, ETEC and E. coli bacteria expressing these nine adhesins, after incubation with serum of the immunized mice, had significant reduction of attachment to Caco-2 cells. These results indicated that anti-adhesin antibodies induced by this adhesin tip MEFA blocked adherence of the most important ETEC adhesins, suggesting this multivalent tip MEFA can be useful for developing a broadly protective anti-adhesin vaccine against ETEC diarrhea.
PRE03 A live attenuated vaccine against *Shigella* and ETEC: characteristics and potency of the ShigETEC prototype strain

Petra Krause, Shushan Harutyunyan, Irene Neuhauser, Michael Aichinger, Valéria Szijártó, Gábor Nagy, Eszter Nagy and Tamás Henics

Eveliqure Biotechnologies GmbH

In both the developed world and endemic countries *Shigella* and Enterotoxigenic *Escherichia coli* (ETEC) remain the two leading bacterial cause of diarrheal disease. Currently, no successful vaccine against bacillary dysentery and ETEC-related diseases is available. It is desirable to design a vaccine that provides protection against both *Shigella* and ETEC. Due to the immunodominant and strictly serotype-specific O-antigen residue of the ~50 serotypes of the four *Shigella* species, it is not possible to elicit cross protection by vaccination with a single serotype. It has also been difficult to select a functional vaccine platform in which to express the two major virulence determinants of ETEC, the heat stable (ST) and labile (LT) toxins for potent immunogenicity and safety. Using the *Shigella flexneri* 2a 2457T strain as a platform, we developed the ShigETEC vaccine with immunostimulatory properties both against multiple *Shigella* serotypes and ETEC. We demonstrated that deletion of the IpaBC tandem from the Ipa gene cluster of the large invasion plasmid provides sufficient attenuation with no detectable loss of immunogenicity. Subsequent removal of the O-antigen component of LPS (∆ipaBC/∆rfbF mutant) led to serotype-independent protection in the mouse lethal lung challenge model. Significant serum IgG titers as well as broncho-alveolar mucosal IgA response were detected in protected animals. Expression of an LT/B-subunit – ST toxoid fusion protein in the *Shigella*-∆ipaBC/∆rfbF mutant elicited both LT and ST specific serum IgG and mucosal IgA responses in vaccinated mice. Finally, Western blot analyses of sera from vaccinated animals revealed considerable cross-reactivity between various *Shigella* and ETEC preparations. To further prove our concept, WaaL O-antigen ligase defective, hence O-antigen negative rough mutants with deleted MxiD or MxiH components of the *Shigella* type III secretion apparatus (∆waaL/∆mxiD and ∆waaL/∆mxiH mutants) completely blocked epithelial invasion in a HeLa in vitro model. Immunization with these double mutant strains elicited complete, serotype independent heterologous protection against virulent *Shigella* strains in the mouse lethal lung challenge model. Our results strongly support ShigETEC as a novel promising vaccine candidate to fight diarrheal diseases caused by *Shigella* and ETEC.
PRE04 Preclinical Evaluation of Combined *Shigella*-ETEC Vaccine Candidates

Eileen Barry¹, Tao Wu¹, Bre-Onna Delaine¹, Aimee Cunningham¹, Christen Grassel¹, Kurt Hanevik², and Myron Levine¹

¹Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland, USA
²Department of Clinical Science, University of Bergen, Bergen, Norway

*Shigella* and ETEC are significant causes of disease in children less than 5 years of age in less industrialized countries. Recent data from the Global Enterics Multicenter Study (GEMS) confirmed that *Shigella* and ETEC were among the top four pathogens causing moderate to severe diarrhea in children in all three age strata examined. There is no licensed vaccine to prevent infection by either of these pathogens. The substantially increased value of a combined *Shigella*-ETEC vaccine that could target common at-risk populations in terms of practical distribution and administration as well as economic implications has recently become more appreciated. We have advanced a platform utilizing live attenuated strains of *Shigella* expressing critical antigens from ETEC to form a multivalent oral formulation. Five live attenuated *Shigella* vaccine strains including *S. sonnei*, *S. dysenteriae* 1, and *S. flexneri* serotypes 2, 3a and 6 that could confer a broad level of protection against *Shigella* infections have been engineered and shown to be safe, immunogenic and protective in preclinical animal studies. The most advanced strain, *S. flexneri* 2a vaccine strain CVD 1208S, has completed Phase 2 clinical trials where it was demonstrated to be safe and highly immunogenic in volunteers. The use of these attenuated strains has been extended by the expression of protective antigens from ETEC to form a combined *Shigella*-ETEC vaccine. Fimbrial and toxoid antigens have been stably expressed from chromosomal loci in the *Shigella* vaccine strains. Assessment of multiple promoter and operon configurations identified strain CVD 1208S-122, expressing ETEC antigens CFA/I and LTB, as a lead candidate which induced robust immune responses to both ETEC antigens and the *Shigella* live vector in preclinical animal studies. Further components include attenuated *Shigella* strains expressing ETEC fimbrial antigens CS1, CS2, C3, CS4, CS5 and CS6 in various combinations. Extensive in vitro studies have confirmed the genetic stability of these constructions. Recent advances utilizing autotransporter technology to efficiently expose antigens on the surface of *Shigella* live vectors have demonstrated unprecedented levels of antibody responses to antigen fusions. The LTB subunit was expressed via fusion to the Sat autotransporter in several configurations in CVD 1208S. Immunization studies in guinea pigs confirmed that optimal surface expression in CVD 1208S(LTB-Sat-6) induced significant anti-LTB responses in 100% of immunized animals after a single dose. A modified ELISA demonstrated that high level surface display correlated with the strongest immune responses. This level of response to a vectored heterologous antigen in all immunized animals is very rarely achieved using live vector technology. Furthermore, all animals responded to the *Shigella* live vector as well and were protected against virulent challenge with the wild type parental strain in the Sereny challenge. Advances in stable, efficient heterologous antigen expression support the development of a multivalent *Shigella*-ETEC vaccine that may confer broad level protection against two important pathogens.
PRE06 From a monovalent to a multivalent synthetic carbohydrate-based vaccine candidate against *Shigella flexneri*


ZH, CL, CG, LM: Institut Pasteur, Chemistry of Biomolecules, 28 rue du Dr Roux, 75724 Paris Cedex 15, France;
FT, AP: Institut Pasteur, Molecular Microbial Pathogenicity, 75015 Paris, France;
PB, JJB: Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain;
BVLN, PA: Institut Pasteur, Structural Microbiology, 75015 Paris, France;
SH, PE: Institut Pasteur, Molecular Biophysics, 75015 Paris, France

Owing to *Shigella* species/serotype diversity and variable geographical distribution, there is an unmet need for a broad coverage vaccine against Shigellosis. *Shigella flexneri*, which comprises multiple circulating serotypes, prevails in developing countries and is of particular concern. Based on the observation that protection against re-infection is mainly achieved by antibodies specific for the O-antigen (O-Ag) moiety of the lipopolysaccharide (LPS), immunogens consisting of synthetic fragments of the putative O-Ag conjugated to carrier proteins have been considered as a possible alternative to detoxified *Shigella* LPS-protein conjugates. Along this line, the development of a multivalent synthetic carbohydrate-based *Shigella* vaccine is a major objective in our laboratory.

Herein, we first highlight the concept of synthetic carbohydrate-based vaccines against bacterial diseases. In doing so, we provide novel data supporting SF2a-TT15, a vaccine candidate against *S. flexneri* 2a, on its way to a phase I clinical trial. We then illustrate the multidisciplinary strategy that we have implemented to identify promising well-defined mimics of the O-Ag from *S. flexneri* 3a, another prevalent serotype. In particular, we report a detailed investigation of the immunodominant role of O-Ag stoichiometric O-acetylation as revealed by chemical synthesis, immunochemistry, physical chemistry, NMR, and X-ray crystallography studies. Next, we describe the rational design, synthesis, and immunogenicity data of the first synthetic carbohydrate-based vaccine candidate against *S. flexneri* 3a. Finally, we discuss preliminary immunogenicity data for a set of *S. flexneri* 2a / *S. flexneri* 3a glycoconjugate combinations and extension to additional serotypes prevalent in the field.
PRE08 Adhesin tips vs. major structural subunits for anti-adhesin vaccines: a comparative study of enterotoxigenic *Escherichia coli* (ETEC) adhesin tip MEFA (multiepitope fusion antigen) and CFA MEFA for antibodies against adherence of ETEC adhesins (CFA/I, CS1-CS6, CS21 and EtpA)

Rahul M Nandre, Xiaosai Ruan, Jerome Nietfeld, Zhenhai Chen, Qiangde Duan, Jiachen Huang, Weiping Zhang

Department of Diagnostic Medicine/Pathobiology, Kansas State University College of Veterinary Medicine, Manhattan, Kansas, USA.

Enterotoxigenic *Escherichia coli* (ETEC) is the most common bacterial cause of children’s and travelers’ diarrhea. Mediated by adhesins, ETEC diarrhea initiates with bacterial attachment to epithelial cell receptors. A vaccine blocking ETEC bacterial attachment should be effective against ETEC diarrhea. However, ETEC strains express over 23 immunologically heterogeneous adhesins, leaving conventional vaccine approaches continuously encounter challenge. Recently, we developed the MEFA (multiepitope fusion antigen) approach and constructed MEFAs targeting the major structural subunits or the adhesive tips of seven to nine important ETEC adhesins, and explored potential anti-adhesin vaccine development. In this study, we subcutaneously immunized mice with the CFA MEFA (major structural subunits) or the tip MEFA (adhesive subunits), with or without toxoid fusion 3xSTaN12S-dmLT, and compared these two MEFAs for immunogenicity and more importantly potency for vaccine development against ETEC adherence. Mice subcutaneously immunized with the CFA MEFA or the tip MEFA developed greater antibody responses to each of seven or nine adhesins. In addition, when co-administered with 3xSTaN12S-dmLT, both MEFAs induced strong antibody responses against all adhesins but also both toxins. In vitro antibody adherence inhibition assays indicted that antibodies derived from the CFA MEFA and the tip MEFA inhibited adherence of bacteria expressing these adhesins equivalently. These results suggested that the CFA MEFA or the tip MEFA, combined with the toxoid fusion, can be developed as broadly protective anti-adhesin vaccines against ETEC diarrhea.
Comparison of sublingual and intradermal delivery routes with ETEC vaccine candidates delivered with LT-based adjuvants.

Milton Maciel Jr. 1, David Bauer 2, Stephen Savarino 1, John Clements 2, Elizabeth Norton 2.

1 Naval Medical Research Center, Silver Spring, MD
2 Tulane University, New Orleans, LA

Introduction: Subunit vaccines are an attractive option for new childhood vaccines, given their propensity for reduced reactogenicity compared to whole killed or live-attenuated vaccines, as well as optimizing the immune response against subdominant antigens. However, subunit vaccines are often limited to specific delivery routes, primarily parenteral injection. Using subunit vaccine candidates from enterotoxigenic Escherichia coli (ETEC), here we compare sublingual (SL) administration, a needle-free mucosal route, to intradermal (ID) vaccination.

Methods: We performed immunizations on groups of BALB/c mice using recombinant proteins derived from the minor and major subunits of class 5a CFA/I fimbrial colonization factor, i.e., CfaEB, or a minor subunit adhesin-toxoid chimera, i.e., CfaE-CTA2/LTB5. Immunizations were performed with antigens alone or combined with the adjuvant dmLT, an R192G/L211A double mutant of the heat-labile enterotoxin (LT) from ETEC. Both aforementioned antigens have shown to be safe and immunogenic in Phase 1 Clinical Trials. We also evaluated the requirement for the B-subunit of dmLT in mucosal surface binding for adjuvanticity and immunogenicity by including CfaEB combined with dmLT-5% lactose (potentially interfering with cell surface binding), the GM1-binding mutant LT-G33D, or isolated LT-A1 domain. Mice were immunized in a prime/boost schedule 3 weeks apart, then euthanized 2 weeks post-boost for sample analysis. These included serum and fecal antibodies (Abs), and recall cytokine responses.

Results: Our analyses revealed that SL immunization generated robust functional neutralizing Abs as judged by hemagglutination inhibition (HAI) assay and serum anti-CFA/I IgG Abs; but only in the CfaEB + dmLT vaccine group. These responses were not seen in SL immunization groups receiving CfaEB antigen alone or CfaEB combined with an altered B-subunit binding formulation (i.e., dmLT-5% lactose, LT-G33D, or LT-A1). This requirement for B-subunit binding to the sublingual epithelium was similarly observed in Chimera and Chimera+dmLT immunization groups, which both had high levels of serum neutralizing Abs, strongly mimicking all ID groups, and the only observable fecal anti-CFA/I IgA responses following SL immunization. In contrast, robust recall IL-17 cytokine responses were detected in all adjuvanted CfaEB SL immunization groups compared to ID groups, including CfaEB plus dmLT, dmLT-5%, LT-G33D, or LT-A1. All CfaEB ID groups developed robust serum and fecal Abs responses, except with CfaEB + LTA1 immunization, where the anti-LT Abs levels were reduced in comparison to the remaining groups

Conclusions: Our results confirmed the importance of route as well as the relevance of the B-subunit of LT-based adjuvants in providing the full effects of dmLT adjuvant properties. In addition, we demonstrated that sublingual immunization with the right formulation is a permissive route for delivering a subunit ETEC vaccine.
Poster Presentations

PRE02 Moving toward a clinical trial of a live attenuated oral vaccine against shigellosis and typhoid fever

Yun Wu¹, Sumana Chakravarty², Minglin Li¹, Jonathan M. Jackson¹, Tint Tint Wai³, Eric R. James², Richard E. Stafford¹, Stephen L. Hoffman¹,², B. Kim Lee Sim¹

Protein Potential, LLC¹ and Sanaria, Inc.², 9800 Medical Center Dr., Rockville, MD 20850, USA.

There are over 125 million cases of shigellosis globally with a significant number of cases caused by Shigella sonnei (Ss) and S. flexneri 2a (Sf2a). Our goal is development and deployment of licensed vaccines for prevention of morbidity and mortality due to shigellosis that 1) induce durable protective efficacy following a rapid immunization regimen, 2) can be distributed without refrigeration, 3) are administered orally, and 4) simultaneously protect against other enteric disease agents. We have exploited the extensive safety record of the live, oral, attenuated Salmonella Typhi vaccine (Ty21a) by utilizing it as a vector to develop a safe, stable, easily administered combination oral vaccine that simultaneously protects against shigellosis and typhoid fever. We have recombineered the Shigella O-antigen gene clusters, including full length wzz O-antigen length control gene, into the Ty21a chromosome to create Ty21a-Ss (TyOraSsTM), which stably expresses S. sonnei form I O-antigen and Ty21a-Sf2a (TyOraSf2aTM), which stably expresses S. flexneri 2a O-antigen. A fully characterized seedbank of Ty21a-Ss was used to immunize mice intranasally. These immunized mice produced significant levels of serum IgG antibodies against both Shigella and S. Typhi. Moreover, mice immunized with Ty21a-Ss were protected against a lethal intranasal S. sonnei 53G infection. Similar data on Ty21a-Sf2a are being generated and will be presented, as will our plans for further increasing the potency of recombinant Ty21a, moving to production of master and working cell banks in compliance with cGMPs, pre-clinical IND enabling studies, IND submission, and design of the first clinical trial.
PRE05 Evaluation of a Tri-valent \textit{Shigella flexneri} Vaccine

Bre-Onna C. DeLaine, Christen L. Grassel, Tao Wu, Eileen M. Barry

University of Maryland, Baltimore

\textit{Shigella flexneri}, a Gram-negative enteroinvasive bacterium, is one of the leading causes of diarrheal disease in children under five in developing countries. The development of an efficacious vaccine has been elusive, in part due to the lack of in vitro correlates of protection and the requirement for serotype specific protection. We performed in vitro and in vivo experiments to assess the ability of two new vaccine candidates to induce immune-associated responses utilizing the well-studied live, attenuated \textit{Shigella flexneri} 2a vaccine CVD 1208S as a standard. The new vaccine strains, CVD 1213 and CVD 1215 (derived from \textit{S. flexneri} 3a and \textit{S. flexneri} 6), represent two prominent \textit{S. flexneri} serotypes. Combined with CVD 1208S, they could provide broad spectrum immunity against \textit{Shigella flexneri} isolates.

Each vaccine strain was engineered with a deletion in the guaBA operon encoding critical enzymes of the de novo guanine nucleotide biosynthesis pathway. The sen and set genes were deleted in appropriate strains. The vaccine strains were characterized in epithelial cell invasion assays, macrophage cytotoxicity assays, and for induction of cytokines associated with \textit{Shigella} induced immune response. Guinea pig immunization studies were performed to assess \textit{Shigella} LPS-specific antibody responses and protection against challenge. Additionally, RNA sequencing was performed to determine transcriptional changes in epithelial cells infected with wild-type or the vaccine strains as well as the bacterial strains in the presence or absence of gastric bile salts.

Using the human HT-29 intestinal cell line, invasion by the vaccines was equivalent to wild-type; however, the attenuated vaccines were unable to replicate intracellularly over a 24-hour incubation period. Vaccine candidates induced similar levels of IL-8 and CXCL-1 secretion from infected HT-29 cells 8 hours post invasion. Infection of human macrophage-like cells with wild-type or vaccine strains resulted in decreased viability that was similar between wild-type and vaccine strains. Guinea pig immunization studies with each individual \textit{S. flexneri} vaccine candidate revealed a robust induction of serotype specific LPS IgA and IgG antibodies; 100\% of the animals responded with a 4-fold or higher increase over pre-immune titers following a single immunization. All vaccinated animals were protected against challenge with the homologous serotype virulent strain. Immunization with a mixed inoculum composed 1208S, 1213 and 1215 resulted in antibody responses against each component \textit{S. flexneri} serotype and 67\% to 100\% protection against challenge with each parental strain. Further evaluation of the antibodies induced by immunization with the live vaccines demonstrated serotype-specific serum bactericidal activity.

The in vitro host responses to the new vaccine candidates are consistent with responses to the well-established CVD 1208S vaccine which has been shown to be safe and immunogenic in clinical studies. Guinea pig antibody responses to the combined immunization remained as strong as responses in animals vaccinated with each individual vaccine. The results of the challenge study demonstrated the vaccines' protective capacity singly and as a mixed formulation. This robust response supports CVD 1213 and CVD 1215 as viable candidates for inclusion in a multivalent vaccine, with CVD 1208S, that could confer broad spectrum protection against \textit{Shigella flexneri} infections."
PRE07 Evaluation of Serotype-independent Immunity Elicited by a T3SS-Based Shigella Subunit Vaccine

Francisco J. Martinez-Becerra, Melissa Pressnall, Olivia Arizmendi, William D. Picking and Wendy L. Picking

Department of Pharmaceutical Chemistry, University of Kansas.

Shigellosis is a gastrointestinal disease of worldwide public health importance for which there is no licensed vaccine. It is also classified as a category B bioterrorism agent. Our group has developed a serotype-independent vaccine based on two proteins of the Shigella type three secretion system (T3SS). These proteins have important roles in pathogenesis and are conserved among virulent Shigella strains. We generated a fusion protein (DB fusion) that comprises the T3SS tip proteins IpaB and IpaD. This vaccine has been shown to be protective in the mouse pulmonary model when administered with the adjuvant dmLT. We propose to use the T3SS vaccine as a model to identify the host immune responses that confer protection against Shigella infection in an LPS independent manner. We hypothesize that using vaccine/infection models we will identify correlates of protection that can be measured in future clinical trials with this vaccine. We determined the type of immunity involved in protection by vaccinating animals using different administration routes and adjuvants. Afterwards, mice were challenged with S. flexneri. We also stimulated dendritic cells with IpaB, IpaD and the combination in the presence of dmLT and measure cytokine release and up-regulation of activation markers. In combining an identification of the correlates of protection with the use of biophysical methods for optimizing vaccine formulation, we hope to establish the basis for evaluating the likely efficacy of this vaccine in protecting humans against shigellosis.
Refinement of a Guinea Pig Intrarectal Shigella Challenge Model and use in Vaccine Efficacy Studies.


1 Subunit Enteric Vaccines and Immunology, Bacterial Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD USA
2 Institut Pasteur, Unité de Pathogénie Microbienne Moléculaire, INSERM U1202, Paris, France
3 Sanofi-Pasteur, Marcy L’Etoile, France

Shigella infection in humans is characterized by the ability of the bacteria to invade the intestinal mucosal epithelium, replicate intracellularly, and spread intercellularly. Animal models that mimic the disease in humans are essential tools for studying Shigella pathogenesis and vaccine efficacy. The established guinea pig keratoconjunctivitis model is commonly used in Shigella vaccine research but as the model does not involve the intestinal tissue, it does not capture all aspects of Shigella pathogenesis. An alternative intestinal guinea pig model has been previously shown to induce rectocolitis in pigs weighing less than 280 grams. However, in most vaccine efficacy studies, animals generally weigh in excess of 375 grams due to treatment regimens. Therefore, to be useful in studies with prolonged immunization and challenge timelines the rectocolitis model was refined to induce rectocolitis in larger and older guinea pigs.

New technical aspects included adjusting the bacterial growth conditions, increasing the inoculum volume and the use of a longer, rectally administered catheter to instill the inoculum farther into the large intestine. Studies were conducted to optimize the intrarectal challenge dose for S. flexneri 2a, S. flexneri 3a and S. sonnei. Each serotype was directly compared to a serotype-matched avirulent Shigella strain. Varying challenge doses were evaluated in animals weighing ≥ 400 grams to achieve an 80% attack rate. A challenge dose of 5 x 10¹⁰ cfu was found to be the optimal dose for S. sonnei and S. flexneri 2a whereas S. flexneri 3a required a half log fewer bacteria.

During dose optimization studies, multiple disease parameters (blood, mucous, diarrhea, inflammation and weight change) were examined to fully characterize the disease. In addition, animals were necropsied to examine multiple sections of the large intestine and rectum to determine the levels of pathology in the mucosal and submucosal layers, crypt distortion, and infiltration of mononuclear cells into inflammation sites. All animals infected with virulent strains of Shigella exhibited some, if not all, outward signs of disease and lost 5-10% of their body weight 24 hours post challenge. Peak disease for all three serotypes was at 48 hours post infection. Necropsy of the animals revealed localized infection of the colonic epithelium characterized by epithelial loss, edema and hemorrhage.

The rectocolitis challenge model was then used to evaluate protective efficacy elicited after immunization with S. flexneri 2a Artificial Invaplex (IVPAR) vaccine with and without adjuvant. Significant protection from intrarectal challenge with S. flexneri 2a, 2457T was achieved after intranasal immunization with detoxified S. flexneri 2a IVPAR (p < 0.0275), intranasal immunization with S. flexneri 2a IVPAR delivered with dmLT (p < 0.005) or without dmLT (p < 0.0275) and finally after subcutaneous immunization with IVPAR delivered with or without Sanofi Pasteur adjuvant (p ≤ 0.05).

In summary, intrarectal challenge of guinea pigs with virulent Shigella spp. is able to induce reproducible rectocolitis that in many aspects mimics human disease. The refined model is relevant for use in Shigella vaccine efficacy studies and should be further investigated using other Shigella vaccine formulations delivered either parenterally or mucosally.
PRE11 Evaluation of Shigella flexneri 2a Artificial Invaplex Formulated for Parenteral Immunization with Deacylated LPS

R.W. Kaminski¹, K.A. Clarkson¹, K.R. Turbyfill¹, M.A. Smith¹, C.R. Strelez¹, A.R. Vortherms¹, D. Jirage¹, H.A. Wenzel², L. Van De Verg², R. Walker², J.D. Clements³, E.V. Oaks¹

¹ Subunit Enteric Vaccines and Immunology, Bacterial Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD
² PATH, Washington, DC
³ Tulane University, New Orleans, LA

Artificial Invaplex (IVPAR) is a subunit vaccine candidate to prevent shigellosis. IVPAR is composed of purified recombinant invasion plasmid antigen proteins (IpaB and IpaC) complexed with lipopolysaccharide (LPS) extracted from wild-type Shigella. IVPAR has been manufactured under current Good Manufacturing Practices (cGMP) and is being evaluated in humans using the intranasal administration route.

One goal of the Invaplex program is to adapt the Invaplex product for parenteral vaccinations. IVPAR, which contains hexa-acylated (wild-type) LPS, may be reactogenic when delivered parenterally, especially if mixed with a potent adjuvant. Therefore, efforts are underway to reduce potential reactogenicity. One such strategy is to assemble IVPAR using LPS isolated from mutated Shigella strains lacking the genes responsible for lipid A acylation (msbB1 and msbB2). Invaplex formulations using LPS isolated from ΔmsbB mutants (InvaplexAR-DETOX or IVPAR-DETOX) were compared to IVPAR assembled with fully acylated LPS in a series of in vitro and in vivo experiments to determine if the mutations affected biological activity, immunogenicity, reactogenicity and efficacy in animal models. Each IVPAR product had similar ratios of IpaB, IpaC and LPS to the total protein content.

The results of in vitro assays demonstrated that IVPAR-DETOX retained the capability to induce self-uptake in fibroblast cells at comparable levels to IVPAR. LPS isolated from ΔmsbB mutants stimulated release of significantly less TNF-α from macrophages as compared to wild-type LPS. Incubation of macrophages with IVPAR or IVPAR-DETOX significantly reduced the amount of TNF-α released into the culture supernatant, as compared to cells treated with purified LPS, even when 10-fold more IVPAR or IVPAR-DETOX was used for stimulation.

The immunogenicity and dose site reactogenicity (edema, erythema and induration) of IVPAR assembled with LPS from wild-type or msbB mutants was evaluated in mice immunized parenterally. Groups of mice were immunized intradermally on days 0 and 14 with three different dose amounts of the IVPAR formulations, with and without the adjuvant double-mutant heat-labile toxin (dmLT R192G/L211A). Comparable levels of LPS- and Invaplex-specific serum IgG and IgA endpoint titers were elicited among all groups indicating no loss of immunogenicity with IVPAR formulations containing deacylated LPS. Mice immunized with IVPAR-DETOX assembled with LPS from the double msbB mutant had the lowest level of reactogenicity among the formulations tested.

The immunogenicity and protective efficacy of IVPAR and IVPAR-DETOX assembled with LPS from the double msbB mutant was then evaluated in the guinea pig rectocolitis model. Guinea pigs vaccinated intranasally with IVPAR or IVPAR-DETOX developed similar Shigella-specific serum IgG and IgA endpoint titers. Moreover, guinea pigs immunized with IVPAR and IVPAR-DETOX were significantly protected after intrarectal challenge with S. flexneri 2a, 2457T (p < 0.05).

In summary, IVPAR-DETOX assembled with purified LPS from mutant msbB strains has reduced pro-inflammatory potential resulting in lower levels of reactogenicity as compared to IVPAR assembled with fully acylated LPS. However, the reduction in reactogenicity did not significantly diminish the biological activity, immunogenicity or protective efficacy of IVPAR-DETOX. The IVPAR-DETOX product has been manufactured under cGMP for use in future clinical evaluations.
PRE12 Expanded Development and Use of the Aotus nancymaeae Shigella Immunogenicity and Efficacy Model.

N. D. Reynolds¹, A. Ranakrishnan ², M. P. Simons¹, M. A. Smith³, K.R. Turbyfill³, J.D. Clements⁴, E. V. Oaks³, R.W. Kaminski³

1. U.S. Naval Medical Research Unit No. 6, Department of Bacteriology, Callao, Peru.
2. Department of International Health, Johns Hopkins School of Public Health, Baltimore, MD. USA
3. Subunit Enteric Vaccines and Immunology, Bacterial Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD. USA
4. Tulane University, New Orleans, LA. USA

*Shigella*, the etiologic agent of bacillary dysentery, causes significant morbidity and mortality throughout the developing world, especially in children. Based on recent epidemiological studies, a vaccine capable of protecting against infections caused by *S. flexneri* 2a, 3a, 6 and *S. sonnei* may be required to cover 80% of the circulating serotypes. To further develop the *Aotus nancymaeae* challenge model for development of a quadrivalent *Shigella* vaccine, dose titration and dose verification experiments were conducted to determine the amount of *S. flexneri* 3a, J17B and *S. sonnei*, 53G necessary to induce a ≥ 75% attack rate in naïve animals. Fecal consistency was examined from day 1 post challenge up to day 10 post challenge. Diarrhea was defined as 2 or more consecutive days of loose stools. The 5x10¹¹ dose produced attack rates that ranged from 75 to 88 percent for *S. sonnei* strain 53G over two trials and 62 percent for *S. flexneri* 3a strain J17B.

In addition to challenge dose determination studies, we sought to determine protective efficacy of *S. flexneri* 2a artificial Invasin Complex vaccine (InvaplexAR) in an *Aotus nancymaeae* model. Three groups of eight Aotus were intranasally (IN) vaccinated with InvaplexAR at a dose of 100 µg, 250 µg, or 250 µg mixed with 25 µg of the adjuvant dmLT. A control group of 14 animals received an IN sham vaccine with PBS alone. Vaccinations were performed on study days 0, 14, and 28.

On study day 49, animals immunized with InvaplexAR (250 µg) combined with dmLT and the PBS control group was challenged by orogastric administration of *S. flexneri* 2a strain 2457T at a dose of 1x10¹¹ cfu/monkey. Animals were observed for 10 days for clinical and diarrheal symptoms. Animals immunized with InvaplexAR (100 and 250 µg without adjuvant) were not challenged due to low serum antigen-specific IgG and IgA. In the group immunized with InvaplexAR (250 µg) combined with dmLT, the incidence of clinical symptoms was significantly less than the PBS control group (p=0.0181). In addition, only two of the eight animals (25%) developed diarrhea compared to six of twelve animals (50%) in the control group, resulting in a protective efficacy of 50% (p=0.37).

Collectively, these results indicate that *S. sonnei*, 53G and *S. flexneri* 3a, J17B can be used in the *Aotus* challenge model. However the lower attack rate of *S. flexneri* 3a strain J17B will require additional animals for adequate statistical power in future experiments. The results of the vaccine trial indicate the potential of the InvaplexAR vaccine to protect against *Shigella* infection in a non-human primate model and the added value of dmLT as an adjuvant for boosting immune responses.
PRE13 Functional complement-fixing antibodies are generated against a *Campylobacter jejuni* capsule conjugate vaccine in non-human primates

Nina M. Schumack 1, Joanna E. Rimmer 2,3, Patricia Guerry 1, and Renee M. Laird 1

1. Naval Medical Research Center
2. University of Birmingham
3. British Royal Air Force

*Campylobacter jejuni* is among the most common causes of diarrheal disease worldwide and efforts to develop protective measures against the pathogen are ongoing. One of the few defined virulence factors targeted for vaccine development is the capsule polysaccharide (CPS). We have developed a capsule conjugate vaccine against a prototype CPS, type HS23/36, that confers 100% protection against diarrhea caused by a homologous strain of *C. jejuni* in a non-human primate (NHP) model; however the mechanisms of protection remain unknown. Other licensed capsule conjugate vaccines against encapsulated gram-negative organisms use bactericidal antibody titers as a correlate of protection. We developed a flow cytometry-based serum bactericidal assay (SBA) to determine if increased levels of complement-fixing antibodies correlate with protection against *C. jejuni*. Sera from NHPs immunized with HS23/36 CPS-CRM197 vaccine showed significantly higher SBA titers when compared with control NHPs. Results were similar to those of a bacteriological-based SBA. All NHPs, irrespective of immune status, that did not develop diarrhea, had higher SBA titers compared with NHPs that developed diarrhea. Importantly, a strong correlation was found between serum anti-CPS IgG and SBA titers in NHPs and similar studies will be conducted in upcoming human clinical trials. This flow cytometry-based SBA assay is much simpler than bacteriological-based assays and should facilitate analyses of functional antibodies against multivalent *C. jejuni* capsule conjugate vaccines.
Shigella is among the most prevalent bacterial pathogens isolated from acute diarrheal episodes in children under 5 years of age in developing countries. The genus Shigella comprises 4 species, including S. dysenteriae, S. flexneri, S. boydii and S. sonnei, and more than 50 serotypes as specified by the composition of the surface polysaccharide O antigen. Due to these serotypic differences and increasing numbers of antibiotics resistance, development of broadly protective vaccine is needed for controlling shigellosis.

We targeted outer membrane proteins of Shigella as a universal Shigella vaccine, and hence constructed mutant strains which have only one unit of O antigen. The mutation shortens the layer of lipopolysaccharide (LPS) on Shigella, allowing other Shigella outer membrane proteins to be better exposed, thereby potentially inducing stronger protective immunity. For example, a conserved outer membrane protein, IcsP which is normally masked by LPS on the cell surface, showed enhanced exposure on mutant strains. Three doses of intranasal immunizations with either live or formalin-inactivated mutant whole cells of S. flexneri 2a induced cross-protection against S. flexneri 2a, S. flexneri 6 and S. dysenteriae 1 in mouse pneumonia model. The addition of dmLT with formalin inactivated mutant whole cells enhanced protective immunity. The experiments using each different mutant strain (based on S. flexneri 6, S. sonnei, S. dysenteriae 2, or S. boydii) are ongoing to select the best formulation for cross-protection.

In conclusion, the mutant strain can be a promising candidate for a universal Shigella vaccine to elicit stronger and broader cross-protective immunity than the strategy of targeting LPS alone.

*Current affiliation: DW Kim- College of Pharmacy, Hanyang University, Ansan, Korea; C Czerkinsky- Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, INSERM, Université de Nice-Sophia Antipolis, UMR 7275, Valbonne, Francec
PRE15 Protection of Aotus nancymae non-human primates from enterotoxigenic Escherichia coli (ETEC) diarrhea by ID or IM immunization with a fimbrial subunit prototype vaccine

Amritha Ranakrishnan 1, Nathanael Reynolds2, Milton Maciel Jr. 3, Michael Prouty3, Marie Garinot4, Jon Heinrichs5, Geneviève Renauld-Mongénie4, Mark Simons2, Stephen Savarino3

1 Department of International Health, Johns Hopkins School of Public Health, Baltimore, MD
2 Department of Bacteriology, US Naval Medical Research Unit No. 6, Callao, Peru
3 Department of Enteric Diseases, US Naval Medical Research Center, Silver Spring, MD
4 Research Department, Sanofi Pasteur, Marcy-l’Étoile, France
5 Research Department, Sanofi Pasteur, Swiftwater, PA

Introduction: Currently, no broadly protective vaccine against enterotoxigenic Escherichia coli (ETEC) exists. A key challenge in the development of new vaccines is improving the intensity and duration of intestinal immune responses and corresponding vaccine efficacy, particularly in children in developing countries. To this end, a number of different vaccine formulations, routes of delivery and adjuvants are actively being pursued and are in various stages of clinical development.

Methods: We utilized the non-human primate, Aotus nancymae challenge model to assess two different TLR agonists as parenteral adjuvants for a prototype anti-ETEC vaccine formulation. The vaccine consisted of the donor strand complemented (dsc) fusion protein of the minor (CfaE) and major (CfaB) subunits of CFA/I fimbria plus the ETEC heat-labile toxin B subunit (LTB). Animals were immunized four times by the intradermal (ID) or intramuscular (IM) routes and orally challenged with CFA/I+ ETEC, strain H10407. Incidence of diarrhea was monitored for 10 days after challenge and serological responses were quantified throughout the immunization protocol.

Results: In the first study, a group of animals (n=10) were immunized ID with dscCfaEB and the B subunit of the heat-labile toxin (LTB) along with a TLR-3 agonist as an adjuvant, while the control group (n=10) received only adjuvant. Following oral challenge, 100% protective efficacy (p<0.05) was observed in the group that received the dscCfaEB vaccine formulation. Protection was accompanied by robust serum anti-dscCfaE and anti-CFA/I IgG and IgA antibody responses as well as functional neutralizing antibodies as demonstrated by the hemagglutination inhibition assay (HAI). In addition, high titer anti-toxin serum antibodies were also observed.

We next sought to investigate the protective efficacy of dscCfaEB when administered by the IM route. In this study, groups of animals (n=8) received the same fimbrial subunit prototype, dscCfaEB+LTB along with a TLR-4 agonist as an adjuvant. The control group (n=8) received only adjuvant. This regimen elicited 80% protective efficacy against CFA/I+ ETEC challenge (p<0.05). IM vaccination also promoted significant increases in serum anti-CFA/I IgG responses, which peaked after the second dose and remained elevated over the course of the study. The serum antigen-specific IgA responses followed similar kinetics, but were lower in magnitude. High titer functional neutralizing antibodies as well as anti-toxin antibodies were also observed.

Conclusions: These studies provide a robust proof-of-principle for dscCfaEB+LTB as a protective antigen against ETEC. In addition, these studies demonstrate that protection against mucosal challenge can be achieved via parenteral vaccination (ID/IM) with two prototype TLR agonist adjuvants. Both adjuvants led to high serum anti-adhesin (CfaE), anti-fimbria (CFA/I), and anti-toxin (LT) IgG and IgA antibody titers, in addition to functional neutralizing antibodies. In exploratory analyses, we found no correlation between these serological measures and protection from diarrhea in this model. New methodologies are currently being developed to investigate the mucosal response in vaccinated animals and address other possible mechanisms of protection present at the mucosa.
PRE17 Evaluation of local skin reactogenicity compared to serum and mucosal antibodies after intradermal ETEC immunization with CfaEB and E. coli heat-labile toxin-derived proteins.

Mark A. Smith¹, Milton Maciel, Jr.², Elizabeth Norton³

¹Naval Medical Research Center, Silver Spring, MD USA
²Walter Reed Army Institute of Research, Silver Spring, MD USA
³Tulane University, New Orleans, LA USA

Introduction. Intradermal (ID) delivery is actively being pursued for a subunit ETEC vaccine containing the adjuvant/immunogen heat-labile toxin (LT) from ETEC or its detoxified mutant, LT-R192G/L211A (dmLT). However, transient injection site side effects (itchiness, hyperemia, and induration) have been observed in ID clinical trials as well as rarer, long-lasting hypo- or hyperpigmentation. LT and dmLT are AB5 proteins with a GM1-binding B-subunit linked to adverse skin reactions. Here, we sought to more fully understand the generation of local skin reactogenicity with LT-based proteins and this relationship to vaccine outcomes.

Methods. We evaluated mice in a vaccine model with the fimbriae colonization protein CfaEB, a pilot protein vaccine candidate against ETEC, paired with dmLT, dmLT + 5% lactose (which binds to the B-subunit), LT-G33D (a GM1-binding mutant), or LTA1 (the enzymatic A1 domain). BALB/c mice were immunized ID twice, 30 days apart, with a Mantoux injection containing 10μg of CfaEB alone or combined with 0.1μg dmLT, dmLT-5%, LT-G33D or 1μg LTA1. Mice were sacrificed at day 2 (n=3) or day 45 (n=5) for blood, fecal and skin collection. Skin samples were processed for H&E histological staining then blindly scored (0-no lesion, 1-minimal, 2-mild, 3-moderate, 4-marked, and 5-severe) and thickness measured. Sera and fecal supernatants were evaluated for anti-LT and anti-CFA IgG or IgA levels by ELISA or functional antibodies by hemagglutination inhibition (HAI) assay.

Results. The most severe skin reactions were observed initially after immunization (day 2). Mice immunized with dmLT or dmLT-5% had significant inflammation (histology median score (hs) of 4) and edema characterized by neutrophil infiltration in the dermis (dermatitis) and subcutis (panniculitis) with mild epidermal hyperplasia. Mice immunized with LT-G33D or LTA1 had moderate inflammation (hs 3) and edema with neutrophils admixed with macrophages and lymphocytes. Mice immunized with CfaEB alone (hs 1.5) had no edema and only mild focal aggregates of neutrophils, macrophages, and lymphocytes. There was no dermatitis or panniculitis in the PBS control mice. By day 45, the only significant skin reactions were observed in the dmLT group (hs 3), but clearly reduced from that seen at day 2 and was characterized in the dermis as no edema but plasma cells infiltration and in the subcutis with moderate edema and neutrophil infiltration. Lower levels of inflammation were also present with dmLT-5% (hs 2), LTA1 (hs 1), and LT-G33D (hs 0.3). All groups immunized with a LT-protein developed significant serum and fecal antibody responses to vaccination. Day 45 histology scores were significantly associated with serum anti-CFA IgG (Spearman’s r = 0.45, P < 0.05) but not serum HAI or fecal anti-CFA IgA. In contrast, day 45 skin thickness measurements were significantly associated with serum anti-CFA IgG (r = 0.67, P < 0.001), serum HAI (r = 0.51, P < 0.004) and fecal anti-CFA IgA (r = 0.38, P < 0.04).
Strategies for broader coverage through combination vaccines
Oral Presentations

CB01 A conjugate vaccine approach to provide protection against *Campylobacter jejuni*, Enterotoxigenic *Escherichia coli*, and *Shigella* sp.

Renee M. Laird¹, Nelum Dorabawila¹, Zuchao Ma², Brittany Pequegnat², Yang Liu¹, Steven Poole¹, Milton Maciel Jr.¹, Frederic M. Poly¹, Michael G. Prouty¹, Mario A. Monteiro², Stephen J. Savarino¹, and Patricia Guerry¹

¹ Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD 20190 and ² Department of Chemistry, University of Guelph, Ontario, Canada

Conjugate vaccines linking carbohydrate antigens to protein carriers are licensed and have been shown to provide protection against many bacterial pathogens including *Haemophilus influenzae* and *Streptococcus pneumoniae*. Despite the successful creation of vaccines against multiple mucosal pathogens, vaccines against enteric pathogens have been more difficult to develop. Here we describe a conjugate vaccine approach using recombinant ETEC fimbrial subunits or heat-labile enterotoxin (LT) as carrier proteins for carbohydrate antigens expressed by *Campylobacter jejuni* and *Shigella flexneri*.

There are 35 known capsule polysaccharide (CPS) types of *C. jejuni*, of which approximately 8 appear to be responsible for the majority of disease. CPS structures and purification methods have been solved for a number of *C. jejuni* CPS types, and we have previously demonstrated CPS-CRM197 conjugate vaccines to be immunogenic in multiple animal models. O-specific polysaccharide (O-SP) conjugates of *S. dysenteriae*, sonnei and flexneri strains have also been described and are immunogenic in both animal models and human clinical studies. There are multiple recombinant ETEC proteins being pursued as vaccine candidates which include proteins involved in bacterial adhesion and the heat-labile toxin. We have recently shown that the CFA/I tip adhesin, CfaE, delivered with a mutant detoxified LT (LT(R192G)), to be immunogenic in humans, however, ETEC adhesin-based vaccines have not yet been explored as carrier proteins. Three *C. jejuni* CPS types (HS23/36, HS3 and HS4) or detoxified *S. flexneri* 2a O-SP (strain 24570) were purified and conjugated to one of three ETEC proteins, an adhesin-pilin (CfaEB) a pilin-pilin (CssBA) fusion from CFA/I and CS6 colonization factors, respectively, and the B subunit of LT (LTB). Subcutaneous immunization of mice with CPS-ETEC or O-SP-ETEC conjugates induced a significant rise in serum IgG titers against both the carbohydrate antigens and the ETEC carrier proteins. Functional antibodies measured by hemagglutination inhibition were present in mice that received conjugate vaccines containing CfaEB carrier protein indicating that adhesin-pilin proteins are able to induce functional antibody responses when covalently linked to carbohydrate antigens. Importantly, when these conjugates were combined into multivalent formulations, we observed high IgG anti-CPS and anti-protein titers that were not significantly different from titers achieved when delivered in a monovalent formulation. These data provide support that a conjugate vaccine approach is a promising platform to provide protection against these three important enteric pathogens.
Systems biology and genomics
Oral Presentations

SB02 A systems approach to the study of ETEC H10407 challenge
Taylor K.S. Richter (1), Yang Song (1), Jane Michalski (1), Anup Mahurkar (1), Owen R. White (1), James B. Kaper (2), Wilbur H. Chen (3) and David A. Rasko (1,2)

(1) The Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA; (2) Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, USA; (3) Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, USA.

Introduction: Enterotoxigenic E. coli (ETEC) is one of the main causes of traveller’s diarrhea and a leading source of diarrhea in the developing world. Many studies have been completed to examine the impact of ETEC infection on the individual, as well as the larger economic impact on populations where this pathogen is endemic. However, recent advances in high-throughput sequencing technologies have opened the possibility to examine the introduction of ETEC on the microbiome of the human gastrointestinal tract.

Methods: Fecal samples were obtained prior to, during, and after ETEC H10407 challenge over a 10-day period. Multiple stools were obtained during the periods of peak diarrhea. An average of 17 stools were obtained from each of six subjects. Fecal samples from an ETEC challenge study were prepared for 16S rRNA, whole metagenome and metatranscriptome sequencing using standard protocols at the Institute for Genome Sciences at the University of Maryland School of Medicine. Analysis of 16S rRNA gene sequencing was completed with established pipelines. Metagenomic analysis was completed using MetaPhlAn and metatranscriptome analysis was completed primarily with HUMAnN and custom bioinformatics analysis pipelines.

Results: The most abundant organisms in each of the samples were identified by 16S rRNA gene analysis and whole metagenome sequencing (WMS). While some concordance exists between these two methods, the whole metagenome sequencing provides a broader picture of the community as a whole. As measured by Shannon diversity index, we did not identify a common trend of alterations to the microbiome correlated to either ETEC challenge or antibiotic treatment. Metatranscriptional studies identified common transcriptional patterns among those individuals with severe diarrhea. A number of pathways such as those involved with bacterial regulation and carbohydrate utilization appear to be indicative of a diarrheal stool that are not observed in pre-challenge stools or individuals with mild to moderate diarrhea.

Conclusions: This is one of the first studies to examine the impact on the microbiome of the complete gastrointestinal tract community before, during and after ETEC challenge and ETEC induced diarrhea, with a comprehensive systems biology approach. These studies begin to provide valuable insights into the impact of the ETEC challenge on the healthy gut microbiome, as well as the alterations in the pathogen transcriptome during the course of disease."
Poster Presentations

SB01 Host genetics factors influence altered susceptibility of Enterotoxigenic *E. coli*: determining the association between FUT2, the Lewis antigens, ABH blood groups and susceptibility to infection

Lynda Mottram
University of Gothenburg

There is strong evidence to suggest changes in host expression of ABH and Lewis antigens could be a major determinant of altered susceptibility to infection. The likely reason for this being blood group antigens can play direct roles in infection by serving as receptors or co receptors for microorganisms, parasites or viruses. A homozygous mutation in Fucotransferase genes leads to the absence of ABH blood groups, and alterations in Lewis antigen expression (non-secretor status). In erythrocytes this is controlled by FUT1, but in epithelial linages and mucosal secretions expression is controlled by FUT2.

Non-secretor status is extremely heterogenous and ethnic specific and is found in approximately 20% of the worldwide population. However in Bangladesh where an enterotoxigenic *E. coli* (ETEC) vaccine trail is currently on going, published studies have shown that as many as 40% of all Bangladeshi’s could be non-secretors. Furthermore, children under the age of two who are living in Dhaka have been found to be more likely suffering with symptomatic ETEC infection if they have non-secretor status, with the explanation for this association being ETEC CFA/I fimbriae binds to a Lewis non-secretor antigen known as Lea. We are currently performing a host genomic association study to evaluate if a single nucleotide polymorphism of FUT2 is directly associated with altered susceptibility to ETEC CFA/I infection, and therefore assess if FUT2 secretor status is playing a direct role in altering susceptibility to symptomatic ETEC CFA/I infection in the Bangladesh population.
Serological cross-reaction between *Shigella dysenteriae* type 4 and *Escherichia albertii* DM104 is caused by inter-species transfer of the O-antigen gene cluster

Faustina Asare and Nils-Kåre Birkeland

University of Bergen, Bergen, Norway

*Escherichia albertii* strain DM104, previously isolated from surface water in Bangladesh cross-reacts serologically with *Shigella dysenteriae* type 4 in a type-specific manner (1), and induces protective immunity to *Shigella dysenteriae* challenge in a guinea pig animal model (2). As strain DM104 has been shown to be avirulent it has a good potential for use as a live vaccine candidate against shigellosis.

Genome sequencing of strain DM104 revealed the presence of a 15 Kb O-antigen gene cluster sharing 85% overall homology with that of *Shigella dysenteriae* type 4, which explains the strong and type-specific cross reaction between these strains and confirms an inter-species transfer of the genes encoding the O-antigen biosynthesis genes. There was no sequence match between the DM104 O-antigen gene cluster and the corresponding region in the *Escherichia albertii* type strain, KF1. One of the DM104 O-antigen genes, *wfeW*, annotated as a glycosyl transferase, turned out to carry sequence motifs belonging to two different Pfam families, and is thus a possible 'hybrid' gene encoding a novel type of glycosyl transferase. Phylogenetically, *wfeW* makes its own branch, distinctly separated from other enteric bacteria. Homologs are found in a few strains belonging to the *Shigella/E. coli* group as well as in the deeply branching Thermotogae phylum and in methanogenic Archaea. Whether this gene encodes the same function in these organisms remains to be seen. The genome sequence analysis of strain DM104 showed that its genome consists of ~ 4.6 Mb, which is slightly larger than that of *Escherichia albertii* KF1, for which it shares 96.1% overall genome sequence homology. The results of this study confirmed the genetic basis for the serological cross-reaction between DM104 and *Shigella dysenteriae* type 4, and support further work with this strain as a live shigellosis vaccine candidate.

References:


Technical, manufacturing, and regulatory challenges in vaccine development and implementation
Poster Presentations

TM01 Development of in vitro assays to document neutralization of LT-mediated toxicity based on its mechanism of action
D. Poncet¹, L. Quemener¹, M. Maciel Jr.³, P. Dinadayala¹, E. Decastro¹, N. Petiot¹, G. Renauld Mongénie¹ and J. Heinrichs²

¹ Sanofi-Pasteur, Marcy L’Etoile, France
² Sanofi-Pasteur, Swiftwater, USA
³ Navy Medical Research Center, Silver Spring, USA

Enterotoxigenic Escherichia coli (ETEC) is responsible for a high diarrheal disease burden especially in children and travelers. After oro-fecal transmission, ETEC reaches the small intestine where adhesion occurs through colonization factors, then the two enterotoxins LT (heat Labile Toxin) and ST (heat Stable Toxin) are secreted and cause aqueous diarrhea. LT consists of five B subunits, which are able to bind the monosialoganglioside GM1 and a single catalytically active A subunit stimulating the intracellular synthesis of cyclic adenosine monophosphate (cAMP), leading ultimately to fluid and electrolyte secretions into the intestinal lumen. Herein we developed and implemented three in vitro assays, representative of the ETEC LT-enterotoxin activity, to document the ability of antibodies generated against rLTB (recombinant B subunit of LT) and/or dmLT (double mutated LT) to neutralize the enterotoxic and cytotoxic effects of native LT: i) GM1-ELISA for binding to GM1 (anti-LT-B), ii) cAMP release by cell lines using fluorescence LANCE Ultra cAMP kit for intracellular enzymatic activity (anti-LT-A) and iii) Morphological Changes of cell lines using the xCELLigence system associated with fluid secretion (anti-LT-A and –B). Optimization of the last two methods was performed. Among different cell lines evaluated, Vero cells were selected. Various toxin sources, including native cholera toxin, and recombinant or native LT, were also evaluated and all demonstrated potent dose-response toxicity. Technical variability was evaluated and statistical analysis demonstrated an acceptable precision value for both methods. When evaluating seroneutralization, unexpectedly high background levels were obtained with pre-immune CD1 mouse sera but not with BALB/c mouse sera in both assays. Seroneutralization of LT toxicity was observed in both assays with hyperimmune BALB/c mouse sera directed against rLTB and dmLT and was consistent with inhibition of LT binding to GM1. Altogether, these 3 assays might be considered as valuable tools to document the functionality of anti-LT antibodies induced by (dm)LT-containing ETEC vaccines.
Vaccine candidates in clinical trials and human challenge models
Oral Presentations

CL01 A Randomized, Double-Blinded, Placebo-controlled, Dose-Escalation, Age-Descending Study to Assess the Safety and Tolerability of Live, Attenuated, Oral *Shigella* WRSS1 Vaccine candidate in Bangladeshi Adults and Children.

1Raqib R, 1Sarker P, 1Anjuman A, 2Fix A, 2Maier N, 1Zaman K, 1Qadri F, 1Talukder K, 3Kaminski RW, 2Van de Verg L, 3Venkatesan MM

1Centre for Vaccine Sciences, icddr,b, Dhaka, Bangladesh; 2Enteric Vaccine Initiative, Vaccine Development Global Program, PATH, Washington, USA; 3Bacterial Diseases Branch, Walter Reed Army Institute of Research, Maryland, USA

**Background:** Shigellosis is a major cause of moderate to severe diarrhea in children ≤ 5 years of age in developing countries. While historically *Shigella flexneri* has been the dominant species in endemic populations, with improved living conditions, *S sonnei* is replacing *S. flexneri* in countries such as Bangladesh. An efficacious multivalent *Shigella* vaccine would greatly limit the burden of diarrheal disease in endemic and the traveler population.

WRSS1 is a live, oral *S. sonnei* vaccine candidate which is unable to spread intercellularly due to a VirG(icsA) deletion. In North American, Israeli and Thai volunteers, a dose of 10^4 CFU was considered safe and immunogenic. The current study was designed to evaluate the safety, clinical tolerability and immunogenicity of WRSS1 in adults and children at icddr,b, Dhaka, Bangladesh.

**Study design and methods:** The study was designed in 2 parts. Part A enrolled healthy adults (n=39) who received one dose of 3x10^4 (A1) or three doses of 3x10^5 (A2) or 3x10^6 (A3) CFU of oral WRSS1 vaccine or placebo. Part B enrolled 5-9 year old children (n=64) who received one dose of 3x10^3 (B1) or three doses of 3x10^4 (B2) 3x10^5 (B3) or 3x10^6 (B4) CFU of vaccine or placebo. Shedding of vaccine strain was assessed by culture and PCR. Immunogenicity was determined by evaluating the frequency and magnitude of antigen-specific antibody secreting cells (ASCs), antibody titers in lymphocyte supernatant (ALS) and serum and stool antibodies to *S. sonnei* LPS and Invaplex.

**Results:** WRSS1 caused minimal to mild and transient reactogenicity events in Bangladeshi adults and children. In adults, the vaccine was excreted for one day after the 1st dose in 10% of the volunteers in cohorts A1 and A2 and in 50% of the volunteers in the A3 cohort. However, children did not excrete the vaccine at any tested dose. Mucosal immune responses in adults were robust at the two highest doses based on ALS antibodies and stool IgA responses. Peak ALS antibody responses were obtained after the 1st dose. LPS-specific serum responder frequencies increased with increasing vaccine doses. LPS-specific stool antibodies continued to rise with increasing doses and remained elevated after the 2nd or 3rd administration of a specific dose. In children the highest immune response was noted in stool followed by responses in serum and ALS.

**Conclusion:** WRSS1 was well tolerated by adults and children at all doses tested. At the highest doses, WRSS1 was excreted by 50% of the adult volunteers who generated significant systemic and mucosal immune responses in a dose-dependent manner. Blood, stool and ALS responses were also seen in children at the highest doses. Lack of excretion in children may indicate lack of colonization that is probably linked to increased episodes of diarrhea, persistent inflammation in the intestine and resistance to colonization. Additional studies are being planned in toddlers.
A Phase 1 Open-label, Dose Escalating Study of Artificial *Shigella flexneri* 2a Invaplex administered intranasally to healthy, adult volunteers

C. Duplessis², C. Porter², Ramiro Gutierrez², M. S. Riddle², T. Lee², K. A. Clarkson¹, H.E. Petersen¹, C.R. Stelez¹, S.C. Sumlin¹, Amanda Lynen², E.V. Oaks¹, K.R. Turbyfill¹, Kris Paolino, Wayne Fornillos R.W. Kaminski¹

¹ Subunit Enteric Vaccines and Immunology, Bacterial Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD

² Enteric Disease Department, Infectious Disease Directorate, Naval Medical Research Center, Silver Spring, MD

**Background:** Shigellosis is a food and waterborne disease. Estimates of *Shigella*-associated mortality range from 0.1 to 1.1 million deaths (60% in children under 5) annually. Additionally, there are an estimated 90 to 165 million *Shigella*-associated of dysentery cases annually in endemic pediatric populations and travelers. In addition to the acute morbidity of diarrhea, shigellosis is linked to several post-infectious sequelae including irritable bowel syndrome (IBS) and reactive arthritis. The intranasal (IN) route of mucosal delivery has shown promise for subunit vaccines administration. The *Shigella* artificial invasin complex (termed, InvaplexAR) contains IpaB, IpaC and *S. flexneri* 2a LPS. The primary objective of this study was to evaluate the safety of *Shigella flexneri* 2a InvaplexAR given by IN immunization. Vaccine safety was actively monitored during vaccination and for 28 days following the third vaccine dose. The secondary objectives were to evaluate immune responses and thus identify a safe and immunogenic dose of *Shigella flexneri* 2a InvaplexAR to advance to preliminary efficacy studies [i.e., expanded Phase 1 or Phase 2b vaccine trials].

**Methods:** The study was an open-label, dose-escalating first-in-human trial in which volunteers received one of four *S. flexneri* 2a InvaplexAR vaccine doses (10, 50, 250 or 500 µg). We have completed the first three cohorts enrolling 27 subjects. The vaccine was administered intranasally (without adjuvant) on Days 0, 14, and 28 in a total volume of 200 µL split equally between both nostrils and delivered with a nasal spray device (VaxINator ™—distributed by Teleflex). Blood, stool, saliva and ocular secretions were collected at specified intervals to examine systemic and mucosal immune responses directed to Invaplex, LPS, IpaB and IpaC. Peripheral blood mononuclear cells (PBMCs) were also collected to determine IgA antibody secreting cells (ASC) and antibody lymphocyte supernatant (ALS) responses.

**Results:** Intranasal immunization with *S. flexneri* 2a InvaplexAR was well tolerated. The overall safety and tolerability profiles were consistent with prior intranasal immunizations. During the 3-dose vaccination series of all cohorts, there were no adverse events that met the vaccination stopping criteria. Most vaccine-related adverse events were of mild severity. Only three subjects reported adverse events of moderate severity. The most commonly observed adverse events were rhinorrhea (0-33%), nasal congestion (11-33%), nasal tenderness or burning (0-33%), and nasal itching (0-33%) most commonly observed in the 4 days following vaccination. The VaxINator ™ consistently delivered 200 µL ± 10% of *Shigella flexneri* 2a InvaplexAR.

**Conclusions:** Intranasal immunization with *Shigella flexneri* 2a InvaplexAR was safe and well tolerated. The AE frequency was comparable to that observed in prior InvaplexNAT investigations, and were stable or decreased in frequency with successive dosing. The VaxINator ™ consistently delivered the intended vaccine volume. Immunological analysis is currently underway and will help inform future clinical evaluations of the subunit *Shigella* vaccine.
Background: Shigella spp. cause severe disease among travelers to and children living in developing countries. Several candidate vaccines against Shigella are in development, but the lack of a clear correlate of protection and challenges with the induction of adequate immune responses among the youngest age-groups in the developing world has hampered Shigella vaccine development over the past several decades. Conjugated vaccines have been shown to be safe and effective for different pathogens (i.e. N. meningitidis, S. pneumoniae, H. influenzae). The bio-conjugation technology, exploited here for the S. flexneri 2a candidate vaccine, offers a novel and potentially cost effective way to develop and produce vaccines against a major pathogen of global health importance.

Methods: Flexyn2a, a novel S. flexneri 2a bioconjugate vaccine made of the polysaccharide component of Shigella flexneri 2a LPS, conjugated to the exoprotein A of P. aeruginosa (EPA), was evaluated for immunogenicity and safety among healthy adults in a single blind, placebo-controlled, Phase I study with a staggered randomization approach. Thirty subjects (12 in each group immunized with 10 μg Flexyn2a with or without aluminum adjuvant and 6 subjects with placebo) received two intramuscular injections 4 weeks apart and were followed for 168 days.

Results: Both formulations were well tolerated; independent of the adjuvant and the number of injections. The overall safety and tolerability profiles were consistent with that of other conjugated vaccines. Both Flexyn2a vaccine formulations elicited statistically significant S. flexneri 2a LPS-specific humoral responses at all time points post immunization. Between-group comparisons of both Flexyn2a active groups did not show statistically significant differences in geometric mean titers of serum IgG or IgA at any post-vaccination time point. Serum antibodies elicited after administration of Flexyn2a were functional as evidenced by bactericidal activity against S. flexneri 2a, 2457T.

Conclusions: The bioconjugate candidate vaccine Flexyn2a has a satisfactory safety profile and elicited a significant humoral response to S. flexneri 2a LPS with or without inclusion of an adjuvant. Moreover, the bioconjugate also induced functional antibodies, showing the technology’s promise in producing an effective candidate vaccine.

This trial is registered at ClinicalTrials.gov (NCT02388009).
CL04 Memory B cell responses of volunteers intradermally immunized with the ETEC fimbrial tip adhesin CfaE plus LT(R192G) followed by oral challenge with CFA/I+ ETEC H10407

Stefanie A. Trop, Sakina Shahabudin, Renee M. Laird, Chad K. Porter, Stephen J. Savarino, Mark S. Riddle, Ramiro L. Gutierrez, and Milton Maciel, Jr.

Naval Medical Research Center, Enteric Diseases Department
Henry Jackson Foundation

Introduction Enterotoxigenic E. coli (ETEC) is a primary cause of infectious diarrhea in developing regions and is a target for multiple vaccine development efforts, including those of the US Navy. Our vaccine development strategy is based on recombinant fimbrial tip adhesins and fimbrillar sub-units to induce antibodies that inhibit ETEC intestinal adherence and colonization. A prototype vaccine comprising CfaE (CFA/I adhesin subunit) administered intradermally (ID) with the antigen/adjuvant LT(R192G) (single mutant of the heat-labile toxin) was recently evaluated in a Phase 2b trial for efficacy against moderate-to-severe diarrhea (MSD) upon challenge with CFA/I+ ETEC, strain H10407. An important goal of immunization is the induction of long-term memory B cells (MBC) that can rapidly respond upon antigen (Ag) re-exposure. Therefore, we evaluated MBC responses to vaccine CfaE and LT following vaccination and challenge, and to CFA/I fimbriae following challenge in vaccinees and naïve controls.

Methods Volunteers were immunized with 25 µg CfaE + 100 ng LT(R192G) on days 0, 21, and 42. Vaccinees and control volunteers were orally challenged with 1.0–1.9 x 10^7 colony forming units of H10407 on day 70. Peripheral blood lymphocytes were isolated from vaccinees on day 0 and from both groups on days 69 and 98. Following a 6-day expansion with polyclonal stimulation, we assessed MBC responses to CfaE and LT post-immunization and -challenge (day 69 and 98), and to CFA/I post-challenge (day 98), compared to baseline levels. Specific IgA and IgG MBC were quantified by ELISpot as antibody-secreting cells (ASC) and presented as Ag-specific/total IgA or IgG ASCs. Correlations between Ag-specific MBC levels postimmunization and MSD were investigated.

Results After vaccination, 24/44 volunteers showed statistically significant higher percentages (Wilcoxon matched-pairs signed rank test) of anti-CfaE IgA MBC (mean±SD: 0.073%±0.098%) compared to day 0 (0.029%±0.045%; P<0.001), while 31/44 generated LT-specific IgA MBC (0.18% ± 0.20% vs 0.094%±0.11%; P<0.001). Vaccinees mounted higher anti-CfaE (42/44; 0.52%±0.72%) and -LT (41/44; 0.92%±1.05%) IgG MBC compared to day 0 (P<0.0001) as well.

Twenty-eight days after oral challenge, naïve controls showed increases in LT-specific IgA (26/39) and IgG (36/39) MBC (0.24% ± 0.22% and 0.81% ± 0.99%, respectively; P<0.01 and P<0.001 vs. day 69), with no changes in CfaE-specific MBC levels. In vaccinees, CfaE- and LT-specific IgG, but not IgA, MBC were further augmented following challenge (P<0.05, P<0.01). CFA/I-specific MBC were generated by both controls and vaccinees, with higher levels of IgA than IgG. Vaccinees that experienced MSD had half the levels of IgG MBC pre-challenge than those who did not (CfaE: mean 0.35% vs. 0.63%; LT: 0.63% vs. 1.1%), though these differences were not statistically significant.

Conclusions ID immunization with CfaE + LT(R192G) induced specific IgG and IgA MBC to both Ags, neutralization of which could prevent ETEC adherence and toxin uptake. Vaccinees without MSD tended to have higher CfaE- and LT-specific IgG MBC levels pre-challenge. ETEC challenge yielded CFA/I-specific MBC, with higher percentages of IgA than IgG, consistent with the oral route of infection. Additional studies are needed to further investigate correlations between Ag-specific MBCs clinical outcomes, MBC longevity, and gut-homing characteristics.

The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

This work was supported by work unit number 643807-A.894.D.A0002. The study protocol was approved by the Naval Medical Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects.
CL05  One step forward towards a phase 1 clinical trial with the first semi-synthetic glycoconjugate vaccine against *Shigella flexneri* 2a, SF2a-TT15

Armelle Phalipon1, Robert van der Put2, Janny Westdijk2, Cécile Artaud3, Dani Cohen4, Jacob Atsmon5, Marie-Lise Gougeon6, Philippe Sansonetti1, Laurence Mulard7

1: Unit Molecular Microbial Pathogenesis, INSERM U1208, Institut Pasteur, Paris, France
2: Institute for Translational Vaccinology (Intravacc), Bilthoven, The Netherlands
3: Center for Translational Science, Clinical Core, Institut Pasteur, Paris, France
4: Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.
5: Clinical Research Center Tel-Aviv Sourasky Medical Center
6: Unit Anti-viral Immunity, Biotherapy and Vaccines, Institut Pasteur, Paris, France
7: Unit Chemistry of Biomolecules, Institut Pasteur, Paris, France

Parenterally-administered detoxified lipopolysaccharide (LPS) conjugates developed in line of the success of capsular polysaccharide-based vaccines have been shown to be a promising strategy to protect adults against *Shigella* infection, while in toddlers their immunogenicity remains to be improved (for a review 1). Both the detoxification and random conjugation processes are known to impact on the immunogenicity of such glycovaccines. To overcome this limitation, and to fulfill the increasing requirements for better-defined and safer molecules to be used in humans, the use of synthetic oligosaccharides (OS) mimicking the epitopes carried on the O-antigen (O-Ag) polysaccharide part of LPS and being the targets of the protective antibody (Ab) response has been proposed as an alternative. Along this line, the synthetic OS-based conjugate, called SF2a-TT15, incorporating a pentadecasaccharide mimicking the SF2a O-Ag, was designed in order to obey to the following rules: (i) the use of carbohydrate haptens suitable for single-point attachment onto a carrier to overcome the limitations due to LPS random chemical modifications and/or detoxification; (ii) the control of various parameters such as the length and nature of the carbohydrate hapten, its loading onto the carrier, and (iii) the choice of the carrier, to allow the design of glycoconjugates with optimal immunogenicity and their compatibility with transfer for evaluation in human (2). Preclinical data of SF2a-TT15 GMP vaccine lot will be presented, including the impact of aluminum hydroxyde on the anti-SF2a-LPS antibody response (3). The design of the ongoing Phase 1 trial will be described.

CL06  Clinical trials of an oral inactivated ETEC vaccine (ETVAX)

Ann-Mari Svennerholm & Anna Lundgren
Department of Microbiology and Immunology, Institute of Biomedicine, University of Gothenburg, Sweden

Background. Although ETEC remains to be a major cause of diarrhea morbidity and mortality in children less than 5 years in the developing world and in visitors to these regions there is no ETEC vaccine for use in humans available yet. Based on our previous studies in animals and humans that mucosal immune responses against LT antigen and colonization factors (CFs) are protective we have developed an oral ETEC vaccine (ETVAX) containing the most prevalent ETEC CFs and an LT toxoid. ETVAX is an oral multivalent vaccine consisting of four inactivated recombinant \textit{E. coli} strains over-expressing CFA/I, CS3, CS5 and CS6, and an LTB related toxoid, LCTBA. Since our recent genomic studies have shown that the vaccine CFs belong to restricted lineages that are prevalent in different geographic locations all over the world and stable during several decades, we believe that ETVAX may have a worldwide coverage during foreseeable time.

Results. ETVAX has been evaluated, alone and together with a promising mucosal adjuvant, double mutated LT (dmLT), for safety and immunogenicity in clinical trials in adult Swedish volunteers. In an initial placebo-controlled, double-blind Phase I study including 4 groups with altogether 129 volunteers the vaccine, given in two oral doses two weeks apart, was found to be well tolerated and safe with no significant difference in adverse events between vaccine and placebo (buffer only) recipients. Furthermore, the vaccine was shown to induce significant intestinal (fecal) secretory IgA and intestine-derived (antibody-secreting cell) ALS IgA responses against all the four vaccine CFs and LTB in more than 80% of the volunteers. The dmLT adjuvant enhanced mucosal immune responses to the CF, i.e. CS6 expressed in the lowest amounts in the vaccine. The vaccine was also shown to induce Th1, Th17 as well as follicular T cell (Tfh) immune responses in a majority of the analyzed volunteers.

In a subsequent Phase I study we could show that ETVAX is capable of inducing a strong functional mucosal immunological memory. This was demonstrated by more frequent, higher and earlier appearing ALS IgA responses to a single oral dose of ETVAX in subjects who had previously, 1-2 years earlier, been given two doses of ETVAX two weeks apart than in previously non-vaccinated (naive) matched volunteers. The responses to the single booster dose were found to be similar, or even higher than after the two initial vaccinations.

Conclusions. Based on these encouraging results ETVAX is now being tested in a large Phase I/II study in age-descending groups (adults, toddlers and infants) in Bangladesh and will be evaluated for protective efficacy in visitors to a highly ETEC endemic country.
Vaccine health economics, investment case for vaccines, and impact assessments

Oral Presentations

HE01 Enteric Vaccines, Ethical Imperatives for Health and the SDGs

David R Curry

Center for Vaccine Ethics and Policy

The Sustainable Development Goals [SDGs/Global Goals] have health threaded through many of the 170+ targets approved by UN member states in September 2015.

While not necessarily explicit in the relevant SDG health targets and indicators, addressing enteric diseases will be a key strategy in achieving many of them, and will make an important impact on the SDGs overall, especially those around poverty and aligned themes. Development of effective enteric vaccines – and their equitable, affordable global deployment – will be a key tactic in this larger context.

Of course, disease burden, developmental impacts on children, economic costs of these diseases, and other factors have long suggested that vaccines for enteric diseases should receive priority in terms of level-of-effort and funding across public and private domains. Indeed, we argue that there is a strong ethical imperative to accelerate this work.

This paper will present a high-level overview of the SDGs/Targets/Indicators with health implications, articulate how enteric disease and vaccines to fight them would factor into achievement strategies, and make the case that there is an ethical imperative to seize the opportunity we have to move the work of enteric vaccines development forward.
# Index of Authors

<table>
<thead>
<tr>
<th>Author Name</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agger, Else Marie</td>
<td>34</td>
</tr>
<tr>
<td>Aichinger, Michael</td>
<td>38</td>
</tr>
<tr>
<td>Akhtar, Marjanah</td>
<td>23</td>
</tr>
<tr>
<td>Alzari, Pedro</td>
<td>40</td>
</tr>
<tr>
<td>Amaya, Erick</td>
<td>9</td>
</tr>
<tr>
<td>Andersen, Peter</td>
<td>34</td>
</tr>
<tr>
<td>Anjuman, A</td>
<td>58</td>
</tr>
<tr>
<td>Ankudinov, I.V.</td>
<td>21</td>
</tr>
<tr>
<td>Aparin, P.G.</td>
<td>21</td>
</tr>
<tr>
<td>Arizmendi, Oliva</td>
<td>45</td>
</tr>
<tr>
<td>Artaud, Cécile</td>
<td>62</td>
</tr>
<tr>
<td>Asare, Faustina</td>
<td>56</td>
</tr>
<tr>
<td>Ashkenazi, Shai</td>
<td>18</td>
</tr>
<tr>
<td>Astrovskaia, Irina</td>
<td>26</td>
</tr>
<tr>
<td>Atsmon, Jacob</td>
<td>62</td>
</tr>
<tr>
<td>Bagamian, Karoun H</td>
<td>14</td>
</tr>
<tr>
<td>Barry, Eileen M</td>
<td>39, 44</td>
</tr>
<tr>
<td>Bassal, Ravit</td>
<td>7</td>
</tr>
<tr>
<td>Bauer, David</td>
<td>42</td>
</tr>
<tr>
<td>Becker-Dreps, Sylvia</td>
<td>9</td>
</tr>
<tr>
<td>Benjamín, William H</td>
<td>34</td>
</tr>
<tr>
<td>Bhuiyan, Taufiqur Rahman</td>
<td>23</td>
</tr>
<tr>
<td>Bialik, Anya</td>
<td>18</td>
</tr>
<tr>
<td>Bicknese, Amelia</td>
<td>11</td>
</tr>
<tr>
<td>Birkeland, Nils-Käre</td>
<td>56</td>
</tr>
<tr>
<td>Blasco, Pilar</td>
<td>40</td>
</tr>
<tr>
<td>Bourgeois, A. Louis</td>
<td>17, 30</td>
</tr>
<tr>
<td>Bowen, Anna</td>
<td>8, 11</td>
</tr>
<tr>
<td>Bravo, Héctor Corrada</td>
<td>26</td>
</tr>
<tr>
<td>Brotons, Ana</td>
<td>35</td>
</tr>
<tr>
<td>Brown, Alexandrea</td>
<td>5</td>
</tr>
<tr>
<td>Brubaker, Jessica</td>
<td>17</td>
</tr>
<tr>
<td>Bucardo, Filemon</td>
<td>13</td>
</tr>
<tr>
<td>Burke, Thomas</td>
<td>16</td>
</tr>
<tr>
<td>Calderwood, Stephen B</td>
<td>23</td>
</tr>
<tr>
<td>Camacho, Ana</td>
<td>36</td>
</tr>
<tr>
<td>Campbell, Davina</td>
<td>11</td>
</tr>
<tr>
<td>Castellano, A</td>
<td>60</td>
</tr>
<tr>
<td>Cenoz, Santiago</td>
<td>35</td>
</tr>
<tr>
<td>Chakraborty, Subhra ..</td>
<td>16, 17, 26, 30</td>
</tr>
<tr>
<td>Chakravarty, Sumana</td>
<td>43</td>
</tr>
<tr>
<td>Chaux, P.</td>
<td>46</td>
</tr>
<tr>
<td>Chen, Wilbur H</td>
<td>19, 25, 54</td>
</tr>
<tr>
<td>Chen, Zhenhai</td>
<td>41</td>
</tr>
<tr>
<td>Chowdhury, Fahima</td>
<td>23</td>
</tr>
<tr>
<td>Coria, Matthew</td>
<td>30</td>
</tr>
<tr>
<td>Clarkson, K.A.</td>
<td>46, 47, 59, 60</td>
</tr>
<tr>
<td>Clements, John D.</td>
<td>29, 42, 47, 48</td>
</tr>
<tr>
<td>Cohen, Daniel</td>
<td>7, 18, 62</td>
</tr>
<tr>
<td>Collier, S</td>
<td>8</td>
</tr>
<tr>
<td>Colomba, Danny V</td>
<td>5</td>
</tr>
<tr>
<td>Cunningham, Aimee</td>
<td>39</td>
</tr>
<tr>
<td>Curry, David R</td>
<td>64</td>
</tr>
<tr>
<td>Czerkinsky, Cecil</td>
<td>50</td>
</tr>
<tr>
<td>de Cerain, Adela López</td>
<td>36</td>
</tr>
<tr>
<td>de Jonge, Marien</td>
<td>34</td>
</tr>
<tr>
<td>Decastro, E</td>
<td>57</td>
</tr>
<tr>
<td>DeLaine, Bre-Onna C</td>
<td>39, 44</td>
</tr>
<tr>
<td>DeNearing, Barbara</td>
<td>17, 18</td>
</tr>
<tr>
<td>Di Paolo, C</td>
<td>60</td>
</tr>
<tr>
<td>Diaz, Yuleima</td>
<td>29</td>
</tr>
<tr>
<td>Dinadayala, P</td>
<td>57</td>
</tr>
<tr>
<td>Donowitz, Mark</td>
<td>27</td>
</tr>
<tr>
<td>Dorabawila, Nulum</td>
<td>53</td>
</tr>
<tr>
<td>Dorman, Alexander</td>
<td>18</td>
</tr>
<tr>
<td>Dougan, Gordon</td>
<td>28, 31</td>
</tr>
<tr>
<td>Duan, Qianqe</td>
<td>37, 41</td>
</tr>
<tr>
<td>Duplessis, C</td>
<td>59, 60</td>
</tr>
<tr>
<td>Dyatlov, I.A.</td>
<td>21</td>
</tr>
<tr>
<td>England, Patrick</td>
<td>40</td>
</tr>
<tr>
<td>Erllich, P</td>
<td>46</td>
</tr>
<tr>
<td>Espinoza, Félix</td>
<td>9</td>
</tr>
<tr>
<td>Estrada, Marcus</td>
<td>33</td>
</tr>
<tr>
<td>Felgner, Philip L</td>
<td>17</td>
</tr>
<tr>
<td>Firstova, V.V.</td>
<td>21</td>
</tr>
<tr>
<td>Fix, A</td>
<td>58</td>
</tr>
<tr>
<td>Fleckenstein, James M</td>
<td>17, 27, 30</td>
</tr>
<tr>
<td>Fornillos, Wayne</td>
<td>59</td>
</tr>
<tr>
<td>Forouzanfar, Mohammad H</td>
<td>5</td>
</tr>
<tr>
<td>Foulke-Abel, Jennifer</td>
<td>27</td>
</tr>
<tr>
<td>Gamazo, Carlos</td>
<td>35, 36</td>
</tr>
<tr>
<td>Gambillara, V.</td>
<td>60</td>
</tr>
<tr>
<td>Garinot, Marie</td>
<td>51</td>
</tr>
<tr>
<td>Gil, Ana Gloria</td>
<td>36</td>
</tr>
<tr>
<td>Gildersleeve, Jeffrey C</td>
<td>30</td>
</tr>
<tr>
<td>Gill, Davinder</td>
<td>31</td>
</tr>
<tr>
<td>Ginsburg, Geoffrey S</td>
<td>16</td>
</tr>
<tr>
<td>Golovina, M.E.</td>
<td>21</td>
</tr>
<tr>
<td>Gonzales, Fredman</td>
<td>13</td>
</tr>
<tr>
<td>Goren, Sophy</td>
<td>7, 18</td>
</tr>
<tr>
<td>Gormley, R.</td>
<td>60</td>
</tr>
<tr>
<td>Gougeon, Marie-Lise</td>
<td>62</td>
</tr>
<tr>
<td>Grass, Julian</td>
<td>11</td>
</tr>
<tr>
<td>Grassel, Christen L</td>
<td>39, 44</td>
</tr>
<tr>
<td>Guerreiro, Catherine</td>
<td>40</td>
</tr>
<tr>
<td>Guerry, Patricia</td>
<td>49, 53</td>
</tr>
<tr>
<td>Gutierrez, Ramiro L</td>
<td>59, 60, 61</td>
</tr>
<tr>
<td>Hanevik, Kurt</td>
<td>39</td>
</tr>
<tr>
<td>Harris, Jason B</td>
<td>23</td>
</tr>
<tr>
<td>Harro, Clayton D</td>
<td>16, 17, 26, 30</td>
</tr>
<tr>
<td>Harutyunyan, Shushan</td>
<td>38</td>
</tr>
<tr>
<td>Heinrichs, Jon</td>
<td>51, 57</td>
</tr>
<tr>
<td>Henics, Tamás</td>
<td>38</td>
</tr>
<tr>
<td>Hochberg, Amit</td>
<td>18</td>
</tr>
<tr>
<td>Hoffman, Stephen L</td>
<td>43</td>
</tr>
<tr>
<td>Hoos, Sylvaine</td>
<td>40</td>
</tr>
<tr>
<td>Hoq, Rubel</td>
<td>23</td>
</tr>
<tr>
<td>Hossain, Lazina</td>
<td>23</td>
</tr>
<tr>
<td>Houben, Diane</td>
<td>34</td>
</tr>
<tr>
<td>Hu, Zhaoyu</td>
<td>40</td>
</tr>
<tr>
<td>Huang, Jiachen</td>
<td>41</td>
</tr>
<tr>
<td>Hurd, Jacqueline</td>
<td>8, 11</td>
</tr>
<tr>
<td>Irache, Juan M</td>
<td>35, 36</td>
</tr>
<tr>
<td>Jackson, Jonathan M</td>
<td>43</td>
</tr>
<tr>
<td>Jaep, Kayla M</td>
<td>6</td>
</tr>
<tr>
<td>James, Eric R</td>
<td>43</td>
</tr>
<tr>
<td>Jimenez-Barbero, J</td>
<td>40</td>
</tr>
<tr>
<td>Jirage, D</td>
<td>47</td>
</tr>
<tr>
<td>Jong, Wouter</td>
<td>34</td>
</tr>
<tr>
<td>Judd, M</td>
<td>8</td>
</tr>
<tr>
<td>Kabuga, Charles Munene</td>
<td>15</td>
</tr>
<tr>
<td>Kaminski, Robert W</td>
<td>22, 24, 46, 47, 48, 58, 59, 60</td>
</tr>
<tr>
<td>Kaper, James B</td>
<td>54</td>
</tr>
<tr>
<td>Khalil, Ibrahim</td>
<td>5</td>
</tr>
<tr>
<td>Khan, Ashraf Islam</td>
<td>23</td>
</tr>
<tr>
<td>Kim, Dong Wook</td>
<td>50</td>
</tr>
<tr>
<td>Kim, Heejoou</td>
<td>50</td>
</tr>
<tr>
<td>Kim, Jae-Ouk</td>
<td>50</td>
</tr>
<tr>
<td>Kim, Min Jung</td>
<td>50</td>
</tr>
<tr>
<td>Kirkcaldy, Robert D</td>
<td>11</td>
</tr>
<tr>
<td>Koley, Hemanta</td>
<td>20</td>
</tr>
<tr>
<td>Korin, Hadar</td>
<td>7</td>
</tr>
<tr>
<td>Kovbasnjuk, Olga</td>
<td>27</td>
</tr>
<tr>
<td>Krause, Petra</td>
<td>38</td>
</tr>
<tr>
<td>Kuhlmann, Frederick M</td>
<td>30</td>
</tr>
<tr>
<td>Kumar, Pardeep</td>
<td>30</td>
</tr>
<tr>
<td>L’vov, V.L.</td>
<td>21</td>
</tr>
<tr>
<td>Laird, Renee M</td>
<td>49, 53, 61</td>
</tr>
<tr>
<td>Lal, Manjari</td>
<td>33</td>
</tr>
<tr>
<td>Larsen, Morten A. G.</td>
<td>29</td>
</tr>
<tr>
<td>Lasierra, Teresa</td>
<td>35</td>
</tr>
<tr>
<td>Lee, Seung Young</td>
<td>50</td>
</tr>
<tr>
<td>Lee, T</td>
<td>59</td>
</tr>
<tr>
<td>Levine, Myron M</td>
<td>19, 39</td>
</tr>
<tr>
<td>Li, Minglin</td>
<td>43</td>
</tr>
<tr>
<td>Li, Shan</td>
<td>26</td>
</tr>
<tr>
<td>Liang, Xiaowu</td>
<td>17</td>
</tr>
<tr>
<td>Ligeour, Caroline</td>
<td>40</td>
</tr>
<tr>
<td>Lin, Jisheng</td>
<td>22</td>
</tr>
<tr>
<td>Lindsay, Brianna R</td>
<td>26</td>
</tr>
<tr>
<td>Liu, Yang</td>
<td>53</td>
</tr>
<tr>
<td>Lurirink, Joen</td>
<td>34</td>
</tr>
<tr>
<td>Lundgren, Anna</td>
<td>23, 63</td>
</tr>
</tbody>
</table>