Vaccine 37 (2019) 4794–4799

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

The way forward for ETEC controlled human infection models (CHIMs)

Kurt Hanevik [a,b,*], Wilbur H. Chen [c], Kawsar R. Talaat [d], Chad Porter [e], Lou Bourgeois [f]

[a] Norwegian National Advisory Unit on Tropical Infectious Diseases, Department of Medicine, Haukeland University Hospital, Bergen, Norway
[b] Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway
[c] University of Maryland School of Medicine, Center for Vaccine Development, Baltimore, MD, USA
[d] Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health, Baltimore MD, USA
[e] Naval Medical Research Center, Forest Glen, MD, USA
[f] PATH, Washington, DC, USA

ARTICLE INFO

Article history:
Available online 29 January 2019

Keywords:
Enterotoxic *Escherichia coli*
ETEC
Controlled human infection models
CHIM
VASE
Workshop
Strain
Selection
Sampling
Standardization
Screening

ABSTRACT

In the absence of good animal models, Controlled Human Infection Models (CHIMs) are useful to assess efficacy of new vaccine candidates against Enterotoxic *Escherichia coli* (ETEC), as well as other preventive or therapeutic interventions. At the 2018 Vaccines Against Shigella and ETEC (VASE) conference, a workshop was held to further review and discuss new challenge model developments and key issues related to further model standardization. During the workshop, invited speakers briefly summarized for attendees recent developments and main agenda issues before workshop participants were divided into four groups for more focused discussions.

The main issues discussed were: (1) whether there is a need for more ETEC strains to test a diversity of vaccine candidates, and if so, what criteria/qualities are desirable in strain selection; (2) how ETEC CHIMs could be more standardized to better support ETEC vaccine development; (3) how volunteer selection criteria and screening should be performed, and; (4) how an expanded sample collection schema and collaborative analysis plan may facilitate a more in-depth assessment of the role of antigen-specific humoral and cellular immune responses in ETEC infection, and provide better insights into ETEC pathogenesis and correlates of protection.

The workshop concluded that additional challenge strains may need to be developed to better support new vaccines and therapeutics that are advancing in the development pipeline. In this regard, the need for a well characterized ST-only expressing ETEC strain was highlighted as a priority given that promising new heat stable toxoid based vaccine candidates are on the horizon. In addition, further standardization of the ETEC CHIMs was strongly encouraged, noting that it may not be realistic to standardize across all strains. Also, intensified volunteer screening may result in higher attack rates, although more stringent eligibility criteria may contribute to a more limited application of the model and diminish its representativeness. Finally, a sampling schedule and priority list for minimum set of samples was also proposed. Future workshops could be held to further refine standards for ETEC CHIMS and to facilitate more collaborative work on stored sample sets from previous and future ETEC CHIMs to maximize the contribution of these trials to our understanding of ETEC pathogenesis and our development of better prevention and control measures for this important pathogen.

1. Background

Controlled human infection models (CHIMs) have been developed as an important tool to assess preliminary efficacy of prototype vaccine candidates for several intestinal pathogens, including norovirus, *V. cholerae*, *Shigella, Campylobacter* and enterotoxigenic *Escherichia coli* (ETEC). CHIMs have the potential to speed up selection of promising vaccine candidates at a lower cost than field trials. It is also increasingly appreciated that CHIMs can be utilized to expand our knowledge of disease pathogenesis and correlates of protection. The VASE (Vaccines Against Shigella and ETEC) 2018 workshop followed up on central topics raised in the 2016 VASE CHIM workshop [1,2] with a specific focus on the ETEC model and new data obtained and analysed over the past two years. In particular, although the ETEC CHIM has been used successfully to evaluate vaccine candidates [3–10], there may be

* Corresponding author at: University of Bergen, Department of Clinical Science, Jonas Lies vei 87, 5020 Bergen, Norway.
E-mail address: kurt.hanevik@med.uib.no (K. Hanevik).

https://doi.org/10.1016/j.vaccine.2019.01.003
0264-410X/

opportunities for model improvement and standardization to include inoculum preparation, sample testing and clinical outcome assessment [11]. This need is highlighted by inconsistent attack rates in several trials [6,12]. Potentially, enhanced screening to exclude individuals resistant to infection may help increase the likelihood of achieving a sufficiently high attack rate among purported naïve individuals [6]. Additionally, we wanted to explore whether new ETEC strains should be developed into challenge strains and used to evaluate ETEC vaccines or treatment alternatives. This workshop brought together a group of researchers with various perspectives and experiences to discuss these issues. This summary article thus reflects the current views of a rapidly evolving landscape related to further development and utilization of ETEC CHIMs.

2. Methods

After a short introduction, this two-hour workshop started with four 10-minute introductions to address four key issues pertaining to ETEC controlled human infection models;

1. Is there a need for more ETEC challenge strains, and if so, what criteria/qualities are desirable in strain selection?
2. What is the rationale for evaluating host susceptibility to ETEC and how such data may be used to refine subject selection criteria while ensuring a representative study population?
3. How could ETEC CHIMs be more standardized to better support ETEC vaccine development, incorporating novel methods of inoculum preparation, recent experience in dose responses, fasting times, and the evaluation of clinical outcomes?
4. How can we improve sample collection schemes and collaborative analysis that facilitate more in-depth assessment of host responses to ETEC infection and provide better insights into how ETEC vaccine candidates may impact on both acute and potentially more long-term consequences of ETEC infections?

After short presentations of these issues, participants were allocated to four different groups, each discussing issues related to one of the four presented topics for 40 min. Groups were moderated by the topic presenter. Group discussions were then summarised and discussed in a 35 min' plenary session.

3. Results and discussion

3.1. Strain selection

The challenge strains are a principal component in ETEC CHIM studies. The characteristics of each strain should be driven by a clearly defined purpose for the challenge study; most of the time, the purpose is for testing vaccines or therapeutics with specific mechanisms of action so strain-specific CHIMs are an important consideration. Strains expressing the most common CFs are often selected as relevant strains, but it could also be important to consider whether the strain expresses those CFs to a certain minimal level in vivo or in association with intestinal epithelial cell in vitro [13–15]. In some cases it could be relevant to conduct 'confirmatory challenge studies' to test the effect of a vaccine/therapeutic with two different strains.

A comprehensive review of strains used was made in 2011 [12]. A few more strains have since been published, so that now 14 different ETEC strains have been tested in at least one human trial (Table 1). ETEC CHIMs have historically been used to better define the parameters of ETEC pathogenesis and immunity and have more recently become a platform for the evaluation of new active or passive interventions for prevention of ETEC as well as new therapeutics for ETEC. Immunological protection against different ETEC strains is dependent upon many factors, including the strain's toxin profile (production of the heat-labile (LT) and/or heat-stable enterotoxins (ST)) and expression of colonization factors (CF) and lipopolysaccharide (LPS) O-antigens. Desired characteristics of ETEC challenge strains may vary according to the purpose of the study. For example, to test a new drug to treat or prevent diarrhea, one may use any strain that effectively causes diarrhea, such as H10407. The group touched upon the importance of selecting a strain from an adult or pediatric diarrhea case to optimize the likelihood for successful testing of a particular vaccine. For a vaccine intended for children in low and middle income countries (LMICs), one would want to test its efficacy against strains that are common causes of diarrhea in these settings. The choice would then be an ST-expressing ETEC strain, preferably also expressing CFA/I or CS6. If testing killed whole cell or live oral ETEC vaccines one may want to avoid the O- and H-antigens of the vaccine candidates to rule out their contribution to strain-specific immunity. It is on this backdrop that the potential value of new ETEC strains is highlighted. For example, an ETEC strain only expressing ST would be of value in assessing the efficacy of an ST-toxoid based vaccine by avoiding the influence of LT. With an ST only expressing ETEC strain, one could also test the contribution of LT-specific immunity, when using dmLT as an adjuvant to other non-LT vaccine components such as the CFs or CF subunits. There was a consensus that CHIMs are not a substitute for field trials or a way to avoid them.

The challenge strain should be fully susceptible to multiple oral antibiotics (current frontline treatment is ciprofloxacin) to facilitate the treatment of volunteers after challenge. The group advocated for strain sequencing, as it offers even more strain-specific information that could prove valuable for not only regulators, but also to provide insight into other parameters (such as safety, antibiotic resistance, antigen discovery, etc). Through whole genomic sequencing, bioinformatics analysis has identified previously undescribed CF loci from ETEC strains that were thought to be CF-negative based on previous methods [16]. Also, through whole genome sequencing, temperate bacteriophages, which could theoretically confer antibiotic resistance, could be identified [17]. Researchers involved in CHIMs should be aware that phage testing may have implications for consistent challenge strain production. In the US, GMP manufacturers must not introduce phages into their facilities, so regulators often ask for GMP lots that undergo phage testing. The group agreed that this warrants further discussion.

Using new ETEC strains in CHIMs may be more complicated than using strains already used in humans, since the regulatory landscape for these strains is in transition. The U.S. FDA, strongly recommends challenge agent(s) to be registered under Investigational New Drug application (IND) and Good Manufacturing Practice (GMP). The Medicines and Healthcare products Regulatory Agency (MHRA) in the UK and the European Medicines Agency (EMA) do not review or approve human challenge study protocols, but they are considered a Non-Investigational Medicinal Product (NIMP) and do require GMP.

3.2. Host susceptibility and volunteer selection

Parameters known to affect host susceptibility include pre-existing immunity, genetic susceptibility, the microbiome, nutritional status, and intestinal mucosal integrity [18–20]. In CHIMs there is already an extensive screen of potential volunteers to address concerns of safety, ethics, results interpretability and practical issues. Currently, ETEC CHIM studies are designed with the intent to carefully select individuals that are healthy, immunocompetent, and have good nutritional status and intestinal mucosal integrity. Yet, the selection of volunteers by even more criteria

Table 1
ETEC strains used in controlled human infection models.

Strain	Toxin	Colonization Factor(s)	Serogroup (if known)	Comment	Ref.
H10407	STh, STp, LT	CFA/I	O78:H11	Most commonly used	[37]
H10407p		*Deleted CFA/I*		*0 of 9 with diarrhea at 10^9 cfu (1979)*	[38]
B7A	STh, LT	CS6, CS21*	O148:H48	Second most commonly used	[39]
E24377A	STh, LT	CS1, CS3	O139:H28	Third most commonly used	[6]
214-4	STp only	CS6	O167:H5	(1976–1981), >70% diarrhea at 10^8 cfu	[40]
B2C	LT, ST	CS2, CS3	O6:H16	single study (1971)	[39]
E2528-C1	LT	CS8, CS14	O25:NM	(1977–79), 40% diarrhea at 10^9 cfu	[41]
TD225-C4	LT	unknown	O75:H9	(1977–81), >50% diarrhea with 10^8 cfu	[42]
H1765	LT, ST	CFA/II	O6:H16	single study (1984)	[43]
LSN03-016011/A	LT	CS17	O8:H-	Two studies done, plus one still blinded	[44]
WS0115A	LT, STp	CS19	O114:H-	single study (2011)	[44]
DS26-1	LT	CS19	O8:H9	single study (2011), no diarrhea with 10^8 cfu	[44]
TW10598	STh, LT	CS2, CS3, CS21*	O6:H16	single study (2014)	[45]
TW10722	STh only	CS5, CS6	O115:H5	>70% with diarrhea at 10^{10} cfu	Sakkestad et al. manuscript
TW11681	STh only	CFA/I, CS21*	O19:H45	Non-diarrheal symptoms 10^6–10^8 cfu	Sakkestad et al. in revision

* Other strains may also express CS21, but may not be tested for this.

may significantly impact the outcomes of the experimental model. In CHIMs with other bacterial enteropathogens immunologic screening parameters have been included as part of eligibility criteria; however, this has not been incorporated in ETEC CHIMs.

Pre-existing pathogen-specific or cross-reactive antibodies could abrogate the diarrheal illness elicited during an ETEC challenge. It is evident from field studies that serum titers of antibodies affects risk for acquiring diarrhea in travellers [18]. A less clear, but similar trend has been shown for the effect of anti CFA/I titers against ETEC disease in a pediatric population in Egypt [21], and anti-CS3 IgA was significantly associated with less moderate to severe diarrhea in an ETEC E24377A model [6]. However, no association was found with anti CS3-IgG nor anti-LT IgG or IgA. More limited studies looking at T cell immunity in ETEC CHIMs suggests a potential role for T follicular helper cells in the risk of diarrhea [22,23].

Going beyond colonization factors and toxins, the ETEC protein microarray may be used to reveal immune responses towards pathovar-specific secreted proteins like EtpA and EatA, as well as the conserved *E. coli* antigens YghJ, FliC [24]. The method has also identified novel antibodies against proteins included on the array that seemingly correlate with disease severity following challenge with ETEC strain B7A (unpublished data).

For some pathogens the expression of certain host factors is known to increase the risk of severity of infection, such as blood group O with *V. cholerae* and *FUT2* secretor status with noroviruses. Blood group seem to play a role for development of some ETEC disease. It has been shown that blood group A human volunteers challenged with ETEC H10407 developed severe diarrhea more frequently than volunteers with other blood groups [20]. Children with Le(a+b−) blood type had significantly higher incidence of diarrhea caused by strains with CFA/I group fimbriae than Le(a−b+) [25]. This was not found in ETEC expressing CS6 [26], indicating that this difference might be strain specific. Microbiome data point to potential differences in some bacterial populations between symptomatic and asymptomatic volunteers challenged with H10407 [19]. Unpublished data were presented that highlight similar differences in those meeting clinical endpoints following B7A CHIM.

Based on the above, volunteers could be selected on several more criteria including: ABO blood group, potential genetic susceptibility markers, absence of protective microbiome, immunosuppression, immunologic screening to enroll only subjects able to mount a vaccine response (based on titers to commonly administered vaccines), pre-existing immunity to ETEC specific antigens, IgA deficiency and previous travel in ETEC endemic countries.

It is presumed that the randomized nature of ETEC vaccine CHIM studies would equally distribute volunteers with variable susceptibilities. The obvious value of doing rigorous screening is to select persons that are more likely to be susceptible to illness in both groups, so that the intervention is the most important factor determining protection against illness. With stricter selection criteria one could remove a set of potential or known confounders and effect modifiers. This may help minimize sample size, and thereby also cost and arguably it would be more ethically prudent; however, one must not dismiss the logistic challenges and cost of increased screening.

The major concern with more extensive screening is that the screened population might not be representative of the final target population. Also, there may be strain-specific criteria that can be problematic to generalise, and defining a new set of inclusion criteria may require the model to be re-validated. However, expanded screening might be appropriate for small studies with limited sample size to answer specific questions early in a vaccine development phase. Another option could be stratified randomization in which these parameters are utilized to construct strata and a randomization scheme is performed separately within each stratum; however, most agreed that the strata-specific criteria are not sufficiently clear to incorporate this as a standardized strategy. Ultimately, stratified analyses and defining some of these metrics *a priori* may be a better alternative to extensive screening.

A side issue discussed was whether increased susceptibility of volunteers could be achieved by setting dietary restrictions during CHIM. It was speculated whether attack rates might increase if yogurt or other probiotics were not allowed on the ward, and that a vegetarian diet would better mimic the situation of a developing country child.

3.3. Standardization of ETEC CHIMs

The inoculum preparation is an important aspect of potential model variability. While the overwhelming majority of studies have been performed using freshly harvested cells from plate grown inocula, at least one experiment with a frozen bulk lot has been done [22]. In further efforts to standardize ETEC inoculum for ETEC CHIMs, a lyophilized B7A challenge strain has been produced under cGMP by PATH with BMGF support, but it has yet to be evaluated in human volunteers (R. Walker, EVI PATH personal communication). The inoculum is currently given with bicarbonate solution before and with the challenge dose, while previously it has been given with milk or with saline. The use of the bicarbonate buffer as the vehicle of delivery has improved the previously observed wide variability in attack rates of challenges and likely is due to better neutralization of gastric acid.

With regard to doses, one would want to give a dose representative of natural infection However, one also needs to give a dose that causes reproducible attack rates of diarrheal disease. Model refinement trials have been done using H10407 given with bicarbonate or CeraVacx buffers at doses between 10^5 and 10^8 focusing on incorporating an overnight fast [11,27]. Similarly, studies with strain B7A compared an overnight and 90 min pre-challenge fast at doses 10^8 to 10^10 [28]. Inoculum preparation and dosing should be standardized and shared across sites. Lyophilized preparations might be attractive from a regulatory perspective, as there would be release criteria associated with vialed product rather than GMP regulations only around cell banks. However, one important concern regarding lyophilized products is the consistency of live/dead bacteria ratio. One of the uncertainties with agar grown inocula is a potential contribution of preformed ST or LT on volunteer symptoms. Presently some sites wash their inocula to avoid this, but some do not. The disease profile in studies using washed and non-washed inoculum appear to be similar, but there are no studies directly comparing the two methods. The question was also raised whether the model of one large bolus of ETEC now used in CHIMS really reflect natural infection in children, where a child most probably is repeatedly exposed to variable, smaller inocula of ETEC (which would be harder to replicate in a challenge model).

An attack rate of 60–70% is commonly used as a target in CHIMs. The reasoning behind this attack rate is to avoid overwhelming, and thereby underestimating, a normally protective vaccine immune response [11]. Inocula of each pathogen should therefore be kept as low as possible to achieve this attack rate target. It would benefit and help standardize sample size calculations for ETEC CHIMs to explicitly agree and accept a target attack rate. Reproducibility of naïve attack rates have been tested in three cohorts receiving 10^7 cfu of H10407 [10]. There is a need for reproducible, well-defined endpoints where one may assess the severity of stool output based on weight of grade-3-5 diarrheal stools and/or by the frequency of passing diarrheic stools during a pre-set time frame following challenges and also account for associated symptoms like abdominal discomfort and fever. Instead, most commonly, the primary endpoints for ETEC CHIM have focused only on stool frequency and/or volume [12]. An alternative endpoint has recently been proposed based on a disease severity score which incorporates stool output with other signs and symptoms important for understanding disease severity [29]. Such secondary symptoms of interest include: maximum 24-h loose stool output, severe diarrhea, diarrhea of any severity, total loose stool output, moderate to severe gastrointestinal symptoms like nausea, fever, vomiting, anorexia, abdominal pain/cramps, time to onset of diarrhea, percent with moderate to severe ETEC illness, and quantitative stool culture (cfu/gram stool). A disease severity score could potentially reduce the sample size required for cohorts [29]. This is extremely attractive as CHIMs are expensive and inpatient unit availability is limited. Use of the score also allows for standardization of clinical outcomes across studies using the same pathogen. It is not clear whether regulatory authorities would accept a disease score as a composite primary endpoint, but such a score should automatically be incorporated into CHIM protocols as a secondary endpoint; eventually one could move to this becoming a primary endpoint for certain target populations.

Clinical outcomes might be affected by the target population. Studies for vaccines meant for travelers utilize an endpoint focused on the frequency of stools that might impact functional activity, whereas for pediatric populations stool volume is more important due to dehydration implications. It would therefore be important to standardize those 'within target population' clinical endpoints across models. For example the ACE527-102 trial [7] endpoint was based on traditional volume/number of stools endpoint, while the VAC 006 trial (ACE527 ± dmLT) (Harro et al. manuscript in preparation) endpoint was solely volume, as PATH's target population are children under the age of 5 years.

Standardization can be important and, can be realistically achievable, with regard to 1. clinical outcomes, 2. inoculum dose and preparation, and 3. target attack rates. The group agreed that ETEC CHIMs should be standardized to the extent possible for each challenge strain and noted that it may not be realistic to standardize across strains nor across pathogens. While the group agreed standardization to a point is important, there was a noted need to remain flexible. An advantage of a relatively standardized protocol and model across multiple vaccines and multiple sites is that it gives greater confidence about down-selecting vaccine candidates and more accurate and realistic comparison of different products/therapeutics. Standardization may also reduce the need for post-hoc analysis.

3.4. Improve sample collection schemes and collaborative analysis

Given the WHO goal of developing "a safe, effective and affordable ETEC vaccine that reduces moderate to severe diarrheal disease and morbidity in infants and children under 5 years of age in LMICs" [30] more can be done to maximize the potential of CHIMs. Detection of early markers of vaccine safety and efficacy would be an important tool in bridging the translational research gap in ETEC vaccine development and possible licensure.

Sample collection, processing and archiving is the cornerstone of this new translational research and should be funded as part of the standard budget for ETEC CHIMs. Blood sampling is usually an important part of every ETEC CHIM to measure immune responses before and after vaccines and/or experimental infections. Recent research into a correlate (or even surrogate) of protection against ETEC has shown interesting associations between activated circulating T follicular cells (cTfh) and antibody in lymphocyte supernatant (ALS) responders to vaccination, as well as a potential early increase in cTfh in ETEC protected individuals [22,31]. While blood is generally being centrifuged to obtain serum/plasma it is often not processed to allow analyses of cellular immunity or transcriptomics. Often budgets will not allow for the more laborious sampling protocols necessary to obtain blood aliquots with protease inhibitors or RNA stabilizing agents or isolation of peripheral mononuclear cells. Sampling in the first hours or days after vaccination is seldom done, but may reveal important early responses to a vaccine or infection that could distinguish susceptible from resistant, or immune from non-immune, subjects, such as early levels of intestinal colonization.

For stool sampling, the level of interest is now elevated above just determining presence/absence of the challenge strain. Samples could be processed or stored to allow for other analysis such as qPCR, transcriptomics and microbiome dynamics. A study examining gut inflammatory responses following infection with *C. jejuni* and re-challenge showed the importance of early sampling where prior infection was shown to blunt inflammation on rechallenge (Baqar S & Tribble D, personal communication). Increased levels of myeloperoxidase and calprotectin were found in stool already in the first day after challenge compared to baseline in subjects challenged with ETEC B7A (Maciel M, personal communication). Recent studies also show that symptomatic disease is associated with increased ETEC shedding [19,27,32,33]. Studies with H10407 have suggested that a threshold level of shedding $\geq 2 \times 10^7$ cfu/gram of stool is associated with a higher likelihood of developing illness ([19,27,32]. The group discussed the question and expressed some concern about how aggressive sampling regimens will be tolerated by volunteers during this period. Pilot studies may be a good way to justify further funding for such sampling. Some studies have also showed the value of saliva collection as a

Table 2
Proposed tiered sampling plan to standardize and harmonize between ETEC CHIMS, and with Shigella CHIMS.

Tier 1 Priority Immunology	Tier 2 Priority Immunology	Samples for Banking
Serum IgG/IgA	Antibody affinity/avidity	Urine
Fecal IgG/IgA	Fecal cytokines	Lacrimal fluid
Antibody in lymphocyte supernatant (ALS)	Toxin neutralization	Stool for microbiome
Antibody secreting cells (ASC)	Hemagglutination inhibition	and transcriptomics
Memory B & T cells	Other adhesion inhibition assays	
Fecal inflammatory markers	ETEC antigen microarrays	
(i.e., myeloperoxidase, calprotectin)	Salivary antibodies	
	Circulating T follicular cells	
	Homing markers (i.e., $\alpha4\beta7$)	
	IgG subclasses	

Table 3
Proposed sampling plan for future ETEC challenge to test ETEC vaccine candidate in a phase 2b trial.

Tentative Study Events	−56	−55	−53	−49	−28	−27	−25	−21	−1	0	1	2	3	4	5	6	7	8	14	28	56	84	180
Vaccinate and Boost	X				X																		
ETEC challenge										X													
Start Antibiotic Therapy (discharge after 2–3 neg stools by culture)															X								
Stool Culture for ETEC (qPCR – optional on archived samples.									X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serology LTB, CFs, EtpA, EatA, ST? IgG/A/M ELISA – B, weekly	X			X	X			X	X								X		X	X	X	X	X
ALS / ASC – B, D7 after Vx & D5 after challenge. No Ag stimulation	X			X					X						X								
Memory B & T Cell – B, D28 after Vx & Challenge (paired with cTfh)	X				X				X											X			X
Stool for Fecal IgA/IgG – weekly	X			X	X			X	X	X							X		X	X	X	X	X
Stool for inflammation markers ELISA – daily, early after challenge									X	X	X	X	X	X	X	X	X						

ALS = Antibody in Lymphocyte Supernatant, ASC = Antibody Secreting Cells B = before, D = day, cTfh = circulating T follicular helper cell.

proxy for mucosal or intestinal sampling [34], as intestinal lavage is a demanding procedure for both volunteers and staff [35].

Specifically, it was proposed that there should be a focused effort to collect samples earlier during challenge to assess immune profiling and transcriptomics (both host and bacterial), intestinal colonization/shedding and disease risk, intestinal inflammation and disease severity, microbiome impact on/of infection and immunization, as well as correlates/surrogates, mechanisms and mediators of immunity. A systems biology approach to ETEC CHIMs identified tentative gene activation profiles associated with reduced susceptibility to ETEC strain H10407 [36]. Early host and bacterial responses after challenge need further study because of potential impact on antigen discovery and events that are associated with reduced colonization and spectrum of illness.

Collecting data on psychomotor vigilance may also prove interesting correlations with diarrheal disease. A recent experiment showed that decreased psychomotor vigilance coincided with increased frequency of stool output during *Campylobacter* challenge (C. Porter unpublished data).

There are several challenges to expand sample collection and standardization in CHIMs. Funding for additional sample collection, processing, testing and archiving can be hard to obtain. In particular, this is true for further exploration of microbiome effects and application of more advanced immunological method to help identify immune correlates and mechanism of protection. There is also a need for better methods to study colonization. Harmonization of sample collection is key to draw as much knowledge from a well-designed experimental vaccination and/or infection as possible. This could include standardization of processing of specimens as well as SOPs for processing and testing of all samples. Sampling schemes are also dependent on on-site facilities and how enrolment is done. Sampling standardization has been initiated for *Shigella* CHIMs and is a good example of a harmonization effort that should be replicated for ETEC. A tiered sampling plan was suggested for future ETEC vaccine CHIMs (Table 2) to set priorities for sample collection (Table 3) and harmonization with *Shigella* CHIMs. There is a need for a working group to guide harmonization and consensus on scope, timing and tiered sampling. This group should work in collaboration with other groups focusing on *Shigella* and *Campylobacter* CHIMs.

4. Conclusion

Overall this workshop resonated enthusiasm for the value of CHIMs for furthering ETEC vaccine development. There are still areas that can be refined and one can identify methodological improvements in every ETEC CHIM performed. Participants were actively engaged in discussions in all four breakout sessions. It was believed that through further refinement and standardization, with expanded sampling regimens, particularly early after challenge, the full potential of ETEC CHIMs can be realized.

Conflict of interest

The authors declared that there is no conflict of interest.

Copyright statement

Disclaimer

The views expressed in this article are those of the author 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 is a US Government work. There are no restrictions on its use. There were no financial conflicts of interests among any of the authors. The workshop was partially supported by PATH.

References

[1] Walker RI, Wierzba TF, Mani S, Bourgeois AL. Vaccines against Shigella and enterotoxigenic Escherichia coli: a summary of the 2016 VASE conference. Vaccine 2017;35:6775–82.

[2] Porter CK, Louis Bourgeois A, Frenck RW, Prouty M, Maier N, Riddle MS. Developing and utilizing controlled human models of infection. Vaccine 2017;35:6813–8.

[3] Evans DG, Evans Jr DJ, Opekun AR, Graham DY. Non-replicating oral whole cell vaccine protective against enterotoxigenic Escherichia coli (ETEC) diarrhea: stimulation of anti-CFA (CFA/I) and anti-enterotoxin (anti-LT) intestinal IgA and protection against challenge with ETEC belonging to heterologous serotypes. FEMS Microbiol Immunol 1988;1:117–25.

[4] Evans Jr DJ, Evans DG, Opekun AR, Graham DY. Immunoprotective oral whole cell vaccine for enterotoxigenic Escherichia coli diarrhea prepared by in situ destruction of chromosomal and plasmid DNA with colicin E2. FEMS Microbiol Immunol 1988;1:9–18.

[5] McKenzie R, Bourgeois AL, Frech SA, Flyer DC, Bloom A, Kazempour K, et al. Transcutaneous immunization with the heat-labile toxin (LT) of enterotoxigenic Escherichia coli (ETEC): protective efficacy in a double-blind, placebo-controlled challenge study. Vaccine 2007;25:3684–91.

[6] McKenzie R, Darsley M, Thomas N, Randall R, Carpenter C, Forbes E, et al. A double-blind, placebo-controlled trial to evaluate the efficacy of PTL-003, an attenuated enterotoxigenic E. coli (ETEC) vaccine strain, in protecting against challenge with virulent ETEC. Vaccine 2008;26:4731–9.

[7] Darsley MJ, Chakraborty S, DeNearing B, Sack DA, Feller A, Buchwaldt C, et al. The oral, live attenuated enterotoxigenic Escherichia coli vaccine ACE527 reduces the incidence and severity of diarrhea in a human challenge model of diarrheal disease. Clin Vacc Immunol: CVI 2012;19:1921–31.

[8] Tacket CO, Reid RH, Boedeker EC, Losonsky G, Nataro JP, Bhagat H, et al. Enteral immunization and challenge of volunteers given enterotoxigenic E. coli CFA/II encapsulated in biodegradable microspheres. Vaccine 1994;12:1270–4.

[9] Levine MM, Black RE, Brinton Jr CC, Clements ML, Fusco P, Hughes TP, et al. Reactogenicity, immunogenicity and efficacy studies of Escherichia coli type 1 somatic pili parenteral vaccine in man. Scand J Infect Dis Suppl 1982;33:83–95.

[10] Harro C, Gutierrez RL, Talaat KR, Porter CK, Riddle MS, Maciel Jr M, et al. Protective efficacy of an enterotoxigenic E. coli fimbrial tip adhesin vaccine given with LTR192G by intradermal vaccination against experimental challenge with CFA/I-ETEC in adult volunteers. 50th United States-Japan Cholera and Related Diarrheal Diseases Conference. Bethesda, MD, 2016.

[11] Harro C, Chakraborty S, Feller A, DeNearing B, Cage A, Ram M, et al. Refinement of a human challenge model for evaluation of enterotoxigenic Escherichia coli vaccines. Clin Vacc Immunol: CVI 2011;18:1719–27.

[12] Porter CK, Riddle MS, Tribble DR, Louis Bougeois A, McKenzie R, Isidean SD, et al. A systematic review of experimental infections with enterotoxigenic Escherichia coli (ETEC). Vaccine 2011;29:5869–85.

[13] Crofts AA, Giovanetti SM, Rubin EJ, Poly FM, Gutierrez RL, Talaat KR, et al. Enterotoxigenic E. coli virulence gene regulation in human infections. Proc Natl Acad Sci U S A 2018;115:E8968–76.

[14] Kansal R, Rasko DA, Sahl JW, Munson GP, Roy K, Luo Q, et al. Transcriptional modulation of enterotoxigenic Escherichia coli virulence genes in response to epithelial cell interactions. Infect Immun 2013;81:259–70.

[15] Fleckenstein JM, Munson GM, Rasko DA. Enterotoxigenic Escherichia coli: orchestrated host engagement. Gut Microbe 2013;4:392–6.

[16] Del Canto F, O'Ryan M, Pardo M, Torres A, Gutierrez D, Cadiz L, et al. Chaperone-usher pili loci of colonization factor-negative human enterotoxigenic escherichia coli. Front Cell Infect Microbiol 2016;6:200.

[17] Davies EV, Winstanley C, Fothergill JL, James CE. The role of temperate bacteriophages in bacterial infection. FEMS Microbiol Lett 2016;363:fnw015.

[18] Holmgren J, Parashar UD, Plotkin S, Louis J, Ng SP, Desauziers E, et al. Correlates of protection for enteric vaccines. Vaccine 2017;35:3355–63.

[19] Pop M, Paulson JN, Chakraborty S, Astrovskaya I, Lindsay BR, Li S, et al. Individual-specific changes in the human gut microbiota after challenge with enterotoxigenic Escherichia coli and subsequent ciprofloxacin treatment. BMC Genomics 2016;17:440.

[20] Kumar P, Kuhlmann FM, Chakraborty S, Bourgeois AL, Foulke-Abel J, Tumala B, et al. Enterotoxigenic Escherichia coli-blood group A interactions intensify diarrheal severity. J Clin Invest 2018;128:3298–311.

[21] Rao MR, Wierzba TF, Savarino SJ, Abu-Elyazeed R, El-Ghoreb N, Hall ER, et al. Serologic correlates of protection against enterotoxigenic Escherichia coli diarrhea. J Infect Dis 2005;191:562–70.

[22] McArthur MA, Chen WH, Magder L, Levine MM, Sztein MB. Impact of CD^{4+} T cell responses on clinical outcome following oral administration of wild-type enterotoxigenic escherichia coli in humans. PLoS Negl Trop Dis 2017;11:e0005291.

[23] McArthur MA, Maciel Jr M, Pasetti MF. Human immune responses against Shigella and enterotoxigenic E. coli: current advances and the path forward. Vaccine 2017;35:6803–6.

[24] Chakraborty S, Randall A, Vickers TJ, Molina D, Harro CD, DeNearing B, et al. Human experimental challenge with enterotoxigenic escherichia coli elicits immune responses to canonical and novel antigens relevant to vaccine development. J Infect Dis 2018;218:1436–46.

[25] Qadri F, Saha A, Ahmed T, Al Tarique A, Begum YA, Svennerholm AM. Disease burden due to enterotoxigenic Escherichia coli in the first 2 years of life in an urban community in Bangladesh. Infect Immun 2007;75:3961–8.

[26] Ahmed T, Lundgren A, Arifuzzaman M, Qadri F, Teneberg S, Svennerholm AM. Children with the Le(a+b) blood group have increased susceptibility to diarrhea caused by enterotoxigenic Escherichia coli expressing colonization factor I group fimbriae. Infect Immun 2009;77:2059–64.

[27] Chakraborty S, Harro C, DeNearing B, Brubaker J, Connor S, Maier N, et al. Impact of lower challenge doses of enterotoxigenic Escherichia coli on clinical outcome, intestinal colonization and immune responses in adult volunteers. PLoS Negl Trop Dis 2018;12:e0006442.

[28] Talaat KR, Bourgeois AL, Porter CK, Maciel Jr M, Gutierrez RL, et al. Re-establishment and use of the ETEC B7A CHIM to assess the efficacy of orally administered bovine serum antibodies against CS6 or whole cell B7A. Vacc Enteric Dis 2017.

[29] Porter CK, Riddle MS, Alcala AN, Sack DA, Harro C, Chakraborty S, et al. An evidenced-based scale of disease severity following human challenge with Enteroxigenic Escherichia coli. PLoS One 2016;11:e0149358.

[30] WHO. WHO Product Development for Vaccines Advisory Committee (PDVAC) Meeting – 2018. WHO; 2018.

[31] Cardeno A, Magnusson MK, Quiding-Jarbrink M, Lundgren A. Activated T follicular helper-like cells are released into blood after oral vaccination and correlate with vaccine specific mucosal B-cell memory. Sci Rep 2018;8:2729.

[32] Lindsay BR, Chakraborty S, Harro C, Li S, Nataro JP, Sommerfelt H, et al. Quantitative PCR and culture evaluation for enterotoxigenic Escherichia coli (ETEC) associated diarrhea in volunteers. FEMS Microbiol Lett 2014;352:25–31.

[33] Vedoy OB, Hanevik K, Sakkestad ST, Sommerfelt H, Steinsland H. Proliferation of enterotoxigenic Escherichia coli strain TW11681 in stools of experimentally infected human volunteers. Gut Pathogens 2018;10:46.

[34] Aase A, Sommerfelt H, Petersen LB, Bolstad M, Cox RJ, Langeland N, et al. IgA from the sublingual compartment as a novel noninvasive proxy for intestinal immune induction. Mucosal Immunol 2015.

[35] Chakraborty S, Harro C, DeNearing B, Ram M, Feller A, Cage A, et al. Characterization of mucosal immune responses to enterotoxigenic escherichia coli vaccine antigens in a human challenge model: response profiles after primary infection and homologous rechallenge with strain H10407. Clin Vacc Immunol: CVI 2016;23:55–64.

[36] Yang WE, Suchindran S, Nicholson BP, McClain MT, Burke T, Ginsburg GS, et al. Transcriptomic analysis of the host response and innate resilience to enterotoxigenic Escherichia coli infection in humans. J Infect Dis 2016;213:1495–504.

[37] Evans Jr DJ, Evans DG. Three characteristics associated with enterotoxigenic Escherichia coli isolated from man. Infect Immun 1973;8:322–8.

[38] Evans DG, Satterwhite TK, Evans Jr DJ, DuPont HL. Differences in serological responses and excretion patterns of volunteers challenged with enterotoxigenic Escherichia coli with and without the colonization factor antigen. Infect Immun 1978;19:883–8.

[39] DuPont HL, Formal SB, Hornick RB, Snyder MJ, Libonati JP, Sheahan DG, et al. Pathogenesis of Escherichia coli diarrhea. N Engl J Med 1971;285:1–9.

[40] Clements ML, Levine MM, Black RE, Robins-Browne RM, Cisneros LA, Drusano GL, et al. Lactobacillus prophylaxis for diarrhea due to enterotoxigenic Escherichia coli. Antimicrob Agents Chemother 1981;20:104–8.

[41] Levine MM, Nalin DR, Hoover DL, Bergquist EJ, Hornick RB, Young CR. Immunity to enterotoxigenic Escherichia coli. Infect Immun 1979;23:729–36.

[42] Levine MM, Rennels MB, Cisneros L, Hughes TP, Nalin DR, Young CR. Lack of person-to-person transmission of enterotoxigenic Escherichia coli despite close contact. Am J Epidemiol 1980;111:347–55.

[43] Evans DG, Graham DY, Evans DJ. Jr. Administration of purified colonization factor antigens (CFA/I, CFA/II) of enterotoxigenic Escherichia coli to volunteers. Response to challenge with virulent enterotoxigenic Escherichia coli. Gastroenterology 1984;87:934–40.

[44] McKenzie R, Porter CK, Cantrell JA, Denearing B, O'Dowd A, Grahek SL, et al. Volunteer challenge with enterotoxigenic Escherichia coli that express intestinal colonization factor fimbriae CS17 and CS19. J Infect Dis 2011;204:60–4.

[45] Skrede S, Steinsland H, Sommerfelt H, Aase A, Brandtzaeg P, Langeland N, et al. Experimental infection of healthy volunteers with enterotoxigenic Escherichia coli wild-type strain TW10598 in a hospital ward. BMC Infect Dis 2014;14:482.