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Clinical endpoints for efficacy studies

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ABSTRACT

Well-established, validated and clinically meaningful primary and secondary endpoints are critical in advancing vaccines through proof of principal studies, licensure and pre-qualification. To that end, the field of vaccine development for *Shigella*, enterotoxigenic *Escherichia coli* (ETEC) as well as other enteric pathogens would benefit greatly from a focused review of clinical endpoints and the use of common endpoints across the field to enable study-to-study comparisons as well as comparative assessments between vaccine candidates. A workshop was conducted to review clinical endpoints from controlled human challenge studies, field studies in naïve adult travelers and pediatric studies in low-middle income countries and to develop a consensus on clinical endpoints for future vaccine trials.

Following sequential presentations on different study designs (CHIM, travelers' efficacy and pediatric efficacy), workshop participants broke into three simultaneous workgroups focused on those study designs to discuss a number of topics key to clinical endpoints specific to each study design. Previously utilized endpoints were reviewed with an eye towards potentially novel endpoints for future studies and consideration of the disease parameters and spectrum of disease targeted for prevention. The strength of support among workshop participants for the use of various endpoints is summarized as are recommendations for additional endpoints to be considered in future studies. It is anticipated that this report will facilitate endpoint determination in future efficacy trials of vaccine candidates.

1. Introduction

As highlighted during the 2018 Vaccines against Shigella and ETEC (enterotoxigenic *Escherichia coli*) conference, multiple vaccine candidates are advancing, or have already advanced, to clinical evaluation in various settings including the controlled human infection model (CHIM), adult travelers to low-middle income countries (LMICs) and pediatric populations in LMICs. The use of well-established, validated and clinically meaningful primary and secondary endpoints for efficacy is critical for advancing vaccines through proof of principal studies, licensure and pre-qualification. The objective of this workshop was to review the clinical efficacy endpoints utilized across these settings, to begin to frame points of concern and areas for additional consideration and where possible develop consensus for endpoints to be utilized in future vaccine trials. The implications of these endpoints on sample size requirements was also considered. Additionally, the use of various scoring systems was reviewed and their merits assessed as potential endpoints in clinical trials of vaccine efficacy. The role of subjective endpoints in addition to (or in lieu of) objective endpoints was considered. In addition to the endpoints of acute disease, discussions also considered the potential for alternative endpoints focused on microbiological and long-term morbidity-based endpoints.

Logistically, the workshop was scheduled as three separate presentations on (1) the CHIM; (2) adult travelers to LMICs; and (3) pediatric populations in LMICs. Following the presentations, there were simultaneous breakout sessions to address suggested points for discussion and questions raised during the presentations and to consider new questions posed during the discussion. Reported below are the results of those presentations and discussions.

2. Workshop presentations

2.1. Controlled human infection model

The CHIM as a method to assess the efficacy of prototype (or final formulation) vaccine candidates presents unique opportunities and challenges making it a topic of a prior VASE workshop [1]. In particular, the CHIM provides an opportunity to assess efficacy following a well-defined exposure of a specific strain and dose

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without the confounding issue of co-infection or co-morbid conditions. The inpatient setting in which CHIM (particularly for *Shigella* and ETEC) are performed provides an opportunity for detailed clinical, microbiological and immunological assessments in a closely monitored setting and enables scheduled and standardized treatments. Clinical endpoints tend to be focused on stool output, in addition to constitutional signs such as fever. The inpatient setting allows for the collection, grading, weighing, and characterization (ie, presence of blood) of stool output. Additionally, the inpatient setting enables standardized observations for clinical findings in a manner that is not practical in other settings.

While these advantages highlight the utility of the CHIM in vaccine efficacy assessment, the models are not without their own challenges. In particular, the setting of exposure, in which a challenge inoculum is administered after neutralization of gastric acidity, may not be representative of natural exposure. Additionally, the inoculum dose is often much higher than what is presumed to be the most likely dose in a natural exposure. Furthermore, the population enrolled in CHIM studies is not reflective of children living in LMICs, a target population for vaccine introduction. Also, due to the high cost of CHIM and limited inpatient facility space, sample sizes are generally small which may yield effect estimates with large confidence intervals.

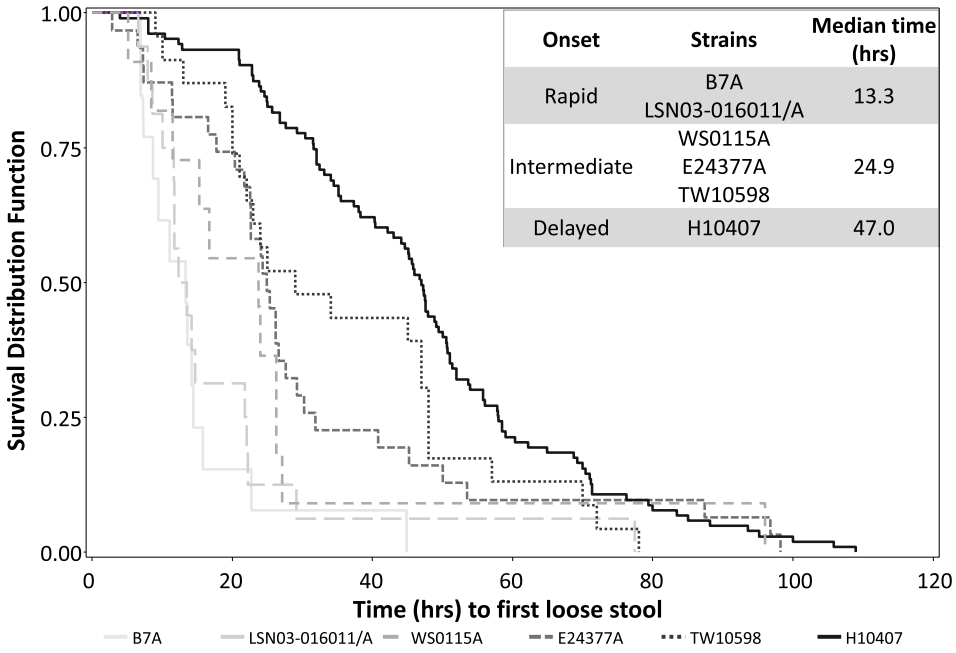
Clinical endpoints for ETEC CHIM have varied over time and institution as previously described, though they have focused predominately on the frequency and volume of stool output [2]. Recently, Porter et al proposed a disease severity score to account for stool output and also include other important clinical signs and symptoms [3]. Importantly, as shown in Table 1, there is variability in the clinical profile of disease in subjects experimentally infected with ETEC that appears to be strain specific with variability in frequency and severity of many endpoints based on subjective reported symptoms such as malaise, abdominal cramps and nausea. Stool-based endpoints appear variable by strain as well with CFA/I, LT+, ST+ strains inducing the highest frequency and volume of stool output. In addition, the time to loose stool onset also appears to vary by strain with some (strains B7A and LSN03-016011/A) associated with a rapid onset of loose stools (median: 13.3 h), some (WS0115A, E24377A and TW10698) with an intermediate onset (median: 24.9 h) and the H10407 strain with a more delayed onset (median: 47.0 h) (Fig. 1). Accordingly, the disease severity score for ETEC CHIM varies across strains with the highest severity score seen in H10407 recipients [3].

Similar to ETEC, the clinical endpoints for *Shigella* CHIM have varied from endpoints based solely on stool volume and/or frequency to those also incorporating fever and/or dysentery (see Table 2). These endpoints are further confounded by the variability in the definitions for each of those components. For example, studies incorporating some measure for blood in the stool have utilized occult blood and/or visible blood in a single loose stool and visible blood confirmed by hemoccult in a single or multiple loose stools. Unlike ETEC, the number of strains assessed in the *Shigella* CHIM is fairly limited and to date has focused predominately on strains 2457T (*S. flexneri 2a*) and 53G (*S. sonnei*) [4].

Variability in these endpoint definitions complicates estimates of anticipated attack rates in subsequent studies. Additionally, estimates of vaccine efficacy can vary substantially depending on the endpoint utilized as highlighted in studies of ETEC [5] and *Shigella* [6,7]. Based on recent calls for standardization of the primary endpoint for *Shigella* CHIM [8], and focused on an attempt to target more moderate-severe disease, a group of researchers with extensive experience in conducting *Shigella* CHIM developed a consensus primary endpoint for future studies which will be published in early 2019. However, no similar effort has been made for ETEC CHIM. In addition to dichotomous endpoints, studies have published on the use of an ordinal disease severity score to be more inclusive the signs and symptoms of diarrheal illness in both *Shigella* and ETEC CHIM [3,8].

Table 1
Frequency and severity of signs and symptoms across 5 strains of enterotoxigenic *Escherichia coli* (ETEC).

Sign/Symptom	Severity	H10407 (n = 132)	E24377A (n = 36)	B7A (n = 16)	LSN03-016011/A (n = 25)	TW10598 (n = 30)
Abdominal cramps	None	28.8%	33.3%	37.5	36.0%	50.0%
	Mild (no disruption of regular activities)	21.2%	25.0%	31.3%	8.0%	23.3%
	Moderate (interferes with regular activities)	28.0%	25.0%	6.3%	28.0%	23.3%
	Severe (prevents regular activities)	28.0%	16.7%	25.0%	28.0%	3.3%
Malaise	None	45.5%	44.4%	81.3%	52.0%	63.3%
	Mild (no disruption of regular activities)	13.6%	16.7%	6.3%	16.0%	33.3%
	Moderate (interferes with regular activities)	21.2%	22.2%	6.3%	12.0%	3.3%
	Severe (prevents regular activities)	19.7%	16.7%	6.3%	20.0%	0.0%
Nausea	None	47.0%	61.1%	50.0%	68.0%	46.7%
	Mild (no disruption of regular activities)	11.4%	22.2%	25.0%	16.0%	46.7%
	Moderate (interferes with regular activities)	12.9%	8.3%	6.3%	0.0%	6.7%
	Severe (prevents regular activities)	28.8%	8.3%	18.8%	16.0%	0.0%
Headache	None	54.6%	72.2%	37.5%	72.0%	60.0%
	Mild (no disruption of regular activities)	19.7%	11.1%	31.3%	20.0%	36.7%
	Moderate (interferes with regular activities)	16.7%	11.1%	18.8%	8.0%	3.3%
	Severe (prevents regular activities)	9.1%	5.6%	12.5%	0.0%	0.0%
Lightheaded	None	65.9%	75.0%	75.0%	88.0%	90.0%
	Mild (no disruption of regular activities)	12.1%	13.9%	25.0%	8.0%	6.7%
	Moderate (interferes with regular activities)	18.2%	2.8%	0.0%	4.0%	3.3%
	Severe (prevents regular activities)	3.8%	8.3%	0.0%	0.0%	0.0%
Vomiting	None (0 episodes)	68.9%	94.4%	87.5%	80.0%	90.0%
	Mild (1 episode over 24 h)	6.1%	2.8%	6.3%	12.0%	10.0%
	Moderate (2 episodes over 24 h)	5.3%	0.0%	0.0%	4.0%	0.0%
	Severe (>2 episodes over 24 h)	19.7%	2.8%	6.3%	4.0%	0.0%
Fever	None (<100.4°F)	81.8%	97.2%	81.3%	100.0%	60.0%
	Mild (≥100.4°F and <101.1°F)	12.9%	2.8%	18.8%	0.0%	40.0%
	Moderate (≥101.1°F and <102.0°F)	4.6%	0.0%	0.0%	0.0%	0.0%
	Severe (≥102.0°F)	0.8%	0.0%	0.0%	0.0%	0.0%



Onset	Strains	Median time (hrs)
Rapid	B7A LSN03-016011/A	13.3
Intermediate	WS0115A E24377A TW10598	24.9
Delayed	H10407	47.0

Fig. 1. Time to onset of loose stools in ETEC CHIM studies across 5 studied strains. Footnote: Toxin and colonization factor profile by strain: B7A: LT+, ST+, CS6, CS21. LSN03-016011/A: LT+, ST-, CS17. WS0155A: LT+, ST+, CS19. E24377A, LT+, ST+, CS1, CS3. TW10598: LT+, ST+, CS2, CS3, CS21. H10407: LT+, ST+, CFA/I.

2.2. Travelers' diarrhea

Travelers' diarrhea is common, affecting 10–40% of travelers on short duration trips [9]. Etiology is subject to geographic variability, but bacterial organisms predominate [10,11]. Vaccines and other countermeasures against travelers' diarrhea can be licensed on the basis of field studies involving leisure traveler and analogous populations such as military and relief personnel; however, in the event that the rarity of the infection precludes a field trial, there is recent precedent with an oral attenuated cholera vaccine for licensure based on pivotal data from CHIM studies [12]. Field studies offer an advantage over the CHIM in assessing vaccine efficacy in an adult population as they offer a natural exposure setting; however, they are not without significant challenges. Among these are a need for *a priori* investments to characterize epidemiology, etiology, and population factors at potential study sites. Logistical factors in study execution, such as the ability to complete the vaccination series ahead of travel exposure, and establishment of high-throughput clinical and laboratory support for microbiologic ascertainment of vaccine preventable outcomes (VPOs).

A review of ETEC and cholera vaccine trials among travelers demonstrates a transition and refinement of clinical endpoints from physician-based criteria to diarrheal event frequency and ultimately, the use of severity of illness measures (Table 3). Interestingly, the 2006 World Health Organization recommendations, recommended the inclusion of clinical endpoints aimed at more severe disease and hospitalization [13]. More recent trials have defined mild, moderate, severe diarrheal episodes, based on daily frequency of loose stool production.

Clinical endpoints and definitions for travelers' diarrhea used in field studies have evolved over the years, from the first published study using physician-defined events [14], to the most recent studies utilizing quantitative assessments of unformed stool in terms of frequency (3 or more unformed stools in a 24hr period being the most often utilized endpoint) [15,16]. One criticism of the frequency-centric approach has stemmed from the observed individual variability of stooling frequency demonstrating the measure can be a poor discriminator of the functional impact of the disease. Moreover, disability measures may be more relevant for certain populations, such as military personnel, where impediments in function may result in mission degradation.

Functional impacts and associated symptoms (fever, cramps, nausea, vomiting) contribute to the disability experienced by the traveler. Increasingly, academic bodies such as the American College of Gastroenterology and International Society of Tropical Medicine guidelines have transitioned to use of combined criteria with 3 or more unformed stools defining the episode and severity grading based on functional impacts to activity [17,18].

2.3. Pediatric diarrhea in low-middle income countries

Four factors to consider in selecting clinical endpoints for phase 3 efficacy studies in pediatric populations in LMICs were presented (Table 4). The first relates the selection of antigenic variants of the organism that are most strongly associated with illness. In the case of ETEC, certain antigenic variants seem to have weak associations with disease among children in LMIC. Examples from two pediatric case-control studies were presented in which ST-expressing ETEC (i.e., ST alone or ST together with LT) with or without a 'major' colonization factor (defined as CFA I, II, or IV) were significantly associated with diarrheal disease whereas any LT-only expressing ETEC were not [19,20]. Clemens et al showed that inclusion of LT-only ETEC as 'vaccine preventable outcomes' diminishes the strength of association between ETEC and illness and thus may result in underestimation of vaccine efficacy [21]. *Shigella* serogroups, serotypes and sub-serotypes, on the other hand, do not appear to substantially differ in pathogenicity.

A second important consideration is to select clinical endpoints of sufficient clinical and epidemiological importance to warrant prevention. Data were presented from the Global Enterics Multicenter Study (GEMS) to support the proposition that medically-attended diarrhea would fulfill this criterion. Two sequential case-control studies were conducted among children 0–59 months of age at study sites in South Asia and sub-Saharan Africa seeking medical care for diarrhea as part of GEMS. The first was a study of

Table 2
Clinical endpoints in *Shigella* CHIM studies.

Reference	Author	Year	Fever (°C)	Dysentery	Diarrhea	Primary endpoint
[38]	Dupont	1969	≥37.8	Bloody mucoid stools	≥2 loose stools in 24 h	Fever, dysentery, diarrhea or severe abdominal cramping
[39]	Dupont	1972	≥37.8	Not stated	≥4 loose stools in 24 h	Diarrhea and fever
[40]	Levine	1973	>37.8	Blood and mucus in stools	≥3 loose stools in 24 h	Diarrhea, fever or dysentery (vomiting also assessed)
[41]	Levine	1977	≥38.3	Blood and mucus in loose stools	≥3 loose stools in 24 h	Diarrhea or dysentery
[6]	Black	1987	≥37.8	1 occult positive loose stool	≥2 liquid stools weighing ≥ 200 g within 48 h, or a single liquid stool of ≥300 g	Diarrhea, fever or dysentery
[42]	Dupont	1989	≥38.0	Hematest-positive or grossly blood stool	≥2 loose stools in 24 h	Fever and diarrhea and severe abdominal cramps/pain or dysentery
[43]	Herrington	1990	>37.8	1 occult positive loose stool	1 loose stool of >300 ml or ≥2 loose stools totaling > 200 ml within a 48 h period	Diarrhea, fever or dysentery
[44]	Kotloff	1992	≥37.8	Gross blood in a single loose stool	≥2 loose stools totaling 200 ml within 48 h or a single 300 ml loose stool	Diarrhea, fever or dysentery
[45]	Tacket	1992	≥37.8	Gross blood in formed or loose stool	≥2 loose stools totaling 200 ml within 48 h or a single 300 ml loose stool	Diarrhea, fever or dysentery
[46]	Mackowiak	1992	>37.8	Not stated	≥2 loose stools totaling 200 ml within 48 h or a single 300 ml loose stool	Not stated
[47]	Kotloff	1995	≥37.8	Gross blood in a single loose stool	≥2 loose stools totaling 200 ml within 48 h or a single 300 ml loose stool	Diarrhea, fever or dysentery
[23]	Kotloff	1995	≥37.8	Gross blood in a single loose stool	≥2 loose stools totaling 200 ml within 48 h or a single 300 ml loose stool	Diarrhea, fever or dysentery
[48]	Munoz	1995	≥37.8	1 loose stool occult positive or with visible blood[1]	≥2 loose stools totaling 200 ml within 48 h or a single 300 ml loose stool	Diarrhea, fever or dysentery
[49]	Coster	1999	≥38.1	Abnormal stool with gross blood	≥2 loose stools totaling 200 ml within 48 h or a single 300 ml loose stool	Shigellosis: oral temp > 38.3 °C, diarrhea OR dysentery, >1 severe intestinal symptom AND > 1 severe constitutional symptom
[50]	Taylor	2006	>38.0	Gross blood in a loose stool on at least 2 occasions	≥2 loose stools weighing 200 g within a 48-h period plus the presence of at least 1 symptom or sign of enteric illness (i.e., abdominal pain or cramps, confirmed blood in the stool, nausea, emesis, excessive gas or flatulence, fecal urgency, anorexia, fever [temperature, 38 °C], and tenesmus) or passage of 1 loose stool weighing 300 g plus the presence of at least 1 symptom or sign of enteric illness	Diarrhea
[7]	Talaat	N/A	>38.0	Gross blood[1] in a loose stool on at least 2 occasions within any 24 h and reportable constitutional symptoms	Mild Diarrhea: ≥2 loose stools weighing ≥ 200 g within 48 h or 1 loose stool weighing ≥ 300 g, not meeting the definition for moderate or severe Moderate diarrhea: 4 to 5 loose stools or 401-800gr loose stools within 24 h Severe diarrhea: ≥6 loose stools or >800 g loose stools within 24 h	Shigellosis: severe diarrhea; dysentery; moderate diarrhea with ≥1 moderate constitutional/enteric symptom
[51]	Frenck	N/A	≥38.0	Gross blood[1] in a loose stool on at least 2 occasions and reportable constitutional symptoms.	Mild Diarrhea: 2–3 loose stools or <400 gr of loose stools within 24 h Moderate diarrhea: 4 to 5 loose stools or 400-800gr loose stools within 24 h Severe diarrhea: ≥6 loose stools or >800 g loose stools within 24 h	Shedding of *S. sonnei* in stool accompanied by moderate-severe diarrhea (and/or dysentery) with moderate fever (≥38.5 °C) or one or more severe intestinal symptoms

[1] Gross blood in loose stool stated to be confirmed by occult test.

acute moderate-to-severe diarrhea (MSD) [19], defined as the presence of either sunken eyes, decreased skin turgor, intravenous hydration, hospitalization, or dysentery; the second study, designated GEMS-1A, evaluated less severe diarrhea (LSD) lacking the clinical findings that characterized MSD (Kotloff, submitted). In addition to prompting medical care, both MSD and LSD were associated linear growth faltering, and *Shigella* and ST-ETEC ranked among the most common etiologic agents. Moreover, according to the Global Burden of Disease estimates, *Shigella* ranks as the third and ETEC as the eighth most common cause of diarrheal mortality [22]. These findings suggest that *Shigella* and ETEC-associated MSD and LSD contribute to a substantial disease burden and poor outcomes, providing justification that prevention of these medically-attended episodes is a worthwhile outcome for a safe and effective vaccine.

The third consideration is to choose endpoints, whether clinical or microbiological, that can feasibly be prevented with a vaccine. The response of volunteers to challenge with wild type *S. flexneri* 2a was presented to illustrate the difficulties that might be encountered if elimination of asymptomatic infection (microbiological protection) were included as an endpoint in a vaccine trial. If a vaccine has a greater protective effect against disease-causing infection than against intestinal colonization per se (akin to the issue of including strains with low pathogenicity as described above), the analysis will underestimate efficacy against symptomatic infection. Prior studies with *Shigella* in which volunteers underwent an initial challenge and were rechallenged with a homologous strain, showed that prior exposure protected against clinical illness but not infection [23]. Similar results have been observed in challenge/rechallenge studies with ETEC [24].

Table 3
Travelers' Diarrhea Vaccine Field Trials: Randomized Controlled Studies.

Vaccine	Population	Primary endpoint	Efficacy determination	Comments
WC-BS [14]	Travelers to Morocco (n = 615)	Physician diagnosed diarrhea (blinded physician judged any stools for "abnormally loose")	ETEC + LT = 60%; p = 0.04	
WC/rBS [52]	Student travelers to Mexico (n = 500)	4 or more LS in 24hr (or 3 in 8hr) plus an additional symptom	50% (95% CI: 14, 71) after second dose	Timing of vaccination
Killed (SBL) ETEC/rBS [29]	Travelers Mexico/ Guatemala (n = 672)	Diarrhea (3 or more LS in 24hr plus symptoms) caused by homologous ETEC (VPO)	More severe (5 or more stools, or moderate sx) VPO ETEC, 77%, p = 0.04	WHO Recommendation: Primary Endpoint against Severe Disease and Hospitalization [13]
Killed ETEC or Cholera Vaccine with rCTB [53]	Travelers to tropics (n = 250)	ETEC Diarrhea (3 or more liquid stools, + ETEC isolated)	ETEC Vaccine: 79% (p = 0.12) ETEC vs. Cholera: 84%	Self-collection of stools. Few (1/12) ETEC strains expressed LT
CVD-HgR [54]	Travelers Asia/ Africa (n = 134)	3 or more LS in 24 h, or 2 LS plus symptoms; Secondary: TD due to LT/ST ETEC	Goal of 50% Efficacy not achieved	Study stopped at interim analysis. Low LT ETEC infections
LT Patch [16]	Travelers Mexico/ Guatemala (n = 170)	Mild TD: 3 LS/24 h Mod TD: 4–5 LS/24 h Sev TD: 6 or more LS/24 h	All Mod-Sev TD 75%, p = 0.007 Severe TD 84%, p = 0.03	Small, pilot site establishment study
LT Patch [15]	Travelers Mexico/ Guatemala (n = 1644)	Mild TD: 3 LS/24 h Mod TD: 4–5 LS/24 h/ETEC LT Sev TD: 6 or more LS/24 h/ETEC LT	Mod-Sev ETEC: 34.6% (95% CI: −2.2, 58.9)	Duration, all-cause diarrhea secondary analyses. Low placebo attack rate

WC: Whole Cell Cholera Vaccine; BS: B-subunit; rBS: recombinant B-subunit; LS: Loose stool; VPO: Vaccine Preventable Outcomes; ETEC: enterotoxigenic *E. coli*; rCTB: recombinant cholera toxin b-subunit

Table 4
Considerations in selecting study endpoints for vaccines studies targeting Shigella and/or enterotoxigenic *E. coli* (ETEC) in pediatric populations living in low-middle income countries.

- Distinguishing 'types' of the organism that are associated with illness
- Selection of clinical endpoints that represent disease warranting prevention
- Selection of clinical endpoints representing manifestations that can be prevented by vaccination
  - Microbiologic: Prevention of asymptomatic infection may not be feasible
  - Clinical:
    - Enteric vaccines seem more efficacious against more severe diarrhea, for which medically attended diarrhea might be a good proxy
    - Vesikari scale identifies important manifestations of rotavirus better than ST-ETEC and *Shigella*
    - Non-bloody diarrhea should be an endpoint for ETEC, while both dysentery and watery diarrhea are suitable for *Shigella*
- Selection of relevant patient population
  - Age:
    - Most disease can be preventing if children are vaccinated by 6 months of age, but the busy infant immunization schedule may require administration at an older age
    - Surveillance up to 36 months of age will capture most disease
  - Risk: *Shigella* and ST-ETEC appear to be distributed across LMIC, so risk-based vaccination not necessary

These findings suggest that targeting so-called "sterile immunity" (prevention of shedding) may not be feasible. Experience with other enteric vaccines suggests that the most severe illness should be targeted, perhaps using medically-attended diarrhea as a proxy. For example, rotavirus vaccines confer superior efficacy against more severe disease, including disease prompting hospitalization, thus illustrating this point (Table 5) [25–28]. Similarly, a first generation inactivated whole cell ETEC vaccine showed significant protection against more severe forms of travelers' diarrhea [29] but did not protect Egyptian children in a Phase 3 trial [30,31]. It is possible that the use of active surveillance to detect cases in the latter trial selected less severe illnesses. Upon review of the ETEC vaccine trial data, the World Health Organization recommended that the primary endpoint should be protection against severe disease and/or hospitalization associated with vaccine-preventable cases [32]. The importance of *Shigella* as a cause of dysentery as well as watery diarrhea suggests that both syndromes should be included as endpoints, whereas for ETEC only watery diarrhea need be included [33]. Of note, whereas the Vesikari scale was developed for and has proven useful to detect more severe illness in rotavirus vaccine trials, it does not appear to be as useful as an endpoint for *Shigella* or ETEC trials [34].

Table 5
Efficacy of rotavirus vaccine in LMIC, by disease severity.

Vaccine[1]	Population	Efficacy against rotavirus gastroenteritis	
		Severe	Any
RV3-neonatal (3 doses) [25]	Indonesia	75% (44–91)	63% (37–81)
RotaTeq® (3 doses) [26]	Africa	39% (19–55)	30% (17–42)
BRV-PV (2 doses) [27]	India	61% (18–81)[2]	23% (13–31)
RotaTeq® (3 doses) [28]	Asia	70% (32–88)[2]	43% (21–58)

[1] RV3-BB: oral, live-attenuated monovalent vaccine; RotaTeq: oral, live-attenuated pentavalent vaccine; BRV-PV: oral, live-attenuated bovine-human pentavalent vaccine.
[2] Very severe (Vesikari score $\geq$ 15).

A fourth consideration in selecting endpoints for phase 3 vaccine trials of *Shigella* and ETEC vaccines in pediatric populations in LMIC is the selection of the most relevant population. In this regard, the peak age incidence and the groups at greatest risk for disease and its sequelae can be examined. The findings from GEMS provide some insight into the target age groups. The proportion of *Shigella* and ETEC episodes that occurred was 3.6% and 11.7% by 6 months of age, 10.5% and 26.4% by 9 months of age, and 20.3%

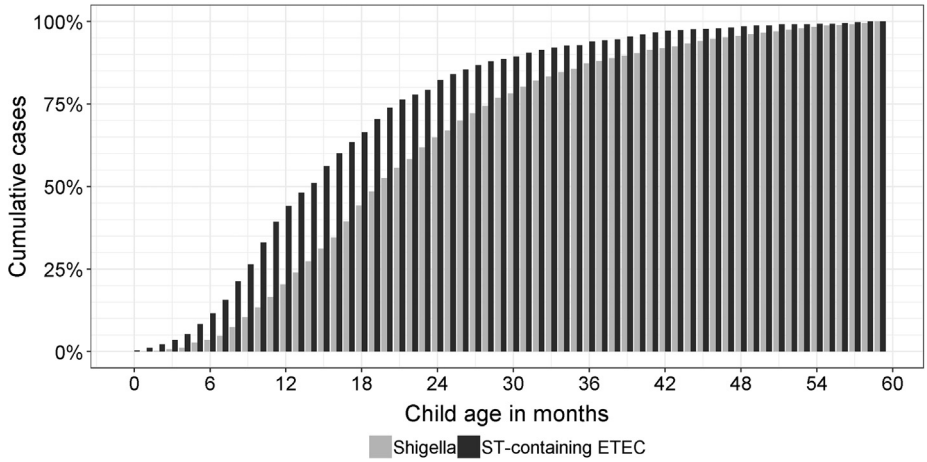


Fig. 2. Cumulative proportion of *Shigella* and ETEC episodes by increasing age in the Global Enterics Multicenter Study (GEMS).

and 44.1% by 12 months of age, respectively (Fig. 2). Most *Shigella* and ETEC episodes occurred before 36 months of age. These findings suggest that a vaccine that confers immunity by 6 months of age would have the maximum impact. Whether that is feasible given the busy routine immunization schedule for infants in LMIC during the first 6 month of life remains to be determined. The group that bears the greatest burden of *Shigella* and ETEC infections and should be the highest priority for vaccination are infants and children living in LMIC.

3. Workshop discussions

The discussion points from the three simultaneous break-out discussions are outlined below.

3.1. Controlled human infection model

The discussion around the clinical endpoints for the CHIM focused first on the lack of consensus in the field. In particular was the discussion of the recent workshop involving multiple institutions to develop a standardized agreed upon primary endpoint of shigellosis for ongoing CHIM with *S. flexneri* and *S. sonnei*. It was noted that no such effort had been made for the ETEC CHIM. This discussion led to a main point that the use of the CHIM is currently two-fold; 1) to down-select vaccine candidates very early in their development path; and 2) as a late-stage development tool. In the first scenario (i.e., candidate down-selection and prioritization), it was felt that clinical endpoints should be those enabling vaccine developers and funders to identify a meaningful pharmacological effect of the vaccine. There was consensus that in this context the utilization of an overall disease severity index or score would have the greatest value. Lack of a biological effect at this stage may be sufficient to halt product development; however, caution was urged against premature halting due to low-level efficacy against what may be an exposure that is orders of magnitude higher than what occurs naturally. The ultimate utility of the CHIM will be based on its ability to predict vaccine efficacy in the field.

For the second scenario (i.e., late stage development with potential support for licensure), there was general agreement that the CHIM endpoint would need to match with the product's labeling. For licensure, the discussion of the recent application of the cholera CHIM for use in travelers was used as an example [12]; however, it was felt that there was a difference in the 'maturity' of the cholera model compared to the current status of the ETEC, *Shigella* (or other enteric) CHIM. Furthermore, it was felt that studies in the primary target population for ETEC/*Shigella* vaccine development (ie, children < 5 yo in LMICs) would be needed and that efficacy in the CHIM would be insufficient to support licensure in pediatric endemic populations. There was a noted opportunity for the potential use of the CHIM to support licensure for other potential target populations, such as adult travelers to LMICs.

Participants agreed that including multiple endpoints (both primary and secondary) is ideal for all CHIM. It was felt that these multiple *a priori* endpoints would enable demonstration of biological effect, enabling appropriate vaccine candidate 'up/down-selection'. Related to secondary endpoints was the recognized value of capturing and analyzing data on shedding post-challenge. In particular, it was noted that for some enteric pathogens, the data suggest quantitative level of shedding is directly correlated to clinical endpoints [24,35]; however, this correlation may depend on the specific vaccine and its presumed mechanism of action.

The question of endpoint prioritization was discussed at some length. Ultimately, the participants concluded that the primary endpoint should be sufficiently severe in the CHIM to reflect severe disease in the target population in order to give vaccine a chance to show efficacy. It was noted that 'severe diarrhea' in challenge model is very different than severe diarrhea in an endemic setting. A discussion ensued emphasizing that the endpoint of moderate-to-severe disease in the CHIM should be guided by what the community of clinical investigators believes to be tolerable prior to early antibiotic treatment and should minimize risk to subjects. It was stated that the principal investigator should maintain an ability to provide early antibiotic treatment based on his/her clinical judgement and that there may be different thresholds of severity in different geographic settings. Furthermore, there was agreement that there should be a balance with the minimum illness to verify that subjects have gotten sick. The participants again noted severity scoring as an important attribute given the ability to observe a biological effect. On a related point was the discussion of atypical illness that may necessitate early antibiotic treatment in a subject that has not met the *a priori* clinical endpoint. It was pointed out that randomized, double-blind, placebo-controlled studies are critically important to minimize potential bias in the management and treatment of these atypical illnesses.

Strain to strain variability in illness presentation was also discussed. It was noted that a clinical endpoint for some strains may not be appropriate for other strains. Because of this variability, it was felt that there may need to be strain-specific endpoints, which may reduce consistency and harmonization in endpoint definitions. Alternatively, it was felt that there could be standardization to a certain extent with the flexibility to slightly modify endpoints as appropriate for the specific strain.

Discussions concluded that there was tremendous value in the CHIM for *Shigella*, ETEC and other enteric pathogens; however, standardization and data- and method-sharing is needed. It was felt that CHIM studies may facilitate vaccine development; however, the models weren't sufficiently robust to serve as 'standalone' studies to demonstrate efficacy. Finally, there was some caution that in some cases CHIM may in fact slow vaccine development; however, it was felt that this could be overcome by appropriate harmonization to ensure consistency in model application across institutions and over time.

3.2. Travelers' diarrhea

Participants in the travelers' diarrhea breakout included basic scientists and vaccinologists, clinicians, microbiologists, civilian, academic and commercial vaccine developers, to include investigators currently conducting a vaccine field trial among European tourists to Benin [36]. Much of the conversation and exchange included comparisons and contrasts with respect to challenges and advantages of performing field trials with military vs. civilian settings. There was discussion and agreement that the determination of clinical endpoints should include impact to activities and function. There was agreement that in addition to the frequency and volume of diarrhea, other symptoms, such as nausea and constitutional complaints, should be included in the endpoint. The specifics as to which complaints should be included has been a key point in other publications on the topic as well as pathogen-specific working groups [1,3,8]; however, consensus-building efforts are still needed. The group agreed that impact on activity was important to describe for both military and civilian traveler populations.

Use of subject disease diaries to capture symptomatology during episodes in real time was discussed. However, several participants noted that the use of diaries can be difficult for subjects, and that it needs to be a simple tool; diaries can be particularly challenging when the trial is conducted among military populations involved in austere and demanding field operations. Use of digital and online diaries has been considered; however, the ability to use online services is also often difficult in locations where military operations and exercises occur.

The ability to interact with the subject during TD episodes is ideal and enhances the quality of data characterizing the clinical illness. Investigators in the ongoing study in Benin, reported the advantage of their clinical site in that subjects are readily available to the research clinic and personnel. Shipboard studies, both in military and civilian populations include accessible populations, but can be subject to low disease rates in the event that itineraries are changed, especially in the case of military Naval forces.

Participants generally agreed that disease scoring algorithms aimed at disease severity should be developed. Also, the group noted the importance of capturing long term sequelae, such as post-infectious irritable bowel syndrome, among outcomes.

The role and challenges of field laboratory capabilities was also reviewed. The military experience can be variable, with some field locations requiring the establishment of field expedient and basic laboratory capability, at some logistic cost, and other locations where partnerships with hospital and public health laboratories is possible. Shipboard facilities again offer an advantage in this regard, but the impact of ship schedule changes on disease incidence has been problematic.

3.3. Pediatric diarrhea in low-middle income countries

The discussion session focused on four issues. The first issue was the selection of antigenic variants to be considered as "vaccine preventable". There was general consensus that all *Shigella* strains should be targeted, but that for ETEC in LMIC, studies should focus only on ST-producing strains.

The second issue was the definition of primary and secondary clinical endpoints for trials involving children in LMICs that represent disease that warrants and can be prevented. The candidate characteristics for inclusion in the primary endpoint included watery diarrhea for both *Shigella* and ETEC vaccines, as well as dysentery for a *Shigella* vaccine trial.

There was general consensus that medically-attended diarrhea detected by passive health center-based surveillance should be a component of the primary endpoint. Some participants raised concerns about the ability of such surveillance to capture cases that do not seek care, which might be the most severe cases. It was proposed that a study could be powered to allow for such non-capture of clinically meaningful cases and that use of busy health centers where *Shigella* and ETEC disease is prevalent could ensure that sample size requirements for medically-attended cases could be met. The group agreed that targeting less severely ill cases in the community could lower measures of vaccine efficacy.

The most appropriate diagnostic tool for identifying microbiologically-proven ETEC and *Shigella* episodes was discussed. Quantitative PCR is more sensitive for detecting infection with the target organisms, but at this juncture has limitations in characterizing the colonization factor antigens of ETEC and *Shigella* sub/serotypes, and cannot be used to assess antibiotic susceptibility profiles.

The group was asked to consider the utility of other factors as secondary endpoints. These included the ability of the vaccine to engender herd immunity, to confer heterologous protection against non-vaccine strains, to identify immune correlates of protection, and to prevent subacute outcomes of *Shigella* and ETEC diarrhea such as linear growth faltering and mortality. There was general support for including these factors as secondary endpoints. The use of growth faltering as a secondary endpoint was discussed in some detail. The general consensus was that change in length/height for age z-score (delta LAZ) would be an important secondary endpoint that captures the ability of vaccination to prevent the adverse sequelae of both symptomatic and asymptomatic *Shigella* and ETEC infection (Nasrin, unpublished) [37]. There was some debate as to the optimal definition of an endpoint related to linear growth and whether it should be expressed as a continuous variable (delta LAZ) or a categorical outcome related to occurrence of mild, moderate, or severe stunting. It was noted that statistical power is dependent on the disease attack rate which must be characterized to choose the optimal field sites. It was noted that while mortality is intriguing as a potential endpoint, it is also likely problematic due to the sample size requirements for a study powered on a pathogen-specific mortality endpoint.

The most relevant populations for field trials and ultimately vaccine implementation was considered. Given the importance of *Shigella* and ETEC across sites in recent epidemiologic studies, there was general consensus that universal vaccination (rather than attempting to target the highest risk areas) were most appropriate. The challenge of determining the optimal age of vaccination was acknowledged.

4. Conclusion

This workshop brought together many of the experts in the field of enteric disease vaccine development with a focused interest on ETEC and *Shigella*. Clinical trials for product down-selection decisions and to support licensure are ongoing in diverse settings and populations and many more are planned in the coming years. Each of these settings offers unique opportunities and challenges for endpoint determination. It is hoped that this report from the

VASE workshop will facilitate vaccine developers in selecting an optimal endpoint for use in their product development.

5. Disclaimer

The views expressed in this article 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 is a US Government work. There are no restrictions on its use.

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Conflicts of interest

The authors declare that there are no known conflicts of interest.

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Appendix A. Supplementary material

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