2020 WHO Product Development for Vaccines Advisory Committee (PDVAC) Virtual Consultation 4: Update on development of Enterotoxigenic E.coli (ETEC) vaccines
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Executive Summary

Rationale for topic: ETEC was identified as a priority pathogen by PDVAC in 2016. The purpose of this session was to review the status of candidates in the pipeline, including the tools, assays and models that are available to facilitate product development. The data from the recent studies with the leading candidate, ETVAX®, including the Phase IIb efficacy study in travellers, were discussed. Of interest were the intended 'next steps' for the paediatric indication and whether facilitation from PDVAC/IVB and the product and delivery research unit (PDR) are needed.

In addition, WHO preferred product characteristics (PPC) for ETEC vaccines have been drafted by an expert working group and are near finalization. This draft was discussed in the context of the current vaccine pipeline.

PDVAC Conclusions and Recommendations:

- *PDVAC unanimously voted that ETEC should remain a priority pathogen for WHO's PDR unit, and PDVAC.*
- *ETEC is one of the main diarrhoea-associated pathogens that causes death and morbidity. In the era of the Covid-19 pandemic, low-income countries are facing an*

ever-greater risk of poverty. We need vaccines and tools that lower the threat of increasing inequity and financial risk.

- *The data from the ETVAX phase 2b study are encouraging, albeit preliminary, and there are other candidates that remain of interest, such as the FTA candidate vaccine. There are a number of candidates moving into the clinic and we need to support the ETEC field to generate data on how the candidates perform in endemic settings and within the major target populations.*
- *Clinical studies need to be expanded to include exploratory endpoints to assess the impact of the vaccine on long-term sequalae, such as height or length for weight z scores. Measurement of macroeconomic aspects, such as impact on health services, to assess the broader effects and value of ETEC vaccines are also important.*
- *The relative importance of clinical endpoints for licensure and policy recommendations for ETEC vaccines needs further discussion, and alignment with policy makers in national immunization programmes. Consensus on a standardized case definition for moderate-to-severe diarrhoea (MSD) and other secondary/exploratory clinical endpoints such as mild diarrhoea, or medically attended diarrhoea is needed.*
- *Investment in generation and collection of data on appropriate secondary and exploratory endpoints in current and planned clinical studies will be needed as these data may refine the choice of primary endpoint for the pivotal efficacy study.*
- *The points highlighted above need to be expanded in the planned ETEC vaccine roadmap manuscript that is being drafted and is intended to be published in early Q4 2020. The roadmap publication will cross reference the ETEC vaccine PPC that will be published on the PDR website.*
- *With respect to the ETEC vaccine PPC, several aspects were discussed, with some recommendations for clarification before finalisation. Implementation of these recommendations are in progress and the PPC is expected to be published on the IVB PDR website in Q4 2020.*
- *There is currently no funding within the PDR unit to support further ETEC-related activities. Resource mobilisation to engage more effectively in this area should be a focus, and the PDR team will seek to collaborate with working groups whenever possible.*

1. Context of the meeting

Enterotoxigenic *Escherichia coli* (ETEC) is one of the leading bacterial causes of diarrhoea, especially among children in low resource settings, as well as travellers and military personnel from high-income countries. It is estimated that ETEC causes about 220 million diarrhoea episodes globally, with about 75 million episodes in children under 5 years of age, and between 18,700 (IHME estimates) and 42,000 deaths (MCEE estimates) in children younger than 5 years. Although mortality rates for ETEC and other diarrhoeal pathogens are declining due to improvements in economic development and availability of safe water and sanitation, these reductions have not been paralleled by significant declines in diarrhoea-associated morbidity, which continue to impact negatively on infant and child health and prosperity in many low- and middle-income countries (LMICs).

Current ETEC vaccine development efforts are focused on inducing antitoxin and anti-colonization immunity. There are both oral and parenteral candidates in clinical studies. The leading whole cell-based vaccine candidate, ETVAX®, is an oral quadrivalent inactivated vaccine, consisting of four *E. coli* strains overexpressing the most prevalent colonization factors, or CFs (CFA/I, CS3, CS5, and CS6) and a hybrid LTB-CTB toxoid (LCTBA) administered with double-mutant heat-labile enterotoxin (dmLT) as both an immunogen and an adjuvant. This quadrivalent candidate has progressed successfully through descending-age studies in Bangladeshi infants, 6-11 months of age, with fractional doses of both vaccine (1/2, 1/4th or 1/8th of the adult dose) and dmLT (2.5 ug, 5 ug or 10 ug) being tested. A Phase I age-descending trial in Zambia in healthy adults and children is underway and a Phase IIb field paediatric efficacy trial is planned in The Gambia. In addition, ETVAX® has just completed a Phase IIb efficacy study in Finnish travellers, demonstrating safety, immunogenicity, and significant protective efficacy of 56% (p=0.025, CI=9-83%) against all severe diarrhoea, independent of pathogen.

2. Objectives of the meeting:

- To provide a status update on candidate and funding landscape for ETEC candidate vaccines
- To evaluate the outcomes of the ETVAX® efficacy study in adult travellers and plans for the paediatric indication
- To determine if ETEC should remain a “priority” pathogen for PDVAC and if yes, whether there are activities that WHO/PDVAC should engage in going forward

3. Meeting summary

3.1 *PDVAC/IVB engagement in ETEC vaccine (Birgitte Giersing, WHO)*

ETEC at PDVAC – A historical perspective:

ETEC was first discussed at PDVAC in 2015; when ETVAX and ACE527 were the most advanced candidates in clinical development. It was revisited in 2016, when a combination vaccine with *Shigella* was proposed as the priority product development strategy. IVB secured funding from BMGF to support vaccine development in late 2016 and the first global stakeholder meeting was held in April 2017 to discuss this combination approach (meeting report, Hosangadi et al, 2019).

The outcomes of the stakeholder meeting were discussed at PDVAC in 2017, when the pipeline for both ETEC and *Shigella* vaccines was relatively robust. The key considerations of a combination strategy were debated, and the importance of defining burden of morbidity (in addition to mortality) to quantify the value of vaccines against these pathogens was reiterated. The combination strategy was considered riskier than development of standalone vaccines due to higher development and procurement cost, potentially lower probability of technical and regulatory success since neither would have been previously licensed, and longer timelines to vaccine access and impact. PDVAC recommended to pursue licensure of both ETEC and *Shigella* individually in the first instance.

In 2018, PDVAC discussed the decline in IHME mortality estimates for ETEC to a level that was considered unlikely to warrant a stand-alone ETEC vaccine. Uncertainty about the robustness/credibility of the enteric pathogen mortality estimates was highlighted, and a call was made to establish a WHO working group to examine the data and methodology to better define model uncertainties, understand the process for generation of estimates, and ultimately make recommendations on the best metrics to serve as priorities going forward. The Burden of Enteric Diseases (BoED) working group (WG) was established in Q3 2018, and the first burden WG meeting was convened in Nov 2018, with the IHME and MCEE modelling groups included. The WG made numerous recommendations to examine data inputs and model assumptions with respect to improving the understanding of under 5 years of age (U5) mortality estimates, published in 2020 (Prudden et al, 2020). The BoED WG reported its work to PDVAC in April 2020, and will soon direct its focus to evaluate and examine data and methodology to generate morbidity burden, to contribute to a full value of vaccine assessment (FVVA). The FVVA is a concept that describes the global value of a prototypical vaccine, including from a LMIC perspective. It aims to articulate the full direct and indirect effects of such a vaccine. Burden is fundamental for describing FVVA for diarrhoeal vaccines, particularly as mortality estimates are decreasing, while morbidity burden remains relatively stable. Defining the FVVA will be crucial to maintain funding and momentum of vaccine development, through licensure, policy consideration, and implementation.

Other parallel cross cutting IVB workstreams that should enable ETEC vaccine development and defining of the FVVA include:

- Development of a Value Attribution Framework for Anti-Microbial Resistance (AMR): a semi-quantitative framework to articulate the value of vaccines for their impact on AMR.
- Vaccine Innovation Prioritization Strategy (VIPS) in Collaboration with GAVI, UNICEF, BMGF, and PATH for novel vaccine delivery technologies and approaches. It aims to achieve equitable coverage for vaccines, through working with vaccine manufacturers.
- Country Assessment for Prioritization in Immunization (CAPACITI), previously called Total system Effectiveness (TSE): A platform to conduct robust communications with country-level stakeholders and understand their R&D priorities.

In addition, IVB, in collaboration with a working group of global experts, and PDVAC, is developing WHO preferred product characteristics (PPCs) guidance for ETEC vaccines (ETEC PPC WG). The primary strategic public health goal was defined as follows: ***to develop a safe, effective, and affordable ETEC vaccine that reduces mortality and morbidity due to moderate-to-severe diarrhoeal disease in infants and children under 5 years of age in LMICs***. The PPC describes important parameters, such as indications, target populations, efficacy, and dose schedule. A draft PPC document was posted for public consultation for 4 weeks in May 2020. The initial draft was considered too broad and has therefore been separated into two documents; a document focused on the key PPC elements, and a complementary 'Roadmap to ETEC vaccines' that identifies the research gaps, to be published in parallel with the PPC in early Q4 2020.

Key questions for PDVAC, at this 2020 meeting:

- Should ETEC vaccines remain a 'priority' for PDVAC and IVB/PDR?
- In addition to the existing cross-cutting activities that PDR/PDVAC undertakes in support of ETEC, are there other specific activities that PDVAC should try to resource, to advance ETEC vaccines?
- Does PDVAC endorse the PPC for ETEC vaccines?

3.2 *Update on the ETEC Vaccine Landscape and Factors Impacting Further Development (Lou Bourgeois, PATH)*

Uncertainty regarding the FVVA of ETEC vaccines has driven unpredictability in the funding environment over the last 4 years, related to:

- Morbidity burden: Does ETEC play a sufficient role in acute illness and the pathogenic pathway leading to environmental enteric dysfunction (EED), stunting, and malnutrition; does ETEC contribute to the AMR threat?

- Technical feasibility: Will candidates (oral or parenteral) be sufficiently immunogenic and protective in the target age-group (6–9 months), in addition to technical challenges in the product development of enteric vaccines in general?
- Do we have the right antigens in candidates to provide broad protection against important ETEC pathotypes?
- Are development timelines adequate to ensure vaccines will be available while they are still needed?

FVVA uncertainty has also led to reconsideration of funders' ETEC investment strategies, with at least one major donor de-prioritizing ETEC. However, despite these issues, other funders have ensured the vaccine portfolio remains robust, with an impressive level of activity and progress since last reviewed at PDVAC in 2018. The ETEC vaccine community remains optimistic that global burden estimates will become more robust and include further etiological evaluation of acute and more long-term morbidity. Continued positive trial results should also help strengthen the case for ETEC as a priority WHO vaccine target.

Currently, there are 5 different vaccine candidates in the clinic or poised to begin clinical development. ETVAX/dmLT is the most advanced ETEC candidate in phase IIb clinical development. There are 2 orally delivered live-attenuated combined *Shigella*-ETEC candidates: the ShigETEC is in phase I and the CVD 1208S-122 prototype vaccine is poised to enter Phase I studies. The ETEC fimbrial tip adhesin (FTA) from NMRC and PATH and the Multi-epitope fusion technology (MEFA) vaccine candidate from The University of Illinois (Illn) and John Hopkins University (JHU) are both parenteral candidates in early clinical and preclinical development, respectively. The MEFA candidate can also be vectored by the Ty21a oral typhoid vaccine. This concept is being developed by developed by Illn and JHU in partnership with Protein Potential.

Recent field, laboratory, and controlled human infection model (CHIM) data indicate that conserved ETEC antigens, like EtpA, EatA, and YghJ, should be considered for inclusion in vaccines, due to:

- Potential improvement in coverage for ETEC strains that lack CFAs, such as ST- or LT-only strains. New proteomic array data profiling mucosal antibody responses to these conserved proteins as well as class 5 fimbriae are encouraging since they suggest vaccine coverage could be broader than expected if vaccines were to include both colonization factor and these new more conserved antigens.

- Expression of EtpA and EatA may help define a broader paradigm for ETEC pathotypes as efforts expand to explore ETEC's contribution to EED, stunting, and malnutrition in LMICs.

- Virulence of LT-only strains expressing major or minor CFAs and/or EtpE and EatA needs further assessment to determine role in ETEC morbidity.

Encouraging results for lead oral (ETVAX) and parenteral (FTA) candidates indicate both are effective at inducing strong mucosal immune responses to key antigens, and the addition of dmLT can improve these responses. With continued success and sustained momentum, ETVAX licensure and WHO prequalification may be possible in about five years. Defining the impact of

ETEC vaccines on reducing morbidity and long term sequelae will be crucial to eventual policy consideration for their utilization. In this regard, new animal models and controlled human infection model (CHIM) data indicate that ETEC strains can trigger elevations in intestinal inflammatory factors (i.e., myeloperoxidase (MPO) and Calprotectin), which are risk factors for EED and stunting. The utilization of these new animal models and ETEC CHIMs will provide early opportunities to assess the impact of ETEC vaccination on intestinal inflammation induced by infection with wild-type ETEC strains.

All lead ETEC candidates are compatible with combination vaccine strategies that may improve their FVVA. Resolving ETEC's status as a WHO vaccine target and maintaining funding is critical to ensure continued progress of the most promising candidates.

3.3 *Using controlled human infection models (CHIM) and preclinical models to improve understanding of ETEC morbidity (Chad Porter, NMRC)*

The results of the first ETEC CHIM study were published in 1971, and there have now been over 1500 participants in ETEC CHIM studies who have received 15 different strains with various colonization factors (CFs) and toxin profiles (LT only, ST only, LT+ST+). These studies were predominantly used to evaluate the preliminary efficacy of vaccines, in addition to measuring the effect of immuno-prophylactics and chemoprophylaxis. CHIMs are increasingly being leveraged to identify novel ('conserved') antigens using proteomic arrays, and for exploration of sub-clinical endpoints beyond the traditional clinical endpoint of moderate-to-severe diarrhoea (MSD).

Enteric CHIMs, including those for ETEC, may not fully recapitulate clinical disease following natural infection, particularly since the studies are performed in different populations. Assessment of signs and symptoms of ETEC infection showed that, in an adult traveller setting, the disease profile is comparable to the disease profile in a CHIM study. While this is different from the target population of children in LMICs, it is reassuring that the disease profile in CHIM studies is similar to what is observed in adults, in the field.

The ETEC CHIM is one of the most advanced and has informed the field in the following ways:

- the diversity of strains studied has facilitated an increased understanding of ETEC strain variability. The clinical disease profile, in particular the incubation period, is quite heterogenous across various ETEC strains of disease ranging from 12 hours to 2-days. Similarly, there is variability in the effect of antibiotics across variable across strains, as well as differences in symptom profile and severity.
- assessment of the role and pathogenicity of ETEC strains that express LT alone: three separate trials with an LT-only ETEC that expresses the colonization factor CS17 demonstrated that increasing diarrhoea attack rates positively correlated with an increasing dose of the challenge inoculum. A passive prophylaxis trial using bovine colostrum from cows immunized with recombinant CS17, where subjects received the anti-CS17 colostrum prior to challenge, were completely protected from diarrhoea following challenge with the CS17 strain. An active vaccination study using a Dukoral-like

vaccine targeting the heat labile (LT) enterotoxin was able to demonstrate a significant effect of anti-LT responses at reducing disease in the CHIM. Taken together, these data support the role of both the toxin and the CF the LT only model of in ETEC disease.

- identification of faecal inflammatory biomarkers: in paediatric field studies, increasing levels of faecal myeloperoxidase have been negatively associated with EDD and subsequent growth shortfalls following infection with specific enteric pathogens, including ETEC. Increased levels were inversely associated with length and weight for age z-scores, as demonstrated in the MAL-ED study. ETEC was also significantly associated with increased myeloperoxidase (MPO) levels. Faecal MPO has also been assessed in the context of the ETEC CHIM. In studies with two separate ETEC strains, both of which express LT/ST, the levels of faecal MPO increased in subjects challenged with both B7A and H10407, most notably in subjects with MSD; however, an increase is also seen in subjects who did not have MSD. This elevation in inflammatory markers is a potential endpoint to assess vaccine effect and may help to identify a vaccine that would have greater health benefit in LMICs.
- elucidation the role of microbiome in infection and pathogenicity: in North American adults exposed to a single ETEC infection, the microbiome has demonstrated resilience; however, repeated infections may cause dysbiosis possibly due to disruption of the mucosal barrier causing modification in adaptive immune response.
- evaluation of subclinical endpoints for ETEC infections, such as cognitive function. Psychomotor vigilance testing, which is a measure of attention status and fatiguability for sleep deprivation, has been measured in young, healthy North American adults. A measurable cognitive deficit as assessed by increased response times was found to be associated with MSD. In addition, an increase in the number of lapses was also observed in subjects with MSD. These effects were observed 2 days after challenge and persisted for 5 days. These findings in the cognitive functions associated with MSD may point to an extra-intestinal effect of enteric infection.
- the role of blood group in the risk of developing MSD: increased risk of MSD was observed with type A and AB blood groups, in particular with ETEC strains expressing EtpA. EtpA interacts with glycans on intestinal epithelial cells from blood group A accelerating adhesion and toxin delivery. These CHIM data are consistent with field studies in paediatric populations linking ABO blood group and secretor status to ETEC illness. (Qadri F, et al. 2007). This association was not observed with ETEC strains not expressing EtpA.

With respect to preclinical ETEC infection models, the *Aotus nancymae* non-human primate (NHP) model has been developed and repeatedly tested with multiple ETEC strains, CFs and toxin profiles. NHPs have been established for multiple strains to study effects of acute diarrhoeal illness and present an opportunity for colonoscopy, pathology, and other investigations. Active vaccination using parenterally administrated subunit candidates demonstrated protection against diarrhoea in the model, highlighting the ability to probe protective epitopes and examine pathogen-host interactions. Passive prophylaxis can also be evaluated.

Murine preclinical models enable investigation of infection on diarrhoea, weight, and inflammatory markers, and can evaluate the role of diet and the role of toxin. The recently developed murine model at University of Virginia has been leveraged to assess the role of faecal MPO. Following a course of antibiotic treatment to disrupt normal microbiota, and subsequent challenge with the ETEC strain H10407, levels of faecal MPO were increased indicating mild-to-moderate intestinal inflammation along with growth impairment, watery diarrhoea, and heavy stool shedding. Animals fed a zinc deficient diet had more pronounced increases of faecal MPO, which was further magnified after ETEC challenge with the H10407 strain. The MPO effect was minimized with animals that were challenged with LT knock-out ETEC, indicating an LT specific role in intestinal inflammation in this model.

3.4 Development of ETVAX®, an ETEC vaccine candidate (Nils Carlin, Scandinavian Biopharma)

ETVAX® is an oral, inactivated multivalent vaccine containing four inactivated *E. coli* strains expressing the most common colonization factors plus an LT toxoid (a chimera of LTB and CTB) and a dmLT adjuvant. Administration of the vaccine together with an adjuvant enhanced the magnitude, breadth and kinetics of the intestinal immune responses in Bangladeshi infants. As an inactivated vaccine, ETVAX potentially lends itself to co-administration with other vaccines. Two indications are being developed:

- *ETVAX® travellers' formulation:* An oral 2 dose vaccine. A full immunization requires 2 doses orally taken at least 1 week apart, with the last dose taken at least 1 week before travel.
- *ETVAX® pediatric indication formulation*: An oral 3 dose vaccine; currently evaluating three vs two doses in clinical trials. It is administrated with buffer, dmLT and 10 ml of water, and is mixed and contained in a single container to be administered safely to children.

Its intended mode of action is to prevent colonization of ETEC bacteria in the intestine and to induce antibodies that neutralize the heat-labile toxin (LT) produced by the bacteria. It is has the potential to protect against at least 80% of all clinical ETEC strains, and even more through potential cross-protection against other CFs as suggested from serological cross-reactivity in adults (S Leach et al 2017) and in children (Qadri & Svennerholm unpublished). The addition of dmLT has been shown to significantly enhance the immune response and LT toxin neutralization. Results from 5 published clinical studies in both target populations detail its immunogenicity and safety, to date.

ETVAX is being developed by Scandinavian Biopharma (SB), together with researchers at the University of Gothenburg and the international non-profit organization PATH. The programme was previously funded by BMGF but is now predominantly funded by the European Union's (EU) Research and Innovation Framework Program, Horizon 2020 through the EDCTP (European and developing countries clinical trial partnership) organization, DFID (UK Department for International Development), and SME Instruments supporting innovative small and medium-sized

enterprises (SMEs) in the health care biotechnology sector. SB also has a cooperative research and development agreement (CRADA) with USAMMDA (U.S. Army Medical Materiel Development Activity).

Clinical development plan:

The OEV-122 Study at iccdr,b, Bangladesh was an age de-escalation study in adults, 2-5 year olds, 1-2 year old, and 6-11 months age groups. Results for frequencies of mucosal IgA antibody responses against the 5 primary vaccine antigens showed that adults had 100% response, and responses decreased with age, and the 6-11 months old age group with 50% response rate. These results prompted the consideration of 3 doses vaccine in infants. Frequencies of faecal IgA antibody responses were (≥2-fold) among infants 6 to 11 months old to ETVAX antigens given placebo or vaccine only, or vaccine with dmLT on day 7 and day 28, indicating that in this age group dmLT potentiates a faster immune response with titres seen after two doses already reached at day 7.

In the recently completed 2-dose placebo-controlled phase IIb study, 743 Finnish adults travelled to Benin following vaccination. The study total duration was 14 days + 6 days after returning home. Preliminary results suggest that

- the safety of the vaccine was excellent, and the overall vaccine immunogenicity was good, with IgA and IgG serum antibody responses to LTB (the B-subunit of ETEC heat-labile enterotoxin).
- the overall median magnitude of the IgA response was 33-fold; however, the overall vaccine take rate (defined as ≥4-fold IgA response to LTB) was lower than expected based on prior studies, 76% versus the 90% seen previously among Swedish adults. The reduced response rate was restricted to cohorts vaccinated during a certain period (P=0.0015) suggesting that the dosing procedure used for mixing dmLT and vaccine in that period was inconsistent and merits further investigation and standardization.
- the attack rate of diarrhoea was higher than expected based on experience from previous traveller diarrhoea (TD) studies. Overall, 61% of the volunteers experienced diarrhoea (according to the classical TD definition). Moderate-to-severe diarrhoea (≥4 loose stools/24 hours and at least one additional symptom) was found in 56% of all volunteers, of whom approximately 60% provided at least one ETEC-positive faecal sample.
- the contribution of ETEC to the diarrhoeal pathogen burden was even more evident when more severe cases (≥16 loose stools/24 hours) were considered; 75% of the pathogens associated with these episodes were ETEC, highlighting its importance as a cause of severe TD. The toxin profiles of all ETEC isolates from diarrhoeal stools were: LT only 34%, LT/ST 32%, and ST only 34%.
- Antibiotic or antisecretory drug treatment was given to significantly fewer vaccine responders than to placebo recipients (p=0.03), indicating that ETVAX reduced the severity of enteric illness.

A secondary objective of the study was evaluation of protective efficacy (PE) against vaccine-preventable outcomes (VPO). The endpoint of 70% PE was not met. Mixed infection with ETEC of another toxin and/or CF phenotype proved surprisingly common at 18% of VPO ETEC cases.

ETVAX® provided significant protection in the context of a higher than expected TD attack rate. The PE against MSD among vaccine responders (≥4-fold seroconversion to LTB) against any ETEC, and allowing for concomitant presence of EAEC, EPEC, EIEC/*Shigella*, *Salmonella* sp., *Campylobacter* sp., and parasites, was 52% (p=0.006; 95% CI=18-72%), representing 25% of all TD. When disregarding vaccine immune response, the protection remained significant (PE=41%, p=0.02; 95% CI=7-63%). Mixed infection with ETEC of another toxin and/or CF phenotype was observed (18% of VPO ETEC cases) and decreased the measured protective capacity slightly. Overall, the PE against diarrhea of any cause (including viral pathogens) affecting daily activities increased progressively with increasing severity of disease, so that among vaccine responders with ≥16 loose stools in 24 hours the PE was 56% (p=0.025, CI= 9-83%) and disregarding the vaccine immune response it was 43% (p=0.05). This represents 22% of all TD.

With respect to the paediatric indication of ETVAX®, an immunogenicity trial is currently being conducted in Zambian children evaluating 1/4 or 1/8 dose in a three-dose schedule (OEV-124). Preliminary data suggest promising immunogenicity results for toxoid and colonization factor antigens in the vaccine. A phase 2b efficacy trial in Gambian children is scheduled to start in September/ October 2020, and a pivotal phase 3 efficacy trial in Zambian children with an all-in-one formulation is being planned to follow the phase 2b in The Gambia.

SB has sought scientific advice from EMA and Swedish medical product agency and has had a PQ advice meeting with WHO. The phase I/II study in Bangladesh conducted under an IND with US FDA, that is now has been transferred to SB.

Manufacturing Progress:

The immunogenicity observed in Zambia and in the Bangladesh study (OEV-122) using fractional doses of the vaccine suggests a favourable cost of goods for use in LMICs settings following licensure and prequalification by the World Health Organization.

SB is partnering with EuBiologics Co., Ltd in Republic of Korea, who developed a prequalified oral cholera vaccine in two different presentations; Euvichol® and Euvichol-Plus®. EuBiologics is increasing its production capacity by building a new vaccine manufacturing facility with 1000L production fermenters and down-stream capabilities. The manufacturer has set aside a production capacity of 8 million doses for ETVAX®, which can be increased by installation of more equipment. Currently production of tech-batches for all components including dmLT followed by GMP production of ETVAX® monovalent bulks is ongoing at Eu Biologics.

ETVAX® is now scaled up to commercial scale, stabile & has a validated process, with 48 months stability of bulk and 60 months stability of vaccine.

Questions to SB:
Q: Is there any information about the thermostability profile of the paediatric formulation?
- Cold chain will be needed, but no formal thermostability studies have been conducted yet.

Q: What about the footprint in the cold chain?
- The vial is 20 ml in size, which is slightly bigger than other (single dose) vaccines, and the dry formulated effervescent powder, LCTBA and dmLT are added to the 10 ml of whole cell bulk in PBS through a frangible seal; thus no addition of water is needed. The all in one formulation can reduce the cold chain footprint.

Q: What is the vaccine schedule; 2 or 3 doses?
- We used a 2-dose regimen, and in the pediatric studies in Zambia, we evaluated a 3rd dose after 90 days as a booster.

Q: Do you have plans to check children's growth, post vaccination? Or to include any other exploratory endpoints in the studies?
- We are planning to follow up the study volunteers with weight measurements in The Gambian study.

Q: What are the epidemiological data for ETEC in the 3 countries where the clinical trials are being conducted?
- We did not conduct specific epidemiological studies, but we have the GEMS data for The Gambia and the pathotypes study in Zambia showed similar patterns of other nearby countries. Numerous studies have been published concerning pathotypes in Bangladesh.

General discussion:

Q1: With respect to the ETEC PPC, how does the proposed immunization strategy of administrating the ETEC vaccines with MR, and dose regimen and schedule relate to the ETEC epidemiology?

The ETEC PPC WG deliberated about the need to protect infants before the age of peak incidence, and how best to align this with immunization opportunities in the EPI schedule. At the same time, the group attempted to avoid being overly prescriptive; the proposals in the PPC are for the primary series to be administrated by the age of 9 months, potentially aligning with the measles vaccination schedule with doses between 6 and 9 months, with a possibility for a booster at 15 months to align with the second measles dose, or later.

Q2: What is the association between cognitive scores and faecal MPO in the CHIM for diarrheal infections?
The samples are available for these analyses, but due to limited resources the MPO levels have not been evaluated in the volunteers of CHIM studies. It's an analysis that would be important to do.

Q3: What do we need to do to get a consensus on endpoints and case definitions?
There was consensus from the working group to use Moderate-to-Severe Diarrhoea (MSD) as the primary endpoint for vaccine studies. There are proposed case definitions for MSD in the literature, as was used in the GEMS and ETVAX studies, but we need a standardized definition. We also discussed including mild diarrhoea, or medically attended diarrhoea, as potential endpoints since these have impact on stunting, and these are proposed as secondary endpoints in the PPC. It will be important to collect data on these in current and planned clinical studies as this data may refine the choice of primary endpoint for the pivotal efficacy study. So, the endpoints could be broadened beyond MSD for clinical studies – and there needs to be more discussion on their associated case definitions.

Q4: Seeking clarification regarding efficacy endpoints of the Benin study – with respect to the 41% efficacy against MSD, are these ETEC associated diarrhoea cases? There was 43% efficacy against diarrhoea of any cause? Why is the % efficacy for all cause diarrhoea higher than for vaccine preventable outcomes?
Confirmed the primary end-point in the study was MSD. There is no clear explanation at this time for the higher efficacy in the all-cause diarrhoea analysis, but there was a high number of co-pathogens, i.e., salmonella and EAEC, in the placebo group which hindered analysis in the vaccine arm and assessment of VPO. The analysis is not yet complete and is ongoing. Plan to complete the CSR by end of Q3 2020, and to publish the data following that.

Comment related to AMR: *One of the fascinating findings is the lower use of antibiotics and anti-diarrheal medications in the vaccine arm, and this will have a great impact for the value of the ETEC vaccine. The need to assess this as an exploratory endpoint is proposed in the ETEC vaccine PPC guidance.*

Comments related to clinical endpoints and case definitions: *It will be important to clarify what data and clinical endpoints are needed to support policy making, and what should be collected*

for research. Policy makers will look for standardized case definitions of MSD, and clarity on how it is measured. Other endpoints can be collected to inform research – such as modelling of disease impact. We should harmonize all of the different definitions to the extent possible, to avoid variable outcomes across studies and candidates.

Q7: Is it worthwhile to make investments in developing consensus around clinical endpoints and case definitions for both traditional primary endpoints and the secondary / exploratory endpoints we've been discussing – and to advocate for including both in future clinical studies to generate evidence that would support policy setting?
Yes – this is very important - and investing in these secondary endpoints could be the most effective way to assess the role and impact of an ETEC vaccine. So called 'secondary' effects could be even greater than the impact on overt diarrhoea (because of there are so many more 'asymptomatic' ETEC infections), especially if we add measure faecal MPO in stools and delta HAZ in clinical studies. Initial and final HAZ and checking MPO in any ETEC+ stools (and perhaps matched controls) is likely much easier and less expensive than all the tracking of diarrheal illnesses.
This 'research gap' will be addressed and expanded on in the ETEC vaccine roadmap manuscript that is being drafted by the ETEC PPC WG.

Comment related to exploratory endpoints and research: *As we think about better ways to quantify ETEC vaccine value, new tools are evolving that can be used to assess their impact on growth in CHIM studies, such as serum-based assays, that can measure and monitor the gut inflammatory status, and other tools to assess growth to help us beyond anthropometry.*

Closed discussion and PDVAC recommendations:

- *PDVAC unanimously voted that ETEC should remain a priority pathogen for WHO's PDR unit, and PDVAC.*
- *ETEC is one of the main diarrhoea-associated pathogens that causes death and morbidity. In the era of the Covid-19 pandemic, low-income countries are facing an ever-greater risk of poverty. We need vaccines and tools that lower the threat of increasing inequity and financial risk.*
- *The data from the ETVAX phase 2b study are encouraging, albeit preliminary, and there are other candidates that remain of interest, such as the FTA candidate vaccine. There are a number of candidates moving into the clinic and we need to support the ETEC field to generate data on how the candidates perform in endemic settings and within the major target populations.*
- *Clinical studies need to be expanded to include exploratory endpoints to assess the impact of the vaccine on long-term sequalae, such as height or length for weight z scores. Measurement of macroeconomic aspects, such as impact on health services, to assess the broader effects and value of ETEC vaccines are also important.*
- *The relative importance of clinical endpoints for licensure and policy recommendations for ETEC vaccines needs further discussion, and alignment with policy makers in national immunization programmes. Consensus on a standardized case definition for MSD and*

other secondary/exploratory clinical endpoints such as mild diarrhoea, or medically attended diarrhoea, is needed.

- *Investment in generation and collection of data on appropriate secondary and exploratory endpoints in current and planned clinical studies will be needed as these data may refine the choice of primary endpoint for the pivotal efficacy study.*
- *The points highlighted above need to be expanded in the planned ETEC vaccine roadmap manuscript that is being drafted and is intended to be published in early Q4 2020. The roadmap publication will cross reference the ETEC vaccine PPC that will be published on the PDR website.*
- *With respect to the ETEC vaccine PPC, several aspects were discussed, with some recommendations for clarification before finalisation. Implementation of these recommendations are in progress and the PPC is expected to be published on the IVB PDR website in Q4 2020.*
- *There is currently no funding within the PDR unit to support further ETEC related activities. Resource mobilisation to engage more effectively in this area should be a focus, and the PDR team will seek to collaborate with working groups whenever possible.*