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Technical product attributes in development of an oral enteric vaccine for infants

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ABSTRACT

Development of an oral enteric vaccine for infants is important for *Shigella* and enterotoxigenic *Escherichia coli* (ETEC) vaccine development. At a recent workshop titled "Technical Product Attributes in Development of an Oral Enteric Vaccine for Infants," at the 2nd International Vaccines Against *Shigella* and ETEC Conference (VASE Conference), the preferred product attributes for development were discussed for these vaccines. The aims of this workshop were to identify gaps and gather opinions from key experts from preclinical, process development, manufacturing, regulatory, and clinical areas to fine-tune and refine key target product attributes for infant oral vaccine development. The workshop used some examples of marketed oral infant vaccines to discuss potential improvements that can be made, such as inclusion of preservatives, multidose vials, and antacid buffer presentation (liquid or lyophilized) in novel oral enteric vaccine development.

1. Introduction

As the childhood vaccination schedule becomes more crowded, it is important to consider needle-free delivery strategies. Oral vaccine delivery avoids many of the challenges of injectable vaccines. Compared to parenteral vaccines, vaccines for oral delivery are relatively easy to administer, have the capacity to induce local mucosal immunity in the intestinal mucosa, and can be potentially produced at a relatively low cost [1]. However, for an oral vaccine to be widely practical, particularly if intended for infants in low- and middle-income countries (LMIC), it will be crucial to have a vaccine formulation and presentation that facilitates use in the target population [2–6]. The challenges and considerations for oral vaccine formulations targeting infants were discussed in a recent workshop titled "Technical Product Attributes in Development of an Oral Enteric Vaccine for Infants," at the 2nd International Vaccines Against *Shigella* and enterotoxigenic *Escherichia coli* (ETEC) Conference (VASE Conference).

The discussion moderated by workshop co-organizers Manjari Lal and Jessica White from PATH aimed to gain a better understanding of product considerations for developing an oral formulation for delivery of ETEC and *Shigella* vaccines to infants. Attendees included academic scientists, industry members, and World Health Organization (WHO) experts. The expert panel members included Richard Walker (PATH), Nils Carlin (Scandinavian Biopharma), Birgitte Giersing (WHO), and Tarun Sharma (Hilleman Laboratories). The discussion was broken into two main categories, formulation and regulatory pathways. Under formulation, discussions included types of antacid buffers, dry versus liquid preparations, dosage volumes, dosage presentations and packaging, and cold chain space requirements. Regulatory aspects focused on thermostability and potential alternatives to oral delivery.

The challenges in optimizing oral presentations is particularly important for vaccines against enteric pathogens like ETEC and *Shigella* that are a substantial threat to public health, especially to children living in low-resource settings [7,8]. Mucosal immunity can play a significant role in protection against enteric pathogens. As antibiotic resistance increases, vaccines against enteric pathogens are important tools in mitigating disease burden [9]. Some of the current candidates for vaccines against *Shigella* and ETEC in development are live attenuated bacteria or inactivated whole cells, both of which require administration via a mucosal rather than a parenteral route [5,10]. In this manuscript, we will attempt to present a summary of the workshop discussion on the oral

Abbreviations: CTC, controlled temperature chain; dmLT, double mutant heat-labile toxin of enterotoxigenic *Escherichia coli*; EPI, Expanded Programme on Immunization; ETEC, enterotoxigenic *Escherichia coli*; FDA, US Food and Drug Administration; LMIC, low- and middle-income countries; VVM, vaccine vial monitor; WHO, World Health Organization.

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vaccine development field and considerations for future vaccines in development.

2. Formulation

2.1. Live versus killed vaccine

An important consideration in developing oral formulations is the number of doses required to provide protective immunity. A strong, long-lasting immune response may be achieved with fewer doses using a live (attenuated) vaccine antigen compared to a killed vaccine antigen. An example of this is Vaxchora® (PaxVax), a live attenuated oral cholera vaccine requiring a single dose versus Euvichol® (EuBiologics) or Shanchol® (EU Biologics and Shantha Biotechnics), oral inactivated cholera vaccines requiring two doses with a minimum of two weeks delay between each dose. The disadvantage of more doses required may be offset by other factors. For example, killed vaccines are generally more stable on storage, unlikely to contain contaminating pathogens, and unlikely to cause disease due to residual disease-causing characteristics. Furthermore, it may also be possible to reduce the number of doses of a killed vaccine required by co-administration with immune stimulating adjuvants resulting in vaccine dose sparing effect(s). The ultimate determination of number of doses required will be based on the efficacy, safety and cost of the final vaccine presentation.

2.2. Protection from gastric acidity

Antacid buffers or enteric coatings are included in vaccine formulation to prevent antigen degradation in passage through the stomach [11]. Live attenuated vaccines must be formulated as a dried preparation, whereas inactivated whole cells such as the current cholera inactivated whole cell vaccines Euvichol and Dukoral® (Valneva Sweden AB) can be formulated as either liquid suspensions or dried formulations. Furthermore, live vaccines require protection from gastric acids in the stomach to prevent degradation of vaccine antigens. Some killed vaccines may not need protection from acid depending on the acid sensitivity of antigens conferring protection, such as acid-resistant *Shigella* O-polysaccharide. However, vaccines based on protein antigens, such as ETEC or the mucosal adjuvant double mutant heat-labile toxin (dmLT) of enterotoxigenic *Escherichia coli*, will require protection against gastric acidity [12].

While enteric coating offers one approach for protecting vaccines transiting the stomach, liquid bicarbonate-based buffers have commonly been used with ETEC and *Shigella* vaccine candidates. In order to maintain buffer capacity, bicarbonate-based buffers require storage as a dry powder and reconstitution prior to administration, increasing the complexity of vaccine preparation by requiring exogenous water and a reconstitution vessel. These are concerns for developing a practical vaccine presentation using bicarbonate-based buffers. Vaccines formulated in this manner often consist of multiple components: a vialed bacterial suspension, a sachet containing bicarbonate buffer, a cup for reconstitution, and exogenous water to which the sachet and vial components are added and mixed prior to administration. In addition, depending on the target age group, a spoon or oral syringe may be required for administration. Vaccines requiring multiple components for storage, transportation and ultimately administration not only increases the overall product cost but also the logistical complexity of packaging and delivery, considering cold chain volume [1]. Novel vaccine packaging and delivery technologies are required to help address some of these challenges. A few of these novel delivery technologies were discussed during the workshop. Previous work at PATH has focused on development of a liquid citrate-based antacid buffer for administration of a live attenuated rotavirus vaccine candidate in small volumes to infants. There may be value in considering a citrate-based antacid or other alternate antacid buffer for enteric bacteria vaccine development because it has demonstrated stability as a liquid and is currently being used in the infant population with licensed rotavirus vaccines [13–15].

2.3. Novel packaging of oral vaccines

In order to improve product shelf life, vaccine antigens may require either drying or packaging separately from antacid buffer [16–21]. Several novel packaging technologies are in development to allow for packaging of dry and liquid vaccine components in one container. PATH has conducted some preliminary work to develop novel packaging for oral delivery to infants where the bacteria could be a vialed product, liquid or lyophilized, to which liquid antacid buffer is added via a plastic squeeze tube that can also serve as a delivery device. In addition to PATH's preliminary device development, several manufacturers have more advanced designs for dry and liquid vaccine presentations [22,23]. For example, Hilleman Labs is developing an all-in-one device with a frangible seal separating two compartments, which can be broken and mixed prior to use. This novel device allows for a single storage and delivery device. A consideration for an all-in-one device is the amount of cold chain volume required. In many cases, the dry antacid component does not require cold chain storage but in an all-in-one device the whole thing would be required to be in the cold chain. This device is in development and would require qualification with the vaccine of interest.

2.4. Optimization of dose volume

The panel members highlighted the importance of dosage volume for administration to infants. Current marketed liquid rotavirus vaccines containing antacid with an oral administration volume less than 2 mL are a gold standard in terms of optimal administration volume to infants. Larger administration volumes may also have a programmatic impact, potentially taking longer to administer to infants [24]. Minimizing the buffer volume should significantly reduce the storage footprint for the vaccine as well as facilitate delivery. In the pursuit of the lowest volume possible for an antacid buffer, the developer must also consider how reducing the volume has significant impact on the osmolarity, which may have detrimental effects on vaccine stability and palatability of the final formulation. For example, the rotavirus formulation, while a low volume, has a very high osmolarity (>1000 mOsm). Some questions were raised around the effect of high osmolarity on antigen stability, potential interaction, or gastric irritation, which will need to be determined on a case-by-case basis. The osmotic stability of each antigen candidate will vary, and formulations will require optimization for each to balance the dose volume with the required buffering capacity.

With a dry buffer approach, such as Dukoral, one of the panel members pointed out that availability and access to clean water for reconstitution could be a barrier to vaccination in resource-limited areas or outbreak situations [25]. Due to both limited access to clean water and significant presentation optimization with Euvichol there has been very little uptake of Dukoral in LMIC contexts. Considerations on understanding physiologic differences between different populations are important for developing a vaccine formulation that will be effective in a given target population, an example being the significantly lower observed rotavirus vaccine efficacy in low-resource settings compared to high-resource settings [26].

2.5. Issues with dry versus liquid presentations

Some oral bacterial vaccines in development are dry lyophilized products stored in a vial or sachet. There were also discussions around PATH's previous work on development of freeze-dried fast-dissolving tablets for the ACE527 live attenuated ETEC vaccine candidate [27]. The tablets formed by conducting freeze-drying in blister sheets disintegrate (less than 60 s) in minimal volumes of water (a few drops) and have a small storage footprint with a potential to replace the lyophilized product in a glass vial. There was, however, a concern raised in maintaining the sterility of blister sheets for a vaccine product since the blister sheets containing the tablets are not sealed in situ in the freeze drier. To this point, it may not be necessary to maintain sterility for an orally delivered product. Currently, US Food and Drug Administration (FDA) guidance does not provide clear specifications around sterility requirements for dry dosage forms intended for oral route of delivery. This is primarily because solid dosage forms typically produced by drying, dehydration and freeze-drying methods have a very low water content and thus prevent microbial growth, potentially rendering it safe. Although there is precedence for FDA approval of several oral liquid vaccines (Vaxchora, RotaTeq® [Merck & Co., Inc.]), sterility restrictions for oral vaccine delivered as a tablet will need to be evaluated on a case-by-case basis based on intended use, patient population, nature of the product and parameters which would affect product safety where the most objectionable would be presence of organisms that pose a threat to patient safety.

Hillchol™ oral cholera vaccine (in clinical development) from Hilleman Labs is an oral tablet formed by milling the freeze-dried vaccine into powder, which is then pressed into a tablet and enteric coated, thereby eliminating the need for a buffer. Water or some other liquid may be used to wash this tablet down. This presentation simplifies delivery and administration to older children and adults; however, it may not be as suitable for administration to infants.

Dried preparations for oral administration to infants must be reconstituted prior to administration, which often requires an exogenous water source. If one component of the vaccine is a liquid antacid buffer as described above, the water component may not be an issue. A second-generation presentation under development at Hilleman Labs consists of a two-chambered presentation, with enterocoated B subunit (rCTB) in one compartment and liquid vaccine in the other compartment, separated by a frangible seal that can be broken by pressing to allow the contents to be mixed and delivered into the mouth. This novel presentation would be suitable for use in infants.

2.6. Regulatory aspects

2.6.1. Thermostability

LMIC have made maintenance of the cold chain a priority [28]. Countries like Bangladesh or many in Africa maintain the cold chain at clinics where children would be receiving vaccinations. Bangladesh is very effective at cold chain handling of vaccines going into the field, but storage capacity is an issue at clinics. In Latin America, the rural populations are getting smaller and smaller and the vaccination centers are more accessible. People are also coming more to the vaccination centers rather than vaccines being taken to rural areas. While there have been significant improvements to the cold chain, it varies by region, and coverage for the last mile is still difficult in many settings. Improving vaccine thermostability will help improve vaccine coverage.

In designing vaccine formulations and presentations, it is important to consider the thermostability of the antigens that will allow vaccines to be kept at temperatures outside of the traditional cold chain of +2 °C to +8 °C for a limited period of time, and as appropriate of the stability of the antigen. It could be useful to encourage vaccine manufacturers to work toward a controlled temperature chain (CTC) approach, in which the vaccine must be able to tolerate ambient temperature of at least +40 °C for a minimum of three days to facilitate delivery to the last mile (campaigns). Some participants remarked that vaccine stability for three days at 40 °C is a "good goal" but we may "waste time" trying to achieve it. There was concern regarding estimating how many children could be reached with a vaccine that only remains stable at ambient temperature for two to three days. The other point that should be mentioned is that vaccine manufacturers are encouraged to look for stability beyond 3 days at 40 °C—Expanded Programme on Immunization (EPI) programs would like thermostability for as long as possible for vaccines to be more impactful. It was noted that vaccines in CTC are for special campaigns, not for EPI. CTC is aspirational, but it is not a requirement at this time for use in children being vaccinated on an EPI schedule.

2.6.2. Delivery devices

For a new vaccine presentation such as Hilleman's two-compartment presentation with frangible seal, changing the primary packaging material is considered by regulatory authorities to be a major change that requires demonstration of programmatic suitability for WHO prequalification (vaccine in device/container) [29]. If the container design is novel, data required for submission include testing for solvent leakage, leachables, compatibility, biological reactivity, toxicity, and long-term stability, along with studies to ensure that the vaccine can be appropriately accessed by the user by conducting an acceptability/usability study. An additional consideration for developers of novel packaging technologies is how to incorporate vaccine vial monitors (VVMs). Using the mono-multi strips of blow-fill-seal (BFS) tubes as an example, five BFS tubes are associated with one VVM at the top of the strip. The action of removing the strip opens the vial and the tube must be administered. This allows the cost of the VVM to be spread across all five doses and further reduces the overall product cost. Manufacturers will have to identify similarly novel methods to incorporate VVM labeling for freeze-dried tablets in blister cards. Manufacturers and developers of novel vaccine delivery devices are encouraged to engage with the WHO prequalification team early on for advice on programmatic suitability. In addition, novel packaging or presentations can be discussed with the Delivery Technologies Working Group, which is co-chaired by PATH and WHO, for feedback [30].

During discussion, one of the questions raised was on potential inclusion of vitamins along with the vaccine and whether this will offer any benefits on impact of illness/chronic inflammation and overall improvement in health. The panel members commented that having multiple indications could become very complicated from a regulatory perspective. Also, the vaccine developer would have to show that inclusion of a nutritional component does not affect the efficacy of vaccine in multiple clinical trials.

2.6.3. Alternatives to oral vaccination

Consideration of alternatives to oral immunization against enteric pathogens illustrates the importance of finding ways to maximally exploit oral immunization strategies. For example, intranasal immunization is feasible as an alternative mucosal route and may avoid some of the buffer concerns associated with oral vaccines. However, this advantage may be offset by safety concerns, and the nasal congestion often seen in children in LMIC may make delivery difficult [31]. Additionally, the sublingual route of administration also avoids the potential problem of gastric acidity. Sublingual vaccine development requires development of an appropriate formulation to allow for uptake of the vaccine and prevention of swallowing or loss of the administered dose [32].

Gram negative whole cell vaccines would be reactogenic if given by parenteral routes, which limits alternate vaccine delivery routes. Subunit vaccines for enteric pathogens could be safely given by parenteral routes, but mucosal immunity is generally poor in response to vaccines given by these routes. It has been noted that prior mucosal exposure to an antigen can prime the immune response to a subsequent exposure via parenteral administration. One could consider a prime-boost strategy if whole cell and subunit vaccines for a pathogen were available. Such a strategy may be of use in improving immune responses in children in LMIC. Needle-free parenteral delivery may be obtained in the future by using microarray patches to deliver antigen intradermally rather than intramuscularly.

3. Conclusions and recommendations

It will be important to begin the development of a final vaccine presentation early so that it can be ready when needed for advanced clinical trials. Cholera vaccine presentations now available range from the single tube containing the cholera vaccine, Euvichol, to the three-component format used for another cholera vaccine, Dukoral, which needs to include a buffer. If the vaccine is to include acid-susceptible materials, it may be possible to develop a two-component system to facilitate storage and delivery. There was certainly a lot of interest in considering how this might be accomplished, and active investigations to meet this challenge are needed now.

There was consensus on targeting a low administration volume, ease of use, and increased shelf life for novel enteric vaccines for oral delivery in infants. The Dukoral presentation has already been optimized for programmatic use and the improved presentation is in high demand highlighting the importance of formulation and presentation considerations [33,34]. The potential benefit of a mucosal adjuvant like dmLT will likely make it difficult to have a one-component system like the cholera vaccines Euvichol and Shanchol, which do not currently require a buffer. Further, optimal formulations may require replacement of bicarbonate buffers with more stable buffer systems such as the citrate-based buffer system that has been developed for rotavirus. Oral vaccine formulations need to be relatively thermostable to ensure maximum coverage in all settings. Early adoption of oral vaccine formulations and presentations that include frequent coordination with regulatory bodies should facilitate arrival at an acceptable product.

The workshop attendees agreed that similar workshops concerning issues relevant to formulations and presentation of vaccines for oral administration would be beneficial to the vaccine community involved in development and applications. This VASE Conference workshop provided a much-needed platform for researchers in the vaccine community to share research progresses and ideas, discuss problems and solutions, and promote interdisciplinary collaborations. The authors hope that this workshop report will stimulate interest among a wide group of key stakeholders involved in development of oral enteric vaccines for infants.

Declaration of interest

The authors report no conflict of interest.

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Authors' contributions

ML and JW jointly facilitated the workshop and drafted the manuscript. All authors attest they meet the ICMJE criteria for authorship. All authors read and approved the final manuscript.

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