Evaluation of Existing and New Typhoid Vaccines
Overview
A number of developers and sponsors of Typhoid vaccines joined with regulatory experts from relevant countries to provide the DCVRN with an up to date view of

- The importance of Typhoid fever disease in the world today.
- The current status of existing Typhoid vaccines
- Current use of Typhoid vaccines in endemic situations and for travelers
- Initiatives intended to enhance and optimize the use of typhoid vaccines
- The set-up of manufacturers of typhoid vaccines in Developing Countries
- The status of clinical studies to support registration and use of these DC manufactured vaccines and other regulatory issues
- New developments with Typhoid Vi-conjugate vaccines
- New developments with Typhoid whole cell (and recombinant) vaccines

Other:
- WHO Recommendation (TRS-927) for Pneumococcal conjugate vaccines
- Use of adjuvants for novel investigational vaccines

The DCVRN has considered these presentations and has prepared a set of issues that are of particular concern for member developing countries where clinical studies need to be considered for conduct approval, or for approval of a product licence. These are attached.
DCVRN’s considerations for clinical evaluation of typhoid vaccines

**Vi Vaccines:**
The ELISA assays for Vi vaccine immunogenicity need to be standardized.

The relevance of the TRS guidance on “synthetic” Vi antigen should be considered.

**Vi Conjugate vaccines (under development)**
The development of Vi-conjugate vaccines holds much promise for improving the safety and immunogenicity of existing vaccines.

The WHO could consider the preparation of TRS recommendations for Typhoid Vi-conjugate vaccines. The potential of synthetic Vi-conjugate antigens should be included.

The WHO TRS for Pneumococcal-conjugate vaccines can be used as a model for guidelines for Vi-conjugate vaccine/s

The establishment of immune correlates of protection should be considered, and their relationship to vaccine efficacy.

Clinical studies should consider the duration of protection and the requirement for booster or revaccination.

**Novel Live Oral Typhoid Vaccines**
The risks to HIV+ and other immuno-compromised individuals (and contacts) should be considered during clinical studies. This would include the level of shedding of the vaccine agent and the risks to contacts and the risks and possibility of vaccine-agent reversion to virulence.

The use of human challenge studies to establish efficacy is considered inappropriate and unethical in developing countries.

**General**
The potential for interference between Typhoid vaccines and concomitant vaccines should be investigated during clinical studies. This should include studies of adults (travelers)

The influence of infant malnutrition, maternal antibodies and breast feeding should be considered during clinical study evaluation.

The possibility that a different vaccination schedule may be needed for residents in endemic areas, from that suitable for transient travelers should be considered.

The development of suitable animal models for evaluation of typhoid vaccines should be encouraged.

Clinical studies should include monitoring of extended adverse events. E.g. Complications occurring after natural typhoid infection of vaccinees (hemorrhage, perforation); or the rate of establishment of the carrier state and duration.

The potential for cross-protection for Paratyphoid A & B, and efficacy of the vaccine in preventing the typhoid-carrier state, should also be considered during clinical studies.

The DCVRN will address a request to the ECBS that suitable standard preparations for Typhoid vaccines and immunogenicity assays should be established. The ELISA assays for Vi vaccine immunogenicity need to be standardized.
Summarized Presentations

Leon Ochai: IVI Seoul. **Epidemiology and burden of typhoid fever**
Currently accepted (Crump et al 2000) that there are about 21.6 million new infections with *Salmonella typhi* per year, with 0.1% mortality. This disease is well established in the most impoverished nations, particularly in South and South-East Asia, Africa and parts of South America. IVI is conducting a prospective surveillance program in the 5 Asian countries considered most at risk, and include typhoid, cholera and shigella disease. The results to date confirm earlier estimates, and reveal that the rate of disease in infants is higher in areas with high disease prevalence. The limitations of the current data were noted.

R Carpis. IVI, Seoul. **Current status of typhoid fever vaccines**
An over-view of currently available vaccines and their properties was provided.
**Vi polysaccharide vaccine.**
- 60-70% efficacy. Simple, stable, easy to manufacture. Many manufacturers exist, including in the developing countries.
- T-cell independent = not immunogenic in young children. Duration of immunity is limited & regular boosters are needed

**Live attenuated TY21a vaccine.**

**New developments**
- **Vi-conjugate vaccine:** Gives ~95% protection. Anamnestic response.
- **Targeted mutant – live oral vaccines.** Single dose, high protection.

G Houillon Sanofi-Pasteur Lyon. **Typhim Vi vaccine**
The originator Vi-vaccine. Registered in 1988 (France) for use from 2 years of age. Injection, 25 µg per dose. Freeze-dried. Revaccination recommended at 3 year intervals until 15 y. Mostly used in travelers. 1 µg/ml of specific antibody is considered protective.

Guido Dietrich: Berna Biotech. **Vivotif – TY21a**
A mutant strain derived from vaccine strain TY2. Vi neg.
Formulated as dried viable bacteria (plus portion non-viable) in oral capsules.
- 3 doses at 2 day intervals. Used widely by travelers.
- Immunogenic: secreted and circulating antibodies, as well as reactive T cells.
- Protective efficacy 62-95%. The liquid formulation is more immunogenic than the capsules. There is Evidence for herd “protection” when widely used.
- A low rate of adverse events occurs  The vaccine cross-protects against Paratyphoid A disease.

Query: Has the contribution to immunogenicity of the non-viable cell content of the vaccine been established? A: there is evidence to suggest that these have no role in development of immunity (GD could offer to provide a review of such evidence)
Kevin Killeen. AVANT USA. TY800
AVANT specializes in the development of live (oral) vaccines, attenuated by directed mutagenesis, including typhoid and cholera.
Target: oral, single dose, safe, effective (rapid and durable immunity), stable.
Strategy: delete virulence genes – can also insert genes to broaden or enhance immunogenicity. Metabolic mutants are less immunogenic.
Phase I completed. Safe, tolerated & immunogenic Antibodies & T-cells. Vi +.

Query: What is the loss of viability during the Drying process? A: ~ 50%.

Dennis Kopecko. CBER-FDA R&D, USA. Multi-functional vaccines
Targeting diarrhoeal diseases. Uses TY21a as vector for inclusion of genes expressing antigens from other pathogens. Favours a liquid formulation. Vi -
Priorities include Shigella vaccines. Spray dried (stable). Or liquid. Use VVMs.
Shigella sonnei & dysenteriae LPS genes expressed in TY21a are protective in a mouse model. Eventual aim will be suitable for inclusion in EPI program.

Query: Could human vaccine-challenge trials be done. A: Some have been accepted in USA, as (whole-cell) typhoid vaccine has no correlate of protection.- probably not in the current ethical climate – but these will give best information..

Jill Makin Emergent Europe. Experimental Single-dose Typhoid vaccine
Use technology developed from Biodefence program. Targeted metabolic mutants (AroC & SSV SPI-virulence) live-attenuated cannot replicate in macrophages.
Formulated as 1-dose, FD, oral (with antacid). Stored at 2-8°C.
Manufacture – cGMP. Full seed strain / manufacture / QC-QA controls.
Pre-clinical: safe in animals.
Phase I completed in 100 adults in UK & USA. Not analysed yet.
Query: Safety of AroC mutants? A: OK also includes SSV mutant.
Query: Safety in HIV+ recipients? A: Will be included in further studies, where relevant.
Query: Is it Vi+ ? A: Yes, but probably unimportant.

Shousun Chen Szu. NIH USA. Enhanced Vi-conjugate vaccine
Vi vaccines have reduced adverse effects vs whole-cell vaccines; proven efficacy in adults; but: short duration immunity & ineffective in young children.
Developed conjugate vaccine using VI + rEPA (exoprotein A from Pseudomonas)
5 lots have been produced (cGMP) for clinical trials as part of a FDA – IND.
Showed ~90% protective efficacy (IgG level of 3.52 ELISA units/ml ~0.5 µg IgG)
Preparing new study using EPI schedule in infants & boost at 12 months.
Query: How was placebo control approved by Ethics. A: Unavoidable as the planned control vaccine was not available. All subjects received vaccine at end.
Query: What is the expected duration of immunity in adults? A: Probably >10 years.

New Idea The structure of Vi antigen is known: O-acetylated poly-galacturonic acid..
Tested O-acetylation of commercial (US$5/g) galacturonic acid. This synthetic Vi antigen cross-reacts with immune sera. A conjugate vaccine using this is in preclinical evaluation. If successful could be used to support a claim for physico-chemical characterization of Vi antigen.
**Zeng Ming. NICBPB, China. The need for Typhoid vaccine in China**

It is known that typhoid causes disease in China. (~ 4 cases/100 000). The incidence of Paratyphoid disease appears to be increasing. The old whole-cell injected typhoid vaccines have been discontinued, but a purified Vi Vaccine has been produced & registered in China. 30 µg/dose. It has been released for use since 2000.

The Lanzhou Institute has started development of Vi-conjugate vaccines. Vaccines against *S. paratyphi A* OSP-conjugate, and OMP candidates are in pre-clinical evaluation in animals.

There is concern that these new vaccines may be too expensive to develop.

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**Nguyen Duy Thuy. CENCOBI, Viet Nam. Vi vaccines in Viet Nam [Dr S Szu]**

Viet Nam manufactures its own Typhoid Vi vaccine. The development steps are described leading to registration, including clinical studies. Immunization of adults has been shown to be effective. An earlier study had shown equivalence of Viet Nam Vi vaccine to the Typhim Vi vaccine in young children.

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**John Clemens. IVI, Seoul. The DOMI Program experience in Asia**

The Diseases of the Most Impoverished program sought to evaluate the suitability of existing typhoid vaccines for inclusion in a possible school-based immunization program in high disease-burden regions.

The outcome was that the existing Vi vaccines would be most likely to be suitable in these circumstances. Based on this, several studies in identified countries indicated that >70% of school children could be protected and the safety was acceptable. Further studies are ongoing to confirm this.

Opinion surveys of potential target populations indicate acceptability of such a program. Cost analysis is in favour of such a targeted vaccination program. Development of antibiotic resistance in circulating strains mandate an urgent response – and vaccines will be a key part of this.

A four-arm controlled study with two clusters will compare the effectiveness of the proposed vaccination strategy. If more effective Vi-conjugate vaccines become available they could be incorporated into any of the planned programs. The availability of oral vaccines, if cost-comparable could also be included.

Query: What would be the intended revaccination interval. A: Every 4y until 15y

Query: Would the putative “herd protection” confound the comparator arms of the proposed studies? A: This has been taken into account in the geographical dispersion of the various clusters.

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**Shousun Chen Szu. NIH USA. Vaccine trials in China**

The establishment of local manufacture of Vi vaccines in China has been supported by the NIH over a number of years. There are now Vi vaccines manufactured in China, Viet Nam, India and Indonesia.

Clinical studies with several of these have shown general equivalence in safety and efficacy.

As the Vi polysaccharide is well characterized in terms of its physico-chemistry, and if the cGMP and the QC/QA systems in use follow international requirements for other polysaccharide vaccines, and all activities (should be) documented to facilitate FDA inspection during IND submission, then it is proposed that the key physico-chemical
parameters can be used as a reliable estimate of vaccine immunogenicity (potency). From other studies it appears that efficacy can be predicted from the immunogenicity (3.52 ELISA units/ml).

If this is acceptable to regulatory authorities, it would simplify the licensing of new production facilities and new (standard) Vi vaccines.

Query: This concept is novel for a biological medicine, and particularly for a vaccine. A: the evidence is available and can be further refined.

Query: Are these vaccines suitable for developing countries? A: The environment may dictate that the doses used would have to be adapted for specific areas.

Query: How is the potency of the live oral (recombinant) typhoid vaccines estimated? A: By viable count, once antigen expression and quality has been established.

**General discussion:**
Concern was expressed regarding the relative prices of the different vaccines used in Asia. It was noted that cost of the vaccine is only one element in the commitment to use a vaccine and each country would make its own calculation.

The source of seed strains for the extraction and purification of Vi antigen was discussed. Although some use the long-known TY2 strain, there is value in using a local isolate to establish a seed stock, as these will have a well known history of freedom from TSE contamination so that registration may be facilitated. The use of *Citrobacter freundii* has been mooted but so far there is no interest.

The establishment of serological correlates of protection was discussed. The comparative assay of potency / immunogenicity is of concern where there is no established International reference standard. Bridging studies of vaccines are difficult to substantiate without clear correlates of protection. The level of 3.52 ELISA units/ml could be considered for use as a correlate of >90% protection, based on the studies described by Dr Szu, above. The WHO should be requested to establish a suitable international standard for Vi (and Vi-conjugate) vaccine immunogenicity assays.

It was noted that the lack of a suitable animal model for typhoid fever has hampered the ability to screen new vaccine candidates. *S.typhimurium* in mice has been used as a model but is unsatisfactory for several reasons. Pre-clinical toxicology tests have been done for some vaccine candidates using animals and also human cell-cultures.

The possibility that typhoid fever could be eradicated by combined use of public health measures, vaccines and appropriate therapy, was discussed. This is theoretically possible given the absence of non-human vectors and current technology but was considered as not yet feasible due to difficulties in access to care by the most affected communities. The problem of long-term human disease carriers was also considered.
**Duncan Steele: WHO / IVR. Challenges of Typhoid Vaccination**
The current WHO position on typhoid vaccination is not generally supportive of widespread programs. The inactivated whole-cell vaccine – now discontinued – has given typhoid vaccines a poor image. The use of other vaccines should be a National decision based on the burden of disease & cost analysis. Integration of typhoid vaccines into the school-age DT vaccine program is proposed. More studies of safety and efficacy are needed, and development of better vaccines is encouraged.

National policies generally favour improved sanitation and water-supply over vaccines. However, the under-reporting of disease, and lack of public awareness of the disease burden results in lack of priority for vaccines.
The epidemiology of the disease, the immune correlates of protection and insensitive diagnostic techniques are uncertainties that hamper improved control strategies.

The best vaccine for the local circumstances must be identified, and further programmatic and financial issues must be resolved before an effective vaccination program can be introduced.

**Luis Jodar: IVI Seoul. Control of Typhoid fever by vaccination**
Following on from the above presentation, The steps taken by the DOMI program to fill the knowledge gaps (see Clemens, above) have resulted in a proposed vaccination strategy for typhoid fever.
It appears that introduction of the vaccine in areas of high disease burden, where Public Health measures are not immediately available would have an impact on disease.

Query: Would such a decision impact on introduction of other new vaccines.
A: WHO should give guidance on local, regional and National priorities.
Query: Does the production capacity exist to meet such a program.
A: It appears that there is substantial capacity for affordable Vi-type vaccines.
Query: The true situation in Africa remains unknow. A: Correct.

**Dennis Kopecko: CBER – FDA – USA. Regulatory Challenges for Clinical Evaluation of Typhoid Vaccines.**
The Federal Regulations for evaluation and licensure of new vaccines was outlined. The IND process was also described, including the requirements for developer-regulator interaction, Pre-clinical studies; and the phases of Clinical Study expected by the FDA. The need for increasing cGMP quality of material for the different stages pre-clinical to clinical study was noted. Stability of potency and preservative must be established. During the clinical studies the importance of Adverse Event reporting, Validated immunogenicity assays, comparison with existing vaccines, and the possibility of challenge studies in humans should be considered.
Query: Paratyphi A & B shoul also be considered during Clinical studies. A: Yes
Query: Is it intended that Vi antigen can be characterized sufficiently to no longer be regarded as a biological. A: Not at that point yet.
Query: Is disease (fever), or bacteraemia a suitable end point in protection studies. A: best studied in human challenge model. Most suitable for travelers-type vaccine.
Query: What are the disadvantages of the live-oral vaccines. A: reversion of mutations – deletion mutants make this almost impossible.
Query: Is there value in conduction Phase I studies in the USA. A: Yes – ensures expert review.
ADDITIONAL PRESENTATIONS

**Luis Jodar**: IVI Seoul. *Production & Control of Pneumococcal Conjugate Vaccines*

The WHO 54th Report – Technical Report Series [927] Guidelines for manufacture and control of pneumococcal conjugate vaccines was summarized. This can be used as an indication of issues that should be addressed in updating the existing Typhoid Vi polysaccharide vaccine guidelines, or in drafting new guidelines for Vi-conjugate vaccines.

The complexities of the multi-valent pneumococcal vaccines were noted. There has been a strong emphasis on physico-chemical characterization of the antigenic components, and potency is based on serological correlates. It is assumed that protection is indicated by similar antibody levels to each of the antigenic components.

Reference materials are being established.

Query: Are all serotype antigens the same. A: This is the assumption.

**Martin Friede**: WHO IVR. *Use of Adjuvants for Investigational New Vaccines*

New adjuvants are of concern to regulatory authorities. Several of the new vaccines under development require more potent adjuvants than those currently in use, due to the small size of peptide components or low immuno-stimulatory properties. New vaccines may also use novel adjuvants to target specific arms of the immune response.

Examples include: AlPhos + Lipid A (HPV vaccine), RC525-MPL analogue (HBV), MF59-squalene (Fluad) and Virosomes (HepA Berna)

Some of these may be of safety concerns in specific populations and should be subject of rigorous pre-clinical and clinical studies.

It has been proposed that the novel adjuvants will be considered as an Active Pharmaceutical Ingredient with a full dossier. Full pre-clinical & toxicology testing on the individual proposed adjuvant would be expected prior to formulation of a vaccine.

Full characterization is required, assays to establish quality, pre-clinical pharm&tox studies, and clear rationale for use with a particular vaccine. Consideration should be given to adjuvant interference with vaccine antigen assay and potency tests. Consistency of production and of efficacy should be proven. The dangers of an auto-immune response should also be studied.

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